

**Crop rotation effects on soil and plant health in a dryland production system
in South Africa**

By

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Declaration

I, Knowledge Mushonga declare that this thesis/dissertation, which I hereby submit for the degree PhD in Plant Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature:



Date:

24/04/2023

Ethics Statement

The study is registered as Ethics Application NAS286/2020 under the title "Crop rotation effects on soil and plant health in a dryland production system in South Africa". The author declares that he has observed the ethical standards required in terms of the University of Pretoria's Code of ethics for researchers and the Policy guidelines for responsible research.

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Abstract

Soil and plant health are key limiting factors of crop production in conventional tillage systems under rain-fed conditions in South Africa (SA). Historical evidence shows that different crop rotation practices result in soil health legacies that are linked to crop type and functional soil biota. On drylands in the eastern Free State (EFS), crop rotation with conventional tillage is commonplace but overlooks soil and plant health. The objective of this study was to highlight how soil and plant health in a conventional tillage field experiment can be assessed for four crop rotation sequences through a description of spatio-temporal changes in soil nutrient composition, pathogen DNA concentration, and crop yields in EFS dryland systems as well as in greenhouses.

A dryland field study on the potato (*Solanum tuberosum*) and maize (*Zea mays* L.) uniformity trials in season 2015/16 and season 2016/2017, indicated that field soils were relatively uniform with no abiotic imbalances. The maize uniformity trial in season 2016/17 provided the baseline data for a long-term field experiment investigating soil and plant health under dryland cultivation in the EFS.

Greenhouse studies of plant-soil feedbacks (PSFs) using two-phase experiments showed indirect effects with positive plant growth recorded for all crop species tested. PSF results suggest that sugar bean (*Phaseolus vulgaris* L.) rotations were strongly associated with specific beneficial microbiota. In terms of PSF crop sequences, sugar bean in sunflower (*Helianthus annuus* L.) rotations offers soil resilience to pathogens associated with sunflower monoculture. Results of pot experiments showed that field-collected soils from Potatoes-Maize-Sunflower-Maize were significantly more suppressive to *Fusarium graminearum* but significantly more conducive to the negative effects of *Rhizoctonia solani* AG 2-2 IIIB on test plant metrics. The low severity of root rot disease in broad beans (*Vicia faba* L.) planted in the field-collected Potatoes-Maize-Maize-Teff

soil, indicate that grass-dominated rotation promotes suppressive soil conditions against *R. solani* AG 2-2 IIIB.

Sunflower seems to have the potential of increasing fungal to bacterial PLFA biomass composition ratios, supporting saprophytic microflora and microbial enzyme activities. Furthermore, the inclusion of sunflower, as the third rotation crop, will possibly create legacies of a less compacted subsoil and a sharp-increase in *R. solani* AG 3-PT population in the following season's maize crop. The incorporation of maize or teff (*Eragrostis tef*) in maize sequences in the final season, increased soil inoculum of *R. solani* AG 3-PT DNA. This suggests that these grasses minimized the resilience of field soils against build-up of *R. solani* AG 3-PT DNA levels within four years. In the final season (2018/19), the soybean (*Glycine max* L. (Merr.)) rotation sequence (Potatoes-Maize-Soybean-Maize) significantly reduced *R. solani* AG 3-PT DNA legacies.

There is an urgent need, in the sandy soils of the EFS under rain-fed conditions, to raise soil pH and increase soil organic matter and micronutrients to assist growers to increase crop yields and improve soil health. The accurate detection and quantification of *R. solani* AG 3-PT would enable farmers to link disease thresholds with the development of effective integrated management plans for dryland crop rotations.

Key words: Crop rotations; dryland crops; soil health legacies; plant-soil feedbacks; *Rhizoctonia solani* AG 3-PT, *Rhizoctonia solani* AG 2-2 IIIB, *Fusarium graminearum*.

CHAPTER 1

Introduction

1.1 Background and Motivation

1.1.1 Soil and Plant Health in Dryland Farming

Janzen et al. (2021) define ‘soil health’ as a metaphor that considers the capacity of the soil to; advance important ecosystem functions beyond ecosystem services and mere soil property composition. Conceptually, plant health focuses on the effects of biological or physicochemical factors in and around the plant and the plant’s nutrition (Savary et al., 2016; NSTF, 2021). The assessment of crop health, involves the monitoring and recording of multiple sets of injuries that may occur simultaneously or successively in the course of a crop cycle or season (Savary et al., 2017). For this study, crop health is defined as any biological or physicochemical factors occurring in and around the plant and the plant’s nutrition that may harm plant form, physiology and performance in a given geographical and temporal setting (Berendsen et al., 2012; Savary et al., 2016; NSTF, 2021).

In dryland crop production systems found in the Eastern Free State (EFS) province of South Africa (SA), crop rotation using conventional tillage is common. Little research has been done on the effects of region-specific crop rotation practice on soil and plant health under dryland systems using conventional tillage management. In dryland systems, soil and plant health including water conservation in the soil profile are currently an absolute priority. There is evidence that conventional intensification of agriculture, such as extensive- and intensive crop rotations, reduce soil biota diversity and food webs, when compared to less intensively used grasslands (Tsiafouli

et al., 2015). A better way forward could be ecological interventions which would minimize soil acidity, compaction and increase soil organic matter content while improving the type and quantities of microorganisms essential for soil and plant health.

Certain crop species are known to induce changes in the legacies of soil physicochemical and biological concentrations, which may in turn influence overall soil health and plant health. Rotations of crops improves soil and plant health by cultivating soil microbial diversity legacies responsible for reducing the impact of crop-specific soil borne diseases (Reinhold-Hurek et al., 2015; Schlatter et al., 2015; Schmidt et al., 2016; van Dam & Bouwmeester, 2016; Pini et al., 2017). In crop rotations, these plant-soil interactions create soil legacies under various microclimatic factors, plant litter inputs and rhizosphere effects which influence the growth and health of subsequent crops (Berendsen et al., 2012).

The plant litter inputs or straw residues that are produced seasonally from crop rotations need to be buried into the plough layer to; avoid the spread and risk of disease (Battaglia et al., 2021; Cherubin et al., 2021), improve soil electrical conductivity and diversity of soil fungal community as well as retain mineral nutrients below-ground (Su et al., 2020). The plant root microbiota (fungi and bacteria) is a fundamental trait in the below-ground trophic interactions between the mutualists (growth-promoting and plant health-promoting bacteria) and antagonists (pathogens, competitors and predators) (Garbeva et al., 2004; Bais et al., 2006; Raaijmakers et al., 2009; Berendsen et al., 2012; Bulgarelli et al., 2013). The fungal community in the rhizosphere serve additional below-ground root functions, through the acquisition of nutrients from soil for plant growth.

In this study, we used the fungal PLFA markers C18:2 ω 6c, C18:1 ω 9t, C18:1 ω 9c, C18:2 ω 6t and C18:3 ω 3c, and bacterial PLFA markers C14:0, i-C15:0, a-C15:0, C15:0, i-C16:0, C16:19, C17:0,

i-C17:0 and C18:17 (Frostegard & Bääth, 1996; Tavi et al., 2013; Willers et al., 2015; Schwab et al., 2017) as biological indicators to estimate soil bacterial and fungal biomass composition.

1.1.2 The Role of Conventional Tillage Systems in Soil Health

Poor soil health in cultivated soils is a consequence of long bare fallow periods, excessive tillage, continuous monoculture, and depletion of soil organic matter (Weil & Brady, 2017). Tillage disturbs the soil physical properties, consequently destroying the habitats and niches of soil biota (Tsiafouli et al., 2015; Bender et al., 2016). In SA, conventional tillage practice threatens soil biodiversity, more than conservation tillage, by promoting rapid decomposition, or the loss of soil and organic matter (Tsiafouli et al., 2015; FERTASA, 2016). We proposed a four-year crop rotation field trial made up of four rotation sequences of strategic dryland crops such as maize (*Zea mays* L.), sugar bean (*Phaseolus vulgaris* L.), soybean (*Glycine max* L. (Merr.)), and sunflower (*Helianthus annuus* L.); on a single site under rain-fed conditions in the Eastern Free State (EFS) Province in South Africa (SA).

In SA, soil organic matter content in cultivated soils has declined to levels that have reduced soil quality and most of the organic matter is respired into the atmosphere as carbon dioxide (Swanepoel et al., 2016). Prolonged crop cultivation under semi-arid conditions in South Africa has a legacy of soils poor in both labile and stable organic matter fractions (Kotzé et al., 2016). However, these soil carbon fractions can be replenished by putting degraded arable land under perennial pastures (Loke et al., 2019). Under extreme loss of organic matter, a perennial grass crop should be included in the rotation system to slow down the mineralization of organic fraction (FERTASA, 2016). Sustainable dryland production should promote organic matter and the associated biological activity caused by microbial diversity (Dixon & Tilston, 2010).

The low organic matter content in sandy soils can also be improved by increasing plant litter inputs and rhizodeposits, which in turn feed the soil decomposer community responsible for the further degradation of the moderately labile and recalcitrant carbon fractions in the litter (stover) inputs (Sait, 2003; Bardgett, 2009; Cherubin et al., 2021). The organic matter content of soils will continue to decline in tilled soils if more favourable conditions are created for oxidation (FERTASA, 2016).

1.1.3 Possible Agroecological Interventions

Agricultural intensification in sandy soils under summer rainfall and temperature requires about 1.5 % organic matter to be productive (FERTASA, 2016). Agricultural intensification has a negative impact on soil biodiversity across different climate and soil conditions (Tsiafouli et al., 2015) despite offering relatively quick, short-term, and visible solutions to farmers (Kleijn et al., 2019). On the other hand, ecological intensification involves the building of networks that support ideal below-ground and above-ground interactions, soil resilience, managing tillage and climate history to ultimately improve soil and plant health in the long-term (Randall & Smith, 2019). So, interventions that are based on ecological principles offer longer-term and more environmentally friendly solutions to farmer problems than intensive agriculture.

In other words, ecological intensification is a farming approach which promotes soil life and ecosystem services, accompanied by a moderate application of anthropogenic inputs; reduced nutrient losses; and sustainable crop production (Bender et al., 2016). Therefore, under ecological intensification, the application of chemical inputs such as fertilizers and pesticides are combined or replaced with beneficial ecosystem processes or services (Kleijn et al., 2019; Baxter, 2021). In this study, repeated measurements of soil and plant health were done at the end of the growing period or plant physiological maturity (at harvest) to understand the changes in soil composition

and crop yield legacies, so that the farmers could decide on the best management options for the next crop. Previous studies in SA indicate that the microbiome in the root zone is influenced seasonally by plant type and growth stages (Oberholster et al., 2018).

The accumulation of crop residues more than decomposition rates have the potential of improving the perennial problem of low soil organic matter in SA soils, better than ploughing practices (Motema et al., 2020). The shift from conventional tillage to no-tillage increases C storage in soil possibly due to reduced rates of soil respiration (Luo & Zhou, 2006). In addition, the fertility of sandy soils according to Jhonson (2009) can be improved by increasing the amount of humus, resulting in better soil aggregation which slows the downward movement of water.

1.1.4 Eastern Free State Dryland Farming

The field trial is done in the region which is characterized by natural flora made up of 20% trees, 70% grass and 10% shrubs (Dube et al., 2019). Crop productivity and food security in the FS Province depends greatly on the risks and impact of climate change in the sandy soils known to have poor water holding capacity, nutrient runoff, well-drained and deep fertile soils ideal for maize production. (Moeletsi & Walker, 2012). Farmers consider drought as the most extreme hazard over most parts of the FS including the wettest parts of the region (Moeletsi & Walker, 2012). A dry climate is associated with less resilient soil systems, especially in semiarid ecosystems where recovery mechanisms rely on the frequency and duration of rainfall events (Seybold et al., 1999). Plant-soil feedbacks hypothetically drive the recovery of soil functions (Zhao et al., 2021a), for example, in annual cropping systems the previous and subsequent crop sequences and management practices can either promote recovery or degradation of soil (Seybold et al., 1999).

Dryland agriculture is characterized by unpredictable risks and complexities associated with limited water resources; variation in climate patterns and trends; land degradation; socio-economic factors; pests and diseases (Savary et al., 2017; Stringer et al., 2017). Therefore, farmers are in need of farming practices that improve crop production under the meteorological and soil characteristics. These studies on region-specific crop production systems make it easier to share and explain the importance of soil and plant health with dryland producers in the EFS Province.

Soil acidity in the EFS has been linked to soil degradation (Schroeder et al., 1994; Fey, 2001; Hoffman & Ashwell, 2013; Uys, 2018). In addition, Tully et al. (2015) describes acidic soils as manifestations of non-erosive soil degradation. Subsoil acidity is very common in higher rainfall areas of SA, while saline soils occur in drylands due to the accumulation of salt in the absence of drainage (FERTASA, 2016). According to Haarhoff et al. (2020), maize production systems in South Africa are often mismanaged and highly degraded. Selecting crop species with low excess cations in their litter inputs, low acid production, and high capacity to take up soil nitrate may be a potential solution to minimizing subsoil acidification (Rengel, 2003).

The EFS is a typical example of a region-specific crop production system under summer dryland receiving high annual precipitation, seasonal and the patterns are variable. The farmer maintained our field as an experimental or treatment field, with their own production systems as a control field. This design also allowed the farmer to compare the differences between the experimental plots and the control, beyond our study. In the same region, studies using metagenomics have shown that long-term conventional tillage practices under dryland conditions resulted in shifts in bacterial diversity and putative function; as they influenced soil pH, mineral content, and organic matter in soils (Dube et al., 2019). A sustainable conventional tillage practice should reduce production risks, contribute to environmental conservation and social responsibility by farmers,

avoid soil and water degradation; as well as maintain or improve crop production and the profitability of the enterprise (FERTASA, 2016).

The study sought to investigate crop rotation effects on soil and plant health by answering the following research question asked by farmers: “Are temporal effects of crop rotation sequences in the EFS moving toward healthier soil under conventional tillage?” In order to answer this question, the farmers in their working field trials must work with researchers in region-specific studies, to establish thresholds or theoretical 'reference states' of soil health, allowing them to monitor the health status of farm soils (Morgan & Cappellazzi, 2021). According to the Soil Health Institute, the assessment of soil health seeks to; reduce soil perturbation, build soil resilience, increase crop diversity, promote plant roots presence, and integrate animal husbandry (Morgan & Cappellazzi, 2021). The goal of the Institute is to "assess soil health through evaluating soil functions of water storage and cycling, carbon storage and cycling, nitrogen cycling, and erosion resistance". Our choices of soil tests or indicators were based on affordability, sensitivity to crop rotation management practices for four years, general use by farmers and easy correlation to fertility and yield (Stott, 2019). The review by Lehmann et al. (2020) provides a comprehensive summary on the relevant soil health indicators for the future and the co-benefits such as soil resilience -and- resistance.

1.2 Research Aim and Objectives

The aim of this study was to assess how different cropping sequences (shown in Table 1.1) will affect soil chemical, physical and microbial properties over time to determine the most optimal crop combinations that will ensure long-term sustainability of production in the EFS.

Table 1.1 Different rotation crops included in a four-year rotation system.

Year	Growing season	Rotation system number			
		1	2	3	4
1	2015/16	Potatoes	Potatoes	Potatoes	Potatoes
2	2016/17	Maize	Maize	Maize	Maize
3	2017/18	Maize	Sugar beans	Soybeans	Sunflower
4	2018/19	Teff	Maize	Maize	Maize
Abbreviation*		PMMT	PMSBM	PMSOYM	PMSUNM
* The bold letter in the abbreviation of the rotation system indicates the crop planted in that year/season.					

Potatoes in year 1 were followed by maize on all plots in 2016/17 (Year 2/ season 2017), when the physical, chemical and microbial observations of the relevant soils commenced. Third year rotation crops were planted in 2017/18 (season 2018) and the 2018/19 summer growing season was designated year 4 (season 2019) of the four-year rotation.

Specific objectives include the following:

- To assess the effects of maize, sugar beans, soybean, sunflower and teff as follow-up crops on soil chemical, physical and microbial properties for the four different rotation systems.
- To start investigating the effects of the different rotation systems on the presence of specific soil-borne plant pathogens of potatoes and other dryland rotation crops.
- The long-term objective of the study is to optimise crop rotation systems for potato production in the Eastern Free State in order to improve: soil health, soil physical and chemical conditions, and profitability of dryland potato production in the region.

This thesis is subdivided into the introduction, literature review, seven working chapters (3 to 9) and a general discussion. Thus the following chapters are presented:

- Chapter 1: Introduction.

- Chapter 2: Literature review.
- Chapter 3: Maize uniformity trial study for baseline soil and plant health status in a dryland system in South Africa.
- Chapter 4: Soil and plant health in the root zone of four crops under dryland field conditions.
- Chapter 5: Plant-soil feedback responses of four dryland crop species under greenhouse conditions. (Published as; Mushonga, K., Steyn, J.M., Swart, W.J., & van der Waals, J.E. (2020). Plant–soil feedback responses of four dryland crop species under greenhouse conditions. *Plant-Environment Interactions* 1:181–195.)
- Chapter 6: Soil resilience in greenhouse rotations of four dryland crop species via plant-soil feedbacks.
- Chapter 7: Region-specific crop rotation effects on soil health and crop yield in a dryland system.
- Chapter 8: Crop rotation effects on soil suppressiveness against *Fusarium graminearum* and *Rhizoctonia solani* AG 2-2 IIIB in bioassays with artificial pathogen inoculation under greenhouse conditions.
- Chapter 9: Impact of region-specific crop rotation legacy effects on soil borne *Rhizoctonia solani* AG 3-PT DNA levels in a dryland production system.
- Chapter 10: General discussion.

CHAPTER 2

Literature Review

2.1 Soil and Crop Health Definitions

Janzen et al. (2021) define ‘soil health’ as a metaphor that considers the capacity of the soil to; advance important ecosystem functions beyond ecosystem services and mere soil property composition. Plant health focuses on the effects of biological or physicochemical factors in and around the plant and the plant’s nutrition (NSTF, 2021; Savary et al., 2016). There are numerous definitions of soil and plant health reviewed in Janzen et al. (2021) and Berendsen et al. (2012), respectively. However, none of the definitions are absolute, since the response to a crop treatment may be different on two different types of soils or on two areas which had different treatment previous to the time of application of the crop.

2.2 Crop Production System– Dryland Farming

Drylands constitute 41% of the land surface on earth (Reynolds et al., 2007). They are situated in areas naturally endowed with high solar energy, rich plant biodiversity and 50 % of the world’s animal husbandry (Stringer et al., 2017). The dryland ecosystem provides many opportunities for agricultural diversification and intensification, and increasing the storage of carbon below-ground (Stringer et al., 2012). Haarhoff et al. (2020) propose that research should focus on conducting diverse crop rotations and quantifying fixed-nitrogen contribution to subsequent crops. For decades, continuous maize monoculture has been the most popular and practiced cropping system across the three major dryland maize producing regions in South Africa including the EFS (Nel et al., 2003). Commercial dryland cultivation was established in SA between 1908 and 1913 (Phillips,

1999) and today, agriculture contributes 3% to SA's economic activity (Stats SA, 2020). Dryland farming improves food security, provides a source of employment and is the main driver of development in developing countries (Stringer et al., 2017). Crop production depends on the grower's strategy for sustainability in specific economic, environmental and social contexts (Swanepoel et al., 2018). Under rain-fed or dryland systems, yields are a function of soil water conservation, crop frequency and crop diversity (Nel & Loubser, 2004). In most summer rainfall regions, the fallow period runs between July and October, about 3 to 4 months long, before planting the succeeding maize crop (Haarhoff et al., 2020). Fallow periods can also last 11 to 12 months to allow build-up of soil water content and reduce risk of crop failure and poor yields. Crop rotation in the summer rainfall area of South Africa showed no yield advantage compared to monoculture cropping in three field trials (Nel, 2005). In other studies, maize yields increased due to rotation under suboptimal or no fertilizer application rates (Nel, 2005).

2.3 Dryland Farming in the Free State province of South Africa

The FS Province occupies 10.6% of SA land area and, is a summer rainfall area receiving 300-800 mm of precipitation. The field trial receives the first summer rains in mid-October to early November and is located in a high maize production regions of the EFS, specifically, Thabo Mofutsanyane district in Petrus Steyn, under the Nketoana municipality (Moeletsi & Walker, 2012). The biome has high summer rainfall and the greatest potential to influence plant-soil legacies, in particular, soil properties and productivity of grasses like maize (Trivedi et al., 2016). The Figure 2.1 study area maps show that the field trial (blue flag and red point) location is geographically situated in a grassland biome of cultivated commercial fields, away from protected areas between the Vals River and the Liebenbergs Vlei River in Nketoana Local Municipality, South Africa.

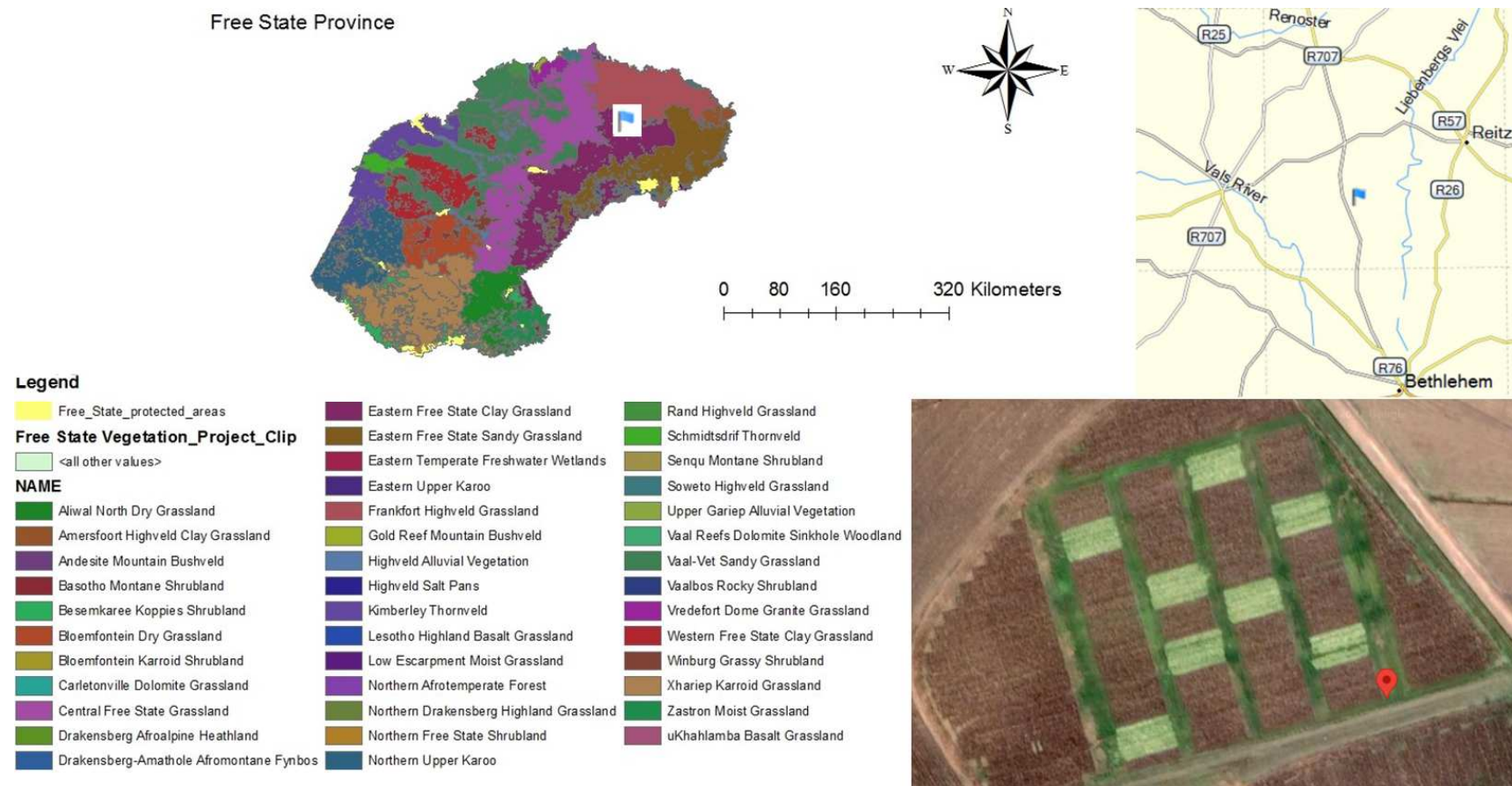


Figure 2.1 Map of the Eastern Free State (EFS) field trial area (blue flag and red point; $S27^{\circ} 53' 45.5''$ $E28^{\circ} 12' 59.8''$, Elevation 1705 m) where the crop rotation trial was studied under dryland conditions in the FS Province of South Africa. Left vegetation map of FS Province, by Mr Knowledge Mushonga using ESRI (2011), right-top map from eTrex Legend® HCx (Garmin International Inc., Olathe, Kansas, USA) and right-bottom map from Google (2021) during the 2019 growing season. The map scale represents the distance for the vegetation type map only (left-map).

The FS region contributes 26.4% of SA's field crops, 15% of SA's gross agricultural income, 7 515 farming units - largest in SA, with 90% of the land being used for agriculture, with 32% arable of 11.6 million ha (Armour, 2010). It should be noted that 14.5% of SA's commercial farming takes place in the FS (Armour, 2010). According to the Directorate Statistics and Economic Analysis (2017a; 2017b) data on the average, the proportion per year per crop in South Africa, the entire FS Province contributes: 36% maize (1987-2017), 26% soybeans, 33% dry beans, and 50% sunflower. These are profitable and low risk crops in the FS region (Nel et al., 2003; Nel & Loubser, 2004; Nel, 2005; Meyer & van Der Burgh, 2015). Our crop rotation experiment was performed as an on-farm level production system, under summer rainfall and dryland conditions of the EFS region. The current status of rain-fed production systems in SA is reviewed extensively in Haarhoff et al. (2020).

2.4 Possible Ecological Interventions toward Soil and Plant Health

The 'Plant Disease Pyramid' adds a fifth dimension – a square pyramid - proposed by Slippers et al. (2020) suggests that the microbiome, society, pathogen, environment and host plant play a critical role in disease development; in particular, the management of soil and plant health through ecological and human social systems. We agree that the important interventions for improving soil health and plant health include building networks that support the ideal below-ground and above-ground interactions, soil resilience, management of tillage, and climate history as proposed by Randall & Smith (2019).

2.4.1 Below-ground and Above-ground Interactions

The soil environment in this study refers either to bulk soil experiencing little or no disturbance and cultivated or disturbed soil subjected to agroecosystem management. The rhizosphere environment is a hotspot of microbial presence; as well as activities in nutrient mineralization, humification, decomposition and microbial biomass, growth and death (Bardgett & Wardle, 2012). Plant growth-promoting bacteria isolated from the rhizosphere are about 2 – 5 % while

the harmful bacteria, are about 8 – 15 % and the remainder constitutes the neutral bacteria (Baker, 1987). Biocontrol bacteria composition is less than 10 % of total rhizosphere bacteria and they show suppressive activity against soil borne pathogens (Weller, 1988). Several commercial products have shown that rhizosphere biocontrol bacteria such as *Bacillus subtilis* reduced disease incidence and severity (Larkin, 2020; Larkin & Brewer, 2020). The rhizosphere bacterial assemblages of crop roots are dominated by *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* (Bulgarelli et al., 2013). The ultimate loss of soil functionality is directly related to the decline in diversity of the soil biota taxa (Tsiafouli et al., 2015).

In the summer, crop cultivation nourishes soil bacteria with rhizodeposits, while in winter; fungi survive as saprophytes, feeding on crop residues (Bardgett & Wardle, 2012). Rhizodeposition appears to fuel an initial substrate-driven community shift in the rhizosphere, which converges with host genotype-dependent fine-tuning of microbiota profiles in the selection of root endophyte assemblages (Bulgarelli et al., 2013). About 64 – 86 % of rhizodeposits are quickly consumed and released as carbon dioxide by rhizosphere microbes (Dixon & Tilston, 2010). Microbial decomposition activities ultimately result in the oxidation of organic compounds in the residues, especially the labile active C, respired into the atmosphere as CO₂ from aerobic soils, or methane in anaerobic soils (FERTASA, 2016).

Plant biomass is affected indirectly by the decomposition process and nutrient dynamics (Bardgett & Wardle, 2012). Decomposers or saprophytes digest and transform organic matter, releasing plant-growth limiting nutrients (Bardgett & Wardle, 2012). These microbes also feed on dead organic material to obtain energy. Free extracellular enzymes and whole bacterial or fungal cells, perform as biocatalysts in the decomposition of crop and soil biota residues into plant-available pool of soluble nutrients, which are easily absorbed from the rhizosphere by organisms (Bardgett & Wardle, 2012). Soil acidity influences nutrient availability, soil

microbial composition, and root growth (Taiz et al., 2018), as well as the beneficial biological processes of nitrogen fixation and mineralization (Fageria, 2016). The low soil fertility in the rhizosphere due to soil acidity slows down microbial and root growth (Taiz et al., 2018).

Microbes produce antibiotics and siderophores to avoid biotic and abiotic stress, respectively (Taiz et al., 2018). Negative plant productivity is due to the accumulation of host-specific pathogens in monoculture whereby the pathogens levels are supported every growing season by the root exudates and litter inputs at the expense of plant-beneficial soil microbiota as a consequence of consecutive sole cropping of the hosts. (Bardgett, 2009). Plant growth promoting rhizobacteria enhance plant defense against soil borne pathogens, by inducing plant systemic resistance and releasing antibiotics (Taiz et al., 2018). The symbiotic relationship between the rhizosphere organisms and crop roots begins at germination, after the seedling exudes toxins to destroy pathogens and rhizodeposits, to attract beneficial organisms (Jhonson, 2009; Berendsen et al., 2012).

Soil biota interactions play a pivotal role in soil health through their catalytic activities in biogeochemical cycling of nutrients such as C and N and the transformation of P and K (Bardgett & Wardle, 2012; Bender et al., 2015). The nutrient cycling activity of decomposers indirectly influences the microbial diversity and plant growth (Bardgett & Wardle, 2012; Veen et al., 2019). Mycorrhizal associations with plant roots, improve phosphorus availability for plant uptake (Bardgett & Wardle, 2012). Nematodes cause direct effects on soil biota and crop yields by feeding upon bacteria and fungi as well as feeding upon roots and forming antagonistic or mutualistic relationships with host plants (Bardgett & Wardle, 2012). Thus nematodes are very good bio-indicators of soil health (Keesstra & Mol, 2011). The Figure 2.2 below indicates plant-soil-microbe interactions, in which the rhizosphere is the front-line defense for roots, against deleterious effects by soil borne pathogens (Weller, 1988; Weller et al., 2002; Bennett et al., 2012).

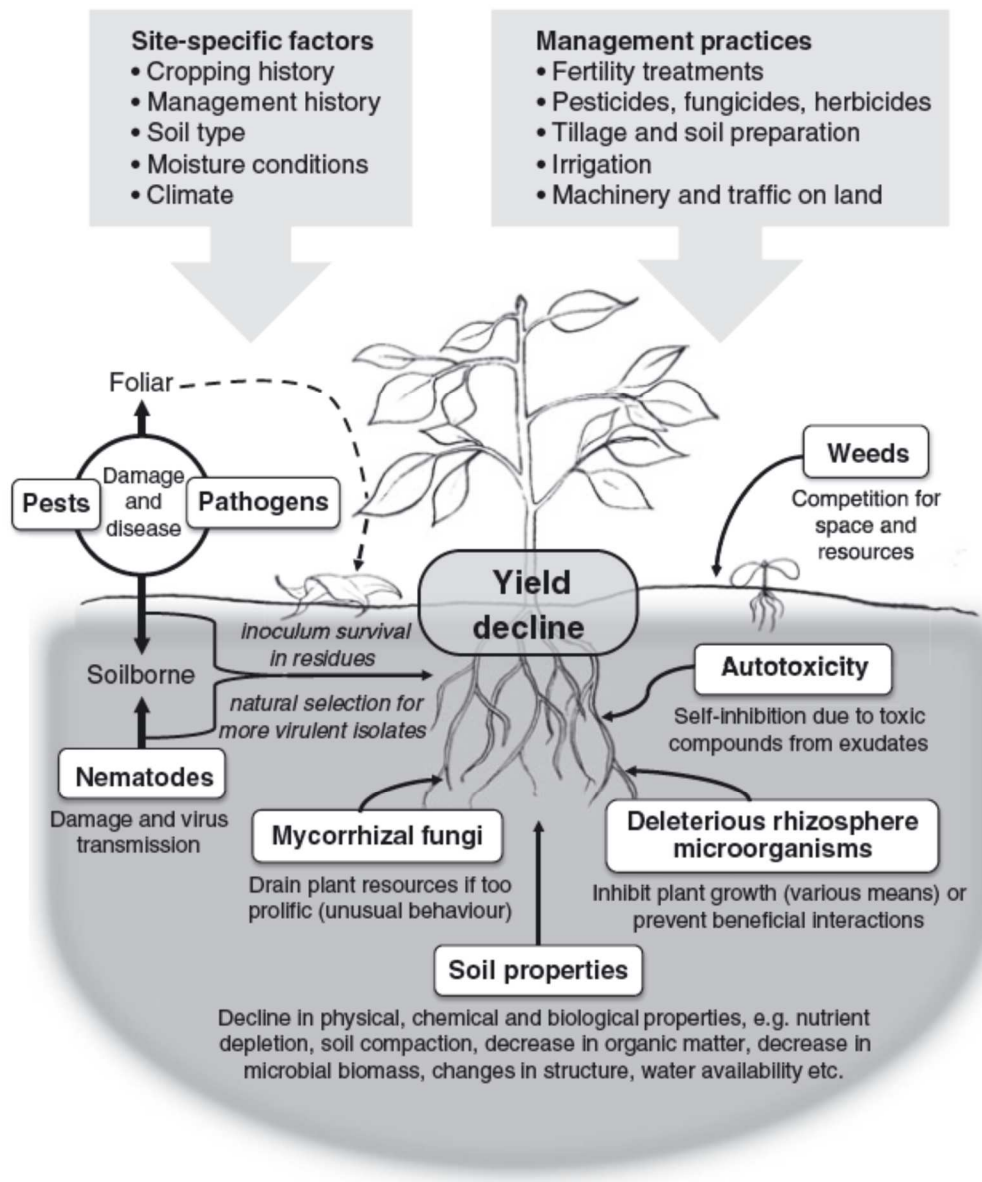


Figure 2.2 The biotic and abiotic factors and their interactions which affect crop yields. Adopted from Bennett et al. (2012).

The rhizosphere microbial interactions are significant for plant nutrient supply, health and quality (Berg & Small, 2009). The net effects of the presence and activity of beneficial, neutral and harmful soil biota, determines positive or negative feedbacks in plant growth responses (Bardgett & Wardle, 2012). The management of crop residues remains the most viable option for building and restoration of carbon storage in cultivated dryland soils. Environmental stressors such as dry/wet or freeze/thaw cycles, also contribute to microbe-mediated nutrient

fluxes (Bardgett & Wardle, 2012). Bardgett (2009) states that palatable plants produce litter inputs of high quality and that crop identity is more influential than crop diversity.

2.4.2 Soil Resilience and Suppression in Agroecosystems

The resilience of soil represents its capacity to perpetuate key biotic interactions and functions even after disturbance (Wall et al., 2012; Bardgett & Caruso, 2020; Benitez et al., 2021). On the other hand, soil resistance represents the capacity of a soil to continue to function without change throughout a disturbance (Seybold et al., 1999; Bardgett & Caruso, 2020). In Figure 2.3 below, soil microorganisms determine the rate and extent of soil recovery after degradation as well as resistance to disturbance (Wall et al., 2012; Shade et al., 2012).

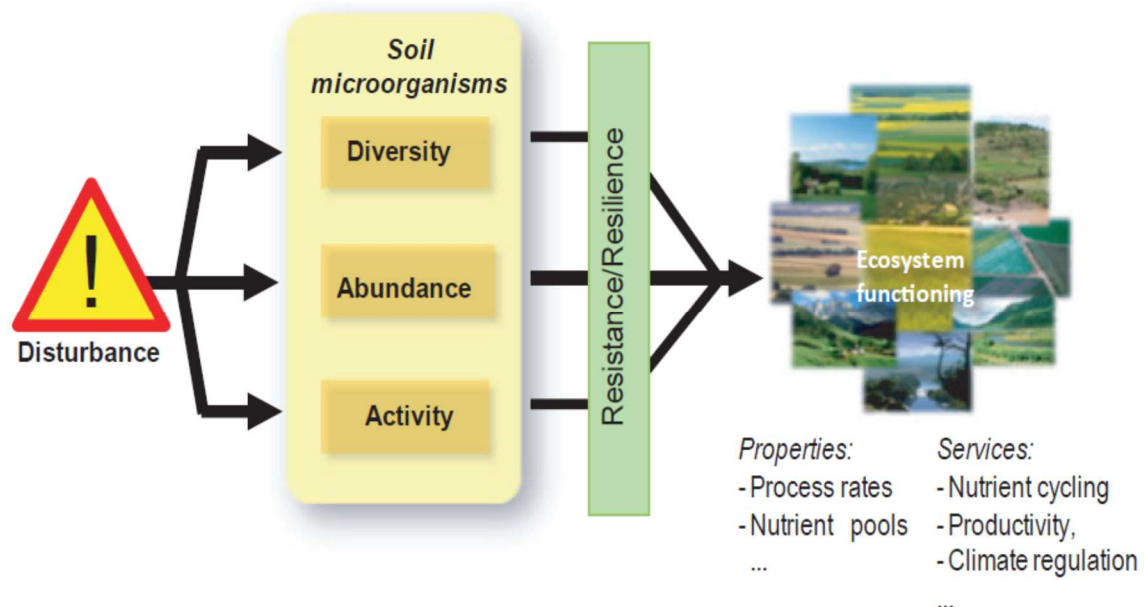


Figure 2.3 The impact of disturbance on soil properties and soil ecosystem services depends on the resilience and resistance of soil microbial community. Adopted from Griffiths & Philippot (2013).

The comprehensive discussion of resilience of soil microbiota is reviewed in Nannipieri et al. (2003), Allison & Martiny (2008) and Griffiths & Philippot (2013). Building soil resilience, involves the maintenance of soil functions, the recovery from degradation, and the improvement to a healthier biological and physicochemical state (Wall et al., 2012; Bardgett & Caruso, 2020). Currently, the impact of changes/thresholds in microbial abundance,

diversity and activity on resistance and resilience of soils are unclear, variable and contradictory because there are no datasets that link microbial diversity, composition and activity to crop productivity (Nannipieri et al., 2003; Bardgett & Caruso, 2020). For example, an increase in enzyme activity reflects nutrient limitation in one agricultural soil, while in another it may reflect soil suppressiveness. For microbial diversity, both low and high diversity can mitigate against community resistance to stress in a similar manner due to functional redundancy (Coyne et al., 2022). Studies have shown that soil functional diversity is a key driver of ecosystem stability (Bardgett & Caruso, 2020). Increased microbial diversity probably provides beneficial services to the crop growth and yield, including plant health through the suppression of phytopathogens and deleterious microbiota (Norris et al., 2023). There is need for sophisticated statistical methods and machine learning approaches to integrate big datasets and predict the soil health property dynamics under different soil conditions.

2.4.3 Management of Agronomic Practice

Soil health provides an overall picture of soil functionality (Arias, 2005). Functional redundancy enables one microbial group to carry out a process at the same rate as another under the same environmental conditions (Allison & Martiny, 2008). According to Van der Putten (2021), the paradox of soil under agricultural intensification is that yields may increase, yet in the long term, soil ecosystems deteriorate to worse levels of soil degradation. In high inputs or intensive farming systems, the yields are increasing but other soil ecosystem services are declining at an equally alarming rate leading to soil degradation, declining soil fertility, pollution of ground and surface water resources, reduced disease suppression and low aboveground diversity (Van der Putten, 2021).

Repeated pesticide and herbicide applications induce changes in soil microbial community but soil health consequences are not yet clear (Zabaloy et al., 2022). Therefore, farmers must be cautioned on the trade-offs in adopting ecological interventions (Castelli et al., 2017). There is

undisputed co-benefits of adopting less intensive no-tillage systems (Bossio et al., 1998; Buckley & Schmidt, 2001; Powlson et al., 2001; Finckh, 2003; González-Díaz et al., 2012; Philippot et al., 2013; Mcdaniel et al., 2014; Bender et al., 2015:2016; Hamid et al., 2017; Swanepoel et al., 2018; Seipelet et al., 2019; Clocchiatti et al., 2020; Debaeke et al., 2021; Adhikari et al., 2021; Ning et al., 2021; Veen et al., 2021; Zhang et al., 2022).

The maize–fallow approach is the only productive fallow system used by conventional tillage producers in SA, although it contributes to serious soil erosion (Haarhoff et al., 2020). The low crop residues and soil water history after rotation with legumes or sunflower, still remains a challenge in maize rotation system in SA (Haarhoff et al., 2020). Monoculture of soybean degrades soils and reduces yields of soybean, however, soybean-maize crop rotation can alleviate the problems accompanying continuous mono-cropping (Liu et al., 2020; Huffman, 2021; Pérez-Guzmán et al., 2021). Monoculture degrades soils through excess application of nutrients, pesticides, herbicides and fertilizers to fields, while the build-up of host specific phytopathogens in monoculture soil reduces crop yields (Bardgett, 2009; Van der Putten et al., 2013). As highlighted earlier, these problems can be alleviated through building of networks that support the ideal below-ground and above-ground interactions, soil resilience, management of tillage, and climate change (Randall & Smith, 2019).

Maize monoculture have a higher gross margin and pose more risks in terms of net cash flow than crop rotation systems. However, Botha & Van Schalkwyk (1999) argue that most farmers prefer rotation systems, on the basis of the economic dogma that higher risks are associated with higher profit returns. Making profits is fundamental to the farming business as it increases the competitiveness and survival of the agribusiness in volatile markets. Studies by Nel & Loubser (2004) suggest that sunflower is a low risk crop in crop rotation systems practiced in the EFS; compared to soybeans and dry beans. Future studies should continue to provide more information on how each crop and rotation sequence, affects production risk.

The low soil organic matter (SOM) in acidic sandy soils in SA seems to be a driver of deficiencies or losses in soil S, Mg, Ca, Zn, Cu and B composition as well as the soil Mn toxicity problem (FERTASA, 2016). The wrong legume and strain of the nitrogen-fixing bacteria partners perform poorly in acidic soils (Jhonson, 2009).

The soil pH regulates microbial community structure, while microbial activity is predominantly driven by the availability of nutrients and substrates (Keesstra & Mol, 2011). Acidic soils reduce bacterial activity and therefore decomposition and nutrient release. The problem of soil acidity in SA manifests as the following;

1. low plant growth,
2. limited yields,
3. reduced availability of macronutrients, and
4. slower rates of soil organic matter decomposition in acid soils than in neutral soils (Foy, 1984; Schroeder et al., 1994; FSSA, 2007).

Lal (2021) proposes that there is an urgent need for 'Carbon (C) farming' where growers can create income in cultivating soil C as a farm commodity that can be traded. However, Mattila et al. (2022) opines that farmers prioritize soil structure and productivity in farming operations but are ignorant of soil C dynamics and they rarely apply the C balance concept. About 67% of the carbon in plant residues and 3% of the C in humus is oxidized to CO₂ annually (FERTASA, 2016). Furthermore, nitrogen fertilization and intensive cultivation increases the release of large amounts of CO₂ from cultivated fields (Luo & Zhou, 2006).

The removal of straw from sandy soils predisposes them to soil health degradation by exposing bare soil to high soil temperatures, soil erosion and poor soil physical structure, consequently hampering the decomposition of litter inputs and carbon balance (Bardgett & Wardle, 2012; Bagnall et al., 2022). In conventional tillage systems, the field soil C: N ratio can be increased by burying the stover in the plough layer (Sait, 2003). The high C: N ratio (80:1 to 120:1) of

cereal stover residues takes a longer time to decompose than legume crop residues with low C:N ratio (25:1) (Stirling et al., 2016). For practical benefits, growers can add carbon inputs based on field plant-litter legacies and/ or importation of carbon sources (Bardgett & Caruso, 2020; Mattila et al., 2022). Organic matter levels should be at par with the soil-plant-climate system to maintain a balance between humification and mineralization processes (FERTASA, 2016). However, there is need for more climate-change studies to establish and gauge these balances through SOM mapping across SA.

2.4.4 Climate Change

Rain-fed systems such as crop rotations under conventional tillage in the EFS are exposed to frost risk, hailstorms, floods, high temperatures from heat waves, inadequate heat units from low temperatures, erratic rainfall patterns, droughts, high-intensity thunderstorms rainfall originating from the hot and humid air from the tropics (Moeletsi & Walker, 2012; Haarhoff et al., 2020). The solution to climate-change challenges in dryland systems, should focus on improving available soil water and; nutrients particularly nitrogen; because it determines crop productivity (Bender et al., 2016). Climate change increase the frequency and magnitude of new plant disease outbreaks (Midgley, 2017). When rainfall patterns delay planting, sunflower is oftentimes planted instead of maize, because it is tolerant to drier soils and requires fewer days to reach maturity. This also reduces the risk of frost-damage and crop failure in the Eastern dryland regions of SA (Haarhoff et al., 2020).

Legumes are less deep-rooted crops than maize and sunflower, therefore they are most sensitive to water stress especially in sandy soil which does not hold much water (Fageria et al., 2010). To improve planning and decision making on climate related problems, Moeletsi et al. (2013) developed the “Free State Maize Agroclimatological Risk Tool (FS-Macrt)” to assist growers and stakeholders, in assessing climatological risk and forecasting for rain-fed maize production systems in the FS. This tool is installed on the computer, the datasets are in excel format and

the modelling algorithms (agroclimatology models, suitability model and risk forecasting model) use historical or already known climate metadata from the national forecasting centres solely for the EFS. The usefulness and accuracy of this tool requires further testing in other regions or systems such as winter-rainfall regions of SA before conclusions are made on its utility in decision making.

2.5 Soil and Plant Health Indicators

2.5.1 Crop Rotation Soil Legacies as Indicators

Soil Health Institute in the North American project to evaluate soil health measurements (NAPESHM) recommends a minimal suite of three measurements namely; 1) soil organic carbon concentration, 2) carbon mineralization potential, and 3) aggregate stability to be widely applied across North America (and likely beyond) (Bagnall et al., 2023). Datasets were collected from 124 long term field sites under crop rotations, tillage and cover crops. In the NAPESH project, they explored more than 30 soil health measurements according to Bagnall et al. (2023). However, they cautioned that it is too early to generalize that these soil health indicators are universal, considering that the current datasets cannot be reused in other regions of the world, and that the indicators were obtained from different scoring and laboratory testing methods which are yet to converge into a; useable, scalable and unified standard soil health assessment tool.

Ideal soil- health indicators should be informative, sensitive, effective and relevant to the grower's needs, although they cannot be managed, or take a long time to change (Lehmann et al., 2020). The review by Lehmann et al. (2020) provides a comprehensive summary on the relevant soil health indicators for the future and the co-benefits such as soil resilience -and- resistance.

2.5.2 Weeds Composition

The reduction or disruption of weed cycles in crop production systems is an effective and practical indicator of soil health (Lehmann et al., 2020). In our study, three weed species *Amaranthus hybridus* L. subsp. *hybridus* var. *hybridus* (Smooth Pigweed), *Senecio consanguineus* DC (Ragwort or Starvation Senecio) and *Taraxacum officinale* (Common Dandelion) were controlled using four crop rotation sequences, at a field scale under rain-fed summer dryland conditions.

Senecio consanguineus is an annual weed of least concern; while *T. officinale* a perennial weed and *Amaranthus hybridus* is an annual broadleaved weed. Neither of these weeds have been evaluated as problem plants in SA as yet (Raimondo et al., 2009). *Senecio consanguineus* only sexually reproduces by seed - is a shallow germinating winter weed that germinates early in spring, forming dense stands at crop harvest (Hugo & Van der Walt, 2016). Control for this weed includes shallow cultivation and the use of a systemic herbicide such as glyphosate, or a contact herbicide like Paraquat, after emergence or harvest (Hugo & Van der Walt, 2016). Moreover, *S. consanguineus* has been recognized as a poisonous plant for both livestock and humans in SA (Dimande et al., 2007).

Amaranthus hybridus is common in summer and winter rainfall cultivated fields in southern Africa. The common pigweed is controlled using registered broadleaved herbicides (Hugo, 2013). The landraces of *A. hybridus*, provide nutritional benefits for livestock and humans (Reinhardt, 2018).

Taraxacum officinale plant materials have potential nematicidal effects against the root-knot nematode *Meloidogyne incognita* (Laquale et al., 2018). We expected that, the cereal grass (*Zea mays* L.), nitrogen-fixing leguminous forbs (*Phaseolus vulgaris* L. and *Glycine max* (Merr.)) and a non-leguminous forb (*Helianthus annuus* L.) will have residual soil legacy

effects that reduce the weed biomass and populations of Smooth Pigweed, Ragwort and Common Dandelion which were a problem in maize rotations.

2.5.3 Plant Health Phytometers using Soil Bioassays

Bioassays or “grow-out” tests are broadly defined as testing methods that involve working directly with test plants; where plants are grown on as “bait” or phytometers and checked for symptoms developing during and after growth or tested using another method for feedback effects (Beales et al., 2019). Plant phytometers are used as indicators of the suitability of the soil environmental conditions for plant growth (Gibson, 2015). The role of phytometers in soil and plant health research include assessing; soil fertility, availability of soil nutrients, plant-soil feedbacks, soil suppressiveness, effects of soil borne pathogens on plants (Gibson, 2015). Most studies use various agricultural crops especially those that are native to the region under study (Mushonga et al., 2020). For example, four dryland crop species namely, *Zea mays* L., *Helianthus annuus* L., *Phaseolus vulgaris* L. and *Glycine max* L. (Merr.) including *Vicia faba* L. were used as phytometers in our region-specific study in the EFS.

Soil inhabiting pathogens are necrotrophic free living saprophytes that produce seedling death, or root rot disease, in agroecosystems, for example, *Rhizoctonia* and some *Fusarium* species (Vega et al., 2019). Damping-off is a non-specific plant disease caused by several pathogenic oomycetes and fungi like *Pythium* spp., *Phytophthora* spp., *R. solani*, *Thielaviopsis basicola* and *Fusarium* spp. In the present study, *R. solani* and *F. graminearum* were chosen because they are the main phytopathological problems for several dryland crops in the FS province (Dube, 2020; Muzhinji et al., 2015). *Fusaria* can survive saprophytically on plant litter residues (Deacon, 2006). This mitosporic fungi produces mycotoxins in stored grains (Deacon, 2006). Muzhinji et al. (2015) found that *Rhizoctonia* isolates of AG 2-2IIIB, AG 4HG-III, and AG R from SA’s potato growing regions were the most aggressive in causing stem canker while AG3-PT, AG5, and AGR in causing black scurf. *Rhizoctonia solani* is divided into 14 (AG 1 – AG

13) somatically incompatible groups, otherwise known as anastomosis groups (AGs) (Ajayi-Oyetunde & Bradley 2018). Also, Muzhinji et al. (2015) described 19 out of 21 binucleate *Rhizoctonia* AG groups (AG A – AG U) in the South African potato industry.

Crop type influences the prevalence and occurrence of a given AG, as a succeeding crop in certain areas (Sparrow et al. 2015; Muzhinji et al., 2015). Maize, potato, St. Augustine grass, *Zoysia* grass, soybean and other bean species as well as sorghum, are preferred hosts for *R. solani* AG 2-2 IIIB (Boine et al., 2014; Muzhinji et al. 2015); and can increase in severity as a result of crop rotation (Thomsen et al., 2018). Of the important potato pathogens, *Rhizoctonia solani* is reported to cause losses of up to 30 % of marketable yield. Commercial production of potatoes can incur up to 50 % loss of quality due to black scurf disease caused by *R. solani* AG 3-PT (Woodhall et al., 2013; Patil et al., 2018). This represents a massive challenge to food security and food safety, so cannot be ignored.

Apart from crop rotation, other cultural methods of controlling *Rhizoctonia* and *Fusarium* diseases, include biocontrol agents such as “*Bacillus subtilis*” to treat seed (Horst et al., 2008; Orozco-Mosqueda et al., 2018; Larkin and Brewer, 2020; Jamali et al., 2020). Cultural techniques also include ploughing and decomposition of host plant litter residues in crop rotations (GRDC, 2008; Bernard et al., 2014; Vega et al., 2019). The dynamics of soil borne pathogen risk and disease potential; were monitored, collected and recorded, at the end of the growing season and when outbreaks occurred in the field, or following break crops in the rotation sequences. In SA, *R. solani* anastomosis group (AG) 3-potato type (PT) is the most predominant AG, in all the potato-growing regions (Muzhinji et al., 2015). *Fusarium graminearum* (Schwabe) is also an economically significant causal agent of seed, seedling, ear and stalk rots, in grasses and cereals (Broders et al., 2007; Horst et al., 2008).

2.6 Practice of Conventional Tillage

Under conventional tillage, the field soil is ripped 50 cm to 75 cm deep in-row before planting maize to alleviate top-and subsoil compaction and pans from previous machinery wheel traffic and tillage operations as well as minimize soil erosion degradation (FSSA, 2007). Primary tillage in conventional practice incorporates plant residues using the share or disc plough, while secondary tillage is done to control weeds and prepare the seedbed (FERTASA, 2016; Khan Dawar et al., 2021). However, conventional tillage exposes the topsoil to wind erosion on sandy soils, resulting in the loss of the organic matter content of the soil (Du Toit et al., 1994) as well as poor root development in subsoils (FERTASA, 2016). In conventional tillage, the seedbed is prepared with the aim of creating a favorable rooting medium in the subsoil conditions by; disturbing the soil structure to allow good root penetration and drainage, breaking surface crusts to avoid soil penetration resistance and disturbing weed below-ground competition (FERTASA, 2016). The urea and ammonia based fertilizers increase pH and ammonia concentrations which inhibit microbial communities (Geisseler & Scow, 2014).

2.7 Soil and Plant Health Analysis

Soil health is a requirement in dryland systems for long-term sustainability, reduced inputs, improved water quality, balanced soils, plant health and improved yields. In addition to soil and plant material testing using wet and dry chemistry, there is a need to detect and geo-reference soil and plant health changes using real-time surveillance of aboveground conditions using drone technologies, remote sensors and soil sensor technologies for the soil-plant environment and microclimate (Swart, 2021). This interdisciplinary approach, allows growers, agronomists, plant health and soil health experts to increase capabilities of replicating both temporal and spatial data necessary for validating time series field experiments (Bardgett & Caruso, 2020). These innovations provide strategic direction and leadership in agronomic

practice while maintaining a sustainable, competitive and profitable agribusiness in the drylands (Tsiafouli et al., 2015; Stringer et al., 2017).

2.7.1 Soil Physicochemical Properties

Soil penetration resistance and bulk density are sensitive soil health indicators which provide inference of management and are; related to soil functions, cheap, easy and reliable (Lehmann et al., 2020). In this study, the *in situ* soil penetration resistance readings were estimated on-site using devices such as Geotron Hand Penetrometer Model P5 (Geotron Systems, Potchefstroom, South Africa) up to 5000 kPa. The *in-situ* soil bulk density was obtained from undisturbed soil samples, from which the bulk density was calculated. There are several extractants of micro- and macro-nutrients in soil tests or analysis, however we chose an acid extractant relevant to acidic soils in South Africa. Compared to other extractants, the Mehlich III Package is the official extractant for the analysis of acidic agricultural soils (Walton & Alle, 2004; Mylavarapu et al., 2014) and provides test results that can be compared to other soil test methods. In addition, the Mehlich III Package is a rapid and cost effective soil test, measuring up to 13 nutrients (P (Bray I/II), Ca, Mg, K, Na, Co, Mo, Fe, Mn, Cu, Zn, B, S, and P) simultaneously (Walton & Alle, 2004).

When the pH (H₂O) is less than 5.5, or pH (KCl) is less than 4.5, it is imperative to determine the soil's exchangeable acidity, acid saturation, lime requirement and phosphate absorption (FERTASA, 2016). In cases where the pH (H₂O) or pH (KCl) is above 7.0, there is an urgent need to test the soil for free lime (FERTASA, 2016). The water extracts of NH₄-N and NO₃-N, represent plant available forms of N, which are relevant for monitoring N cycling by microbial indicators of soil health (Nielsen & Winding, 2002). These tests will allow follow up on the impact of pulses such as sugar beans and soya beans, in building N levels; or non-pulses (maize and sunflower), in depleting N. To perform N-fixation, pulses rely on microbes including *Rhizobium* and *Cyanobacteria*, which are microbial indicators of soil health. As such, there is

need to monitor soil health in terms of N-cycling. During N-cycling, organic N is mineralized to NH_4 by soil microbiota; while biological nitrogen fixation (BNF), involves the reduction of N_2 gas to NH_4 (Souza et al., 2015). Ammonium is either used as microbiota biomass; or oxidised to NO_2 then to NO_3 leading to loss of N by leaching (Nielsen & Winding, 2002).

2.7.2 Soil Biological Properties

2.7.2.1 Soil Active Carbon

The soil active C represents the organic matter fraction that is readily available as a C energy source for soil microbial community food webs (Bardgett, 2009). This carbon metric is very sensitive to management effects and more closely linked to biologically mediated soil properties; such as respiration, microbial biomass and aggregation; than measurements of soil organic C (Weil et al., 2003).

The rate of CO_2 release from the soil is a biological process related to the level of microbial activity; in the same application the Solvita test provides an indication of soil microbial biomass; as well as estimates of potential release of nutrients such as nitrogen from organic matter (Stirling et al., 2016).

2.7.2.2 Soil Microbial Respiration using the Solvita CO_2 Test

The Solvita CO_2 test estimates the microbial activity in the soil and it is positively or highly related to soil fertility (Haney et al., 2008; Brinton, 2016). There are three Solvita tests, namely the; (i) basal CO_2 test, (ii) CO_2 -burst test (used in current study) and (iii) labile amino-nitrogen test. Each test has a brief incubation lasting no more than 24 hours and the tests are designed to be used under normal conditions in any area of the world for any crop (Brinton, 2016). Both the basal CO_2 and CO_2 -burst tests are used to indicate soil microbial activity and use a 24-hour incubation in a sealed jar with a color changing gel-imbedded probe to measure CO_2 produced by soil microorganisms during the incubation period (Haney et al., 2008; Brinton, 2016).

The quantity of CO₂ produced is directly correlated to the quantity of potentially mineralizable nitrogen. The tests are capable of measuring CO₂ concentration in the jar air space between 0 – 3 %. On the other hand, the field CO₂ test is a simpler version of the laboratory CO₂-burst test. Basal CO₂ test requires that a fresh, roughly sieved (4mm) 40g soil sample is incubated with little to no prior processing and the results indicate the basal respiration rate under standardized conditions. In the CO₂-burst laboratory test, the samples are passed through a 2mm sieve and dried to 1-3% moisture, and when soils are rewetted, microorganisms produce a burst of CO₂ that is quantified by the probe color transition (Haney et al., 2008; Brinton, 2016). Several field studies have accepted this soil microbial respiration measurement as an index of soil microbial activity (Haney et al., 2008); as well as an emerging indicator of soil health (Cardoso et al., 2013; Brown, 2015; Fungenzi, 2015; Stott, 2019).

2.7.2.3 Soil Microbial Enzyme Activity using the Fluorescein Diacetate (FDA) Method

The soil microbial activity test using the fluorescein diacetate (FDA) method; is an important quantitative analysis that captures “microbial hotspots” in terms of the enzyme activity caused by microbes in the soil (Schumacher et al., 2015). The FDA tests the activity of microbial enzymes produced by fungi and bacteria. In healthy soils, the breakdown of FDA is very rapid due to a high level of microbial enzyme activity not observed in soil with low microbial diversity (Stirling et al., 2016).

The availability of soil P depends on microbial mobilisation activities; the wet and low temperature conditions during winter, lowers soil microbial activity, and lead to P deficiency (Sait, 2003). In addition, soils with high enzymatic activity are disease suppressive (Stirling et al., 2016). The FDA test is ideal for estimating the microbial activity in soils (Jiang et al., 2016).

2.7.2.4 Soil Organic Matter

Soil organic matter (SOM) supports a great microbial diversity that; produce nutrient-rich humus to suppress populations of soil borne pathogens and also protect nutrients from erosion

or leaching (Sait, 2003; Bender et al., 2016; Stott, 2019). The FAO (2017) reported that the soil organic C component that contributes to soil health is 45 – 60% of the SOM based on estimates by Ramesh et al. (2015) and Lal (2016). The most important source of soil organic matter is provided by plant biomass or litter inputs (Stirling et al., 2016).

2.7.2.3 Soil Community Biomass using Phospholipid Fatty Acid Analysis

Phospholipid fatty acid analysis (PLFAs) is a biochemical technique, which gives fungal to bacterial biomass composition ratio and active biomass of microbes (Frostegård & Bååth, 1996; Ladygina & Hedlund, 2010; Tavi et al., 2013). However, the PLFAs do not address the taxonomic and functional variation due to biotic interactions and environmental and spatial processes (Louca et al., 2018). The patterns and swinging ratios of these lipid biomarkers in soils across space or time have been linked to nutrient cycling, organic matter decay and carbon sequestration (Willers et al., 2015; Stott, 2019; Zaeem et al., 2019; Pérez-Guzmán et al., 2021). The relatively high fungal to bacterial PLFA biomass ratio is associated with reduced rates of nutrient mineralization, while a relatively low fungal to bacterial PLFA biomass ratios associates with high rates of mineralization (Bardgett & Wardle, 2012). Higher proportions of fungi are also associated with an increase in carbon sequestration (Bender et al., 2016). Soil microbial biodiversity is another important indicator of soil health which can be measured in space and time (Bossio et al., 1998).

2.7.2.4 Soil borne Pathogen DNA Content using Quantitative PCR Analysis

One of the most popular methods for plant disease diagnostic applications is the probe-based real-time PCR assay or quantitative polymerase chain reaction (qPCR) method which uses probes. This method couples PCR amplification with the generation of fluorescence using probes which are cleaved during detection of specific DNA sequence of the targeted plant pathogen (Boonham et al., 2016). Quantification of soil borne pathogen DNA copies using the qPCR technique is important in the management of soil and plant health (Bilodeau, 2011). However, this tool does

not provide information on the environment, human mismanagement and susceptible plant components of the square disease pyramid (Slippers, 2020).

The qPCR analysis only confirms the presence of the pathogen DNA in a field soil sample (Sidstedt et al., 2020). The presence of DNA in the soil correlates to infestation, which implies that the soil is at risk of disease development (Stirling et al., 2016). However, absence does not guarantee soil health for plant health since pathogen DNA copies may be from dead or non-pathogenic strains (Bilodeau, 2011). In addition, a qPCR assay does not conclude the health status of soil or plant because it only targets one genus (inclusivity) and omits other pathogenic organisms (exclusivity) (Loreti et al., 2018). In this study, the utility of the qPCR tool was improved by conducting parallel *in vitro* assays and soil bioassays (Gatch & du Toit, 2015). Further studies into viability qPCR testing, standardization and validation will enable this technique to detect the inactive and active soil borne pathogens. Other useful DNA-based methods for determining microbiomes around plant roots are yet to be linked with functional diversity, hence caution must be taken before making firm conclusions.

2.7.2.5 Crop Yields

The expectations were that a cereal grass (*Zea mays* L.), nitrogen-fixing leguminous forbs (*Phaseolus vulgaris* L. and *Glycine max* (Merr.)) and a non-leguminous forb (*Helianthus annuus* L.), would have soil legacies linked to the plant type and functional groups (Garbeva et al., 2004). Legumes build nitrogen legacies in the soil that drive positive plant-soil feedbacks (PSF), whereas *H. annuus* and *Z. mays* are net consumers of nitrogen that drive negative PSF (Cortois et al., 2016; Cesarano et al., 2017). Overall crop yield and yield components were used to estimate the impact of conventional tillage practice on soil health in crop rotations (Honeycutt, 2017). The crop residues of legumes are rich in nitrogen and when left on the aerobic, warm and moist soil surface, they are decomposed slowly by aerobic soil microbiota

to release N which is rapidly converted into plant-available $\text{NH}_4\text{-N}$ and consequently assist in the judicious use of nitrogen-containing fertilizers (Lambers et al., 2008; Sait, 2018).

2.8 Soil Legacies according to Crop Type

Plant-soil feedbacks give a mechanistic understanding of the direct and/or indirect effects of crop rotation soil legacies on crop production that are linked to the plant type and functional groups (Kumar et al., 2021; Mushonga et al., 2020; Randall & Smith, 2019). Conceptually, farmers must deliberately plant a different crop from the different family/type, in the subsequent season on the same field; to reduce the negative impacts of nutrient-depletion and the build-up of pathogens and pest inoculum pressure (Randall & Smith, 2019).

2.8.1 Maize (Cereal Grass) Soil Legacies

Maize (*Zea mays* L.) thrives best in grassland biomes of South Africa, but poorly on alkaline sandy soils (Smith, 2016). The C₄ photosynthetic plants such as maize have a high turnover of carbon and their root exudates are rich in amino acids, sugars and organic acids (Baudoin et al., 2003). These simple soluble substrates provide nourishment for pioneer saprophytic fungi (Deacon, 2006). Soil carbon legacies of a grass monoculture favour bacteria, actinomycetes and fungal biomass growth more than a legume and a forb; grass species influence belowground microbial diversity and carbon allocation in the rhizosphere by producing higher quantity of plant litter inputs and root exudates than herbaceous plant species (Ladygina & Hedlund, 2010). Maize will likely enrich the soil with a balanced microbial community and diversity (Baudoin et al., 2001) and minimize removal of soil nitrogen from sandy soils with low organic matter.

2.8.2 Sunflower (Non-Leguminous Forb) Soil Legacies

Sunflower (*Helianthus annuus* L.) has a strong 2.02m taproot, finely branched and extensive root system that consumes mineral nutrient reserves in the soil (Jaffar et al., 1993); and the long rooting as a response to water stress (Fageria, 2013). Sandy soils under sunflower

cultivation are prone to soil surface crusting, rapid drying and infestation with nematodes (Nel, 2005). In addition, the high exchangeable acidity, soil compaction, nematodes, excessively wet soils; restrict the plant's potential to use moisture and nutrients. Sunflower deep rooting system enables uptake of subsoil water and mineral resources while the topsoil is left in temporary "fallow" since uptake of solutes is driven by drainage water available in the subsoil. However, the soil nutrient and water uptake activities by sunflower are not straightforward because root trait activities are difficult to assess *in situ* (Freschet et al., 2021). Sunflower is effective in absorbing and assimilating K, and it has the highest requirement for B compared to all crops cultivated in SA (FSSA, 2007). Sunflower is drought-tolerant crop which can be cultivated in marginal soils, with little or no fertilizer input requirements, and under dryland production areas in SA (Meyer & Van Der Burgh, 2015; Mathews, 2016; Mathews, 2017; Maluleke, 2021). In agroecological intensification systems, sunflower is a potential strategic crop due to its low-input requirements in nitrogen, potassium, phosphorus, water and pesticides (Debaeke et al., 2021).

2.8.3 Sugar bean and soybean (Nitrogen-Fixing Leguminous Forbs) Soil Legacies

Legumes create higher soil acidification legacies than non-legumes due to the excessive uptake of cations over anions due to N-fixation and low uptake of NO_3 (Rengel, 2003). In order for the legume roots to activate the nodulation process, they exude amino N and flavanoids in larger quantities than non-leguminous grasses such as wheat (Mukerji et al., 2006). Legumes produce high N legacies and thus have a lower C: N ratio (< 20 compared with > 30 for grasses) (Mukerji et al., 2006). The actinomycetes *Frankia* also makes a huge contribution of soil N legacies in-addition to the contribution made by symbiotic N fixers (Mukerji et al., 2006). Phosphorus deficiency restricts symbiotic N-fixation and successful nodulation (Mukerji et al., 2006), but for growers with good access to manure, the soil P and N levels are often very high (Mattila et al., 2022).

Legumes can fix higher than 10 – 40 kg of N per ha, which is available to the next crop in the rotation sequence, lowering N fertilization; depending on the soil type, climate, previous crop yield and tillage system (Lambers et al., 2008; FERTASA, 2016). Sugar bean (*Phaseolus vulgaris*) should be cultivated in soils with high fertility, pH (KCl) 4.8 to 5.5, acid saturation less than 10 % (FSSA, 2007). Sulphur is important fourth element after NPK for legume protein synthesis and oil content as well as for N-fixation by the *Rhizobium* bacteria (FSSA, 2007). Soybean (*Glycine max*) plants respond well to residual soil fertility but it is sensitive to high salt concentrations, and removes more S than most grains (FERTASA, 2016). It is important to use good quality commercial inoculants of *Rhizobium japonicum*, since no effective N-fixing occurs naturally in soils of SA (van der Hoven, 2018). Site-specific maize-legume rotations improve grain yield and protein content of the subsequent maize crop (Nel et al., 2003). In the Nel et al., (2003) rotational cropping study, the hypothesis supposed that dry bean, lupin, sunflower and fallow has no effect on the yield of the follow-up wheat crop while soybean and wheat has no effect on the yield of maize conducted on an acidic Avalon soil type at Bethlehem in the EFS from 1992 to 2001.

2.8.4 Crop Rotation Residual Soil Legacies

The root exudates composition of maize is mainly made up of organic acids such as citric acid and tartaric acid, and this is superior to legumes in C substrates, due to low photorespiration metabolism (Fageria, 2016; Li et al., 2020; Huffman, 2021). Vancura & Kunc (1989) suggest that rhizosphere microbial enzyme activity is crucial for plant nutrition and health, including soil microbial community growth and development. However, the grain yield of maize after succeeding legumes in rotation seems unrelated to nitrogen enrichment of the soil (Nel et al., 2003). The cultivation of maize in monoculture, or narrow rotations, homogenizes soil conditions and support certain weed populations (Sait, 2003) and low nutritional quality fodder (Zaeem et al., 2021). Under long-term soybean and maize production systems, the soil total N

and total C compositions; influence legacies on SOM (Pérez-Guzmán et al., 2021). Both maize and sunflower remove more plant N, P and K than legumes from soil (FERTASA, 2016). In agroecosystems, plants are known to change soil properties via rhizodeposition during their growth and development (Dube et al., 2016) including litter inputs after harvest (Bardgett, 2009).

CHAPTER 3

Maize Uniformity Trial Study for Baseline Soil and Plant Health Status in a Dryland System in South Africa

3.1 Abstract

Background: Soil chemical, biological and physical properties influence crop growth by supplying crops with nutrients and optimum medium for root growth. Historical soil legacies of field plots/blocks must be quantified to account for differences in soil fertility from past soil amendments and possibly homogenized for effective use in new experiments.

Aims: The purpose of the uniformity trial study was to assess field heterogeneity before conducting repeated observations in long-term experiments. Spatial and temporal variations in soil properties can lead to increased topsoil heterogeneity and yield variability between field plots which in turn affects the precision of field trials under various plant production situations. Conceptually, variability in yields within a season on a ‘blank’ trial or uniform field planted with the same crop cultivar and managed similarly is a manifestation of soil heterogeneity. The objective of this study was to get reference data to establish predictors or indicators of soil health and to test ‘dummy’ or zero (same maize variety) treatment effects. The ‘dummy’ treatments represented one maize variety that was planted in all the plots but disguised as four different varieties.

Methods: The ‘blank’ trial was divided equally into four ‘dummy’ crop treatments across eight blocks and planted with the same maize cultivar by the same planter on the same day in a randomized complete block design (RCBD). At the end of the season, topsoil samples were collected from the root-zone and analysed using standard soil tests. A total of 36 soil indicator

results plus quantities of soil-borne pathogen DNA were analysed using quantitative real-time PCR. Yield corrected for 12.5% moisture and its components were obtained using a combine harvester and the crop cut method, respectively.

Results: The field soils were acidic and sandy with adequate %Ca, P, S, Mg, Fe, B, Ca:Mg, (Ca+Mg)/K, but had suboptimal levels of NO₃-nitrogen, NH₄-nitrogen, Mg:K, Ca, Na, K, % acid saturation, Mn, Cu and Zn. On the other hand, the %Mg, %K, %Na, S-Value, and Al levels were above optimum levels in the soil. Solvita values of CO₂ averaged at 4.78 ±0.23 ppm, typical of soil respiration in dry sandy soils with little or no organic matter associated with very low to moderately low soil biological activity. There were no statistically significant variations between blocks in soil factors, yield or its parameters ($p > .05$). Blocking dummy treatments into randomized complete blocks showed that there were no significant differences in soil properties ($p > .05$) or yields ($p > .05$). Spatial variation in soil health properties composition indicated that the positive spatial eigenfunctions in blocks 1 and 5 were likely due to % acid saturation, P Bray II (mg/kg), P (mg/kg), salt-replaceable acidity (cmol(+)/kg), density (g/cm³), %Na, clay (%) levels. However, the overall spatial variation was statistically different for all combined spatial and environmental fractions ($p < .05$).

Conclusion: The RCBD layout reduced variance and spatial overlaps in the eight field blocks, since the field was relatively uniform with no imbalances in soil factors. Confounding spatial differences can be managed using covariate analysis in comparing treatments within blocks on this field when necessary. The study provided the baseline data for a long-term field experiment investigating soil and plant health under dryland cultivation in the Eastern Free State, South Africa.

Key words

Zea mays L. (Maize); uniformity trial; soil properties; spatial variability; dryland farming; ANOVA.

3.2 Introduction

Maize (*Zea mays* L.) is the major grain produced in South Africa with production under dryland conditions estimated to be 3 tonnes per ha (Directorate Statistics & Economic Analysis, 2017b). The cardinal maize-producing regions include the Free State, Mpumalanga and North West provinces, which contribute a fifth of national total production. They rely mainly on rain-fed systems with less than 10 % produced under irrigation (Directorate Statistics & Economic Analysis, 2017a). Maize yields in a dryland farming system vary largely due to climate, soil fertility, soil quality, soil health, pathogens and management practices (Loughin et al., 2007, Sainju et al., 2015).

A uniformity trial or ‘blank’ trial is defined by Scheiner & Gurevitch (2001) as an experiment of plots without the application of treatments. A uniform field must have minimum point-to-point heterogeneity in soil factors (Perrier & Wilding, 1986). These large scale and on-site farm experiments need land or new fields which are difficult to find and fund, although uniformity trials are effective in evaluating field uniformity (Forster, 1937). Our uniformity trial study area is located in the grassland biome of the Eastern Free State region of Petrus Steyn in South Africa, which is dominated by grass species (Smith, 2016). We hypothesized that the ‘blank’ trial will quantify variation of a dryland soil environment and reveal confounding effects likely to influence soil health before introducing treatments. The health status of soil, and consequently that of a plant, depends on the interaction of the soil’s physical, chemical and biological properties.

Field experiments require optimum sample size, plot size, plot shape, randomization, replication and blocking (Zhang et al., 1994; Hoshmand, 2006; Gibson, 2015) to improve uniformity or reduce heterogeneity of soil factors. This improves the precision of field experiments in quantifying spatial and temporal scale soil variability, in general, when testing the impact of new cultivars, pesticides, fertilizers, other products and crop management

regimes in long-term agronomy trials (Cochran, 1939; Swearingin & Holt, 1976; Loughin et al., 2007). *Fusarium graminearum* depresses yields in most cultivated cereals and soybeans by causing severe diseases that lead to yield losses between 30 and 70% (Bai & Shaner, 1994). This soil borne pathogen was selected and tested because it is one of the main disease agents of stem-rot in cereal and potato cropping systems in the EFS and southern Africa (Millard et al., 2016). Also, maize supports the survival of this pathogen before a susceptible host is planted in cereal crop rotations (Sparrow et al. 2015; Rivas-Franco et al., 2019; Gimeno et al., 2020; Crippin et al., 2022; Cao et al., 2023; Savignac et al., 2023).

Failure to detect and adjust for spatial variability of soil properties can undermine the precision of a well-designed and carefully conducted field experiment (Robertson et al., 1999). Ordination techniques such as principal component analysis (PCA) explore correlations and minimize dimensions needed for developing models based on the most predictive variables. Uniformity trials give prior qualitative and quantitative understanding of the field environment, its history, and soil heterogeneity. Management practices also result in soil variability, for example, heavy farm machinery causes on-site physical degradation of soil through compaction leading to reduced microbial activity, root colonization, and water availability (Randall & Smith, 2019). Soil strength measurements using penetrometers are essential in determining soil compaction.

Soil biological activity in this study was estimated using the Solvita CO₂-burst test, which evaluates the microbial mineralization process indicative of the level of biological soil function that releases plant nutrients (Sciarappa et al., 2017). The amount of CO₂ produced is strongly correlated with anaerobic N mineralization, the activity and biomass of the living soil microbial community in the crop root zone (Haney et al., 2015; Sciarappa et al., 2017). The active carbon, also known as the reactive carbon or permanganate oxidizable carbon, is a fraction of the soil organic matter pool that is oxidizable in the presence of potassium permanganate in solution.

This carbon is most readily degradable by microorganisms as well as that bound to soil minerals. The level of microbial activity is indicative of the amount of active organic matter that is broken down and nutrients being released (Franzluebbers et al., 2000; Brown, 2015). Dry soils restrict mineral nutrient solubility, soil microbial biomass and activity.

Controlling spatial variation of soil factors in agricultural field trials is the most important step to compare treatments efficiently and accurately (Hoefer et al., 2020). The cause and degree of field variability is either natural, man-made or both. For example, unequal fertilizer distribution result in heterogeneity problems, which can be minimized by sampling in the plough layer in tillage systems (Westernman, 1990). According to Fey (2001), soil acidity is a national problem limiting crop production in South Africa. The FERTASA annual congress of 2018 raised concerns that soil fertilization is causing high soil acidity in the Free State (Uys, 2018). Previously, Hoffman & Ashwell (2013) described acidic soils as a manifestation of non-erosive soil degradation. Soil acidity lowers plant growth, limits yields and reduces the availability of macronutrients (FERTASA, 2016).

In this study, soil health represents a balanced state of soil properties that nourish soil biodiversity and protect the soil biotic community from harmful factors and mismanagement (Vieweger & Döring, 2015), while plant health refers to any biological or physicochemical factors occurring on a crop rhizosphere that may harm plant form, physiology and performance in a given geographical space and temporal period (Savary et al., 2016). The main objective of this study was to demonstrate spatio-temporal variation in soil physicochemical and biological properties across a dryland field using the statistical (univariate and multivariate) data analysis. This would allow identification of patches of residual soil heterogeneity likely to influence or interact with treatment effects in long-term soil and plant health studies. In addition, depending on the objectives of the study, growers can group and remove plots (adjacent or distant) from field data analysis in an orthodox and unbiased manner (Forster, 1937; Cadet et al. 2003).

3.3 Materials and Methods

3.3.1 Field Site Description and Sample Collection

The field site occupied a total area of 2.98 ha of arenosol greyish sandy soil (FAO, 2005). This field is situated in the Eastern Free State/EFS region of Petrus Steyn in South Africa (S27° 53' 45.5" E28° 12' 59.8", Elevation 1705 m). The uniformity trial was conducted on this field using a randomized complete block design (RCBD), divided into a grid of 32 rectangular plots with each plot covering a 35 m × 21.91 m (24 rows wide in the North-South direction) or 766.85 m² area across eight blocks (the number of replications of each 'dummy' treatment level equals the number of blocks) equally spaced by 10 m buffer zone strips in the North-South direction. This design was convenient for mechanized planting and harvesting. Plant population was 30000 plants/ha and fertilizer amounting to N = 120 kg/ha, P = 20 kg/ha, K = 6 kg/ha was applied. A Sonop weather system was mounted on site (S27° 53' 46.8" E28° 12' 56.5", Elevation 1693 m) to capture and record climate data in real-time. The field experiment was managed uniformly and planted with the same maize cultivar on the same day following standard practice of conventional tillage in the EFS by the same skilful grower using the same planter on 24 November 2016 (see Table 1.1 in Chapter 1). This way, all the plots had no applied treatments, as such, zero treatment effects ('dummy' treatments) with differences in soil properties or crop productivity were expected to be approximately zero across plots.

Potatoes in year 1 were followed by maize on all plots in 2016/17 (Year 2/ season 2017), when the physical, chemical and microbial observations of the relevant soils commenced. Third year rotation crops were planted in 2017/18 (season 2018) and the 2018/19 summer growing season was designated year 4 (season 2019) of the four-year rotation. In this chapter, the season 2016/17 maize uniformity data will be used for analysis of the field trial (refer to Table 1.1, Chapter 1). The RCBD design (Figure 3.1) was also balanced in allocating replicates to 'dummy' treatments and blocks (representing either S1 to S8 or Blo 1 to Blo 8 series).

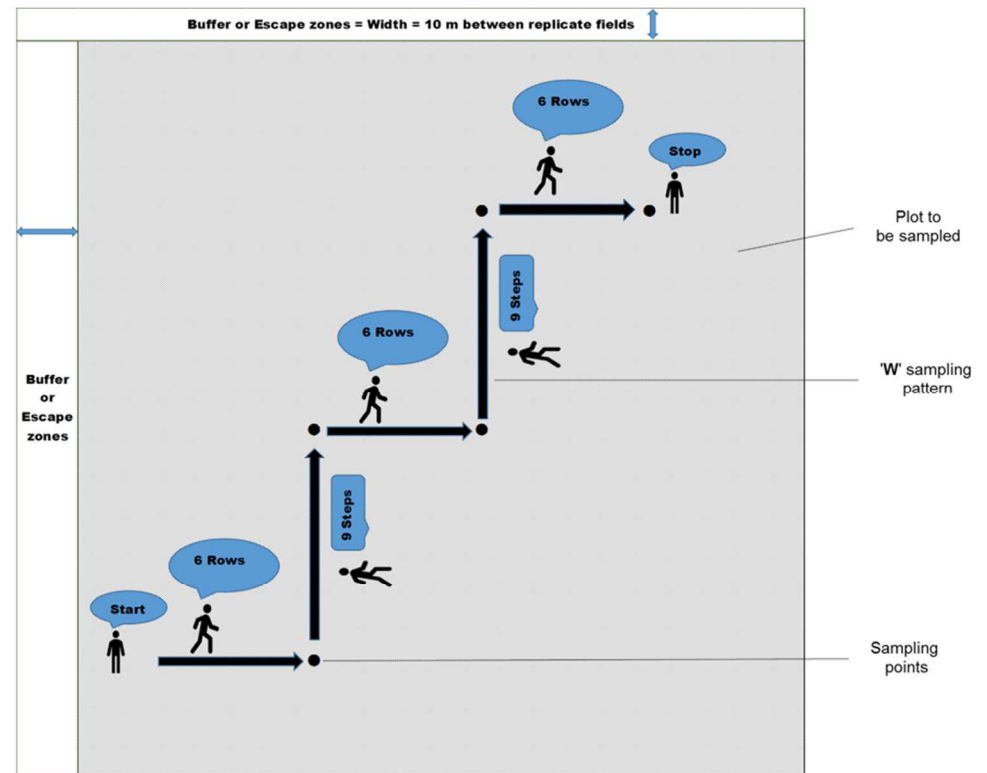
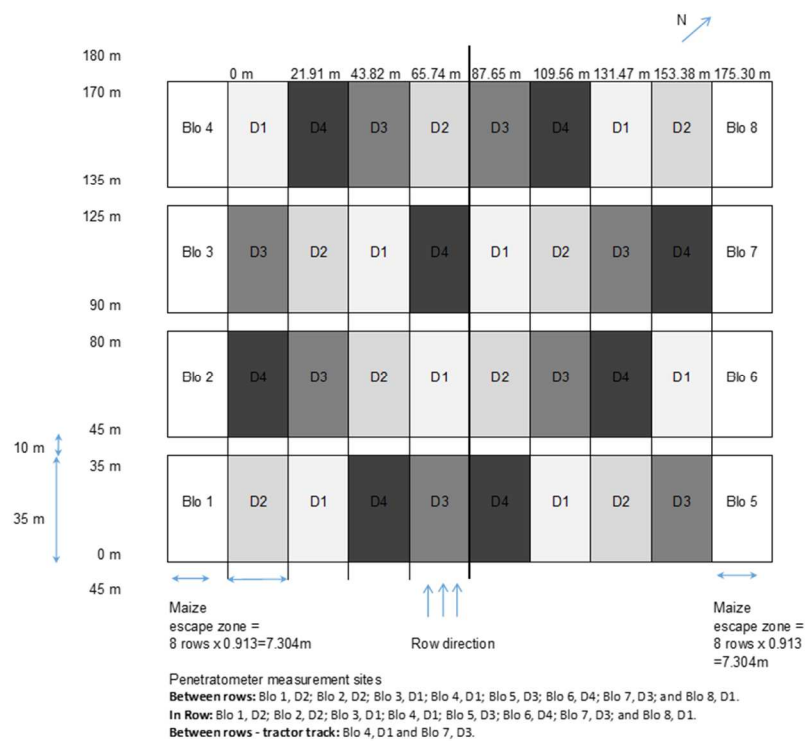


Figure 3.1 Illustration of a randomized complete block design of the uniformity trial (left), with each of the four ‘dummy’ treatments (D1 is white, D2 is grey, D3 is light-black, and D4 is black) allocated once in the eight replications/blocks (Blo 1 to Blo 8) and the random sampling of soils and plant materials through a walk in a ‘W’ sampling pattern across the plot unit (right).

Field soil sampling was done in a spatial W-shaped pattern covering 2 m² per plot area at physiological maturity at the R growth stage (194 days after planting). Rhizosphere soil samples were recovered by brushing-off soil attached to grub up maize plant roots whereas composite soil core samples were collected at the 0 – 20 cm depth using a 5 cm diameter by 8 cm height soil core. Pseudo-replication in sampling for plant health analysis was avoided by sampling from only 3 plants per replicate/block, followed by pooling these samples into one composite replicate/block sample sent for downstream DNA extraction and amplification steps. Composite soil core samples and rhizosphere soil samples were put into separate individual sterile zip-lock plastic bags, stored at 4 – 5 °C for soil analysis and – 80 °C for soil pathogen DNA analysis, respectively. Maize (*Zea mays*) cultivar was an early medium-maturity cereal grass grown under dryland climate conditions. After 7 months 3 days, the maize crop was harvested from all blocks using the same combine and driver on the same day, recording on average 12 % grain moisture content after full physiological maturity on 27 June 2017.

3.3.2 Crop-cut Method of Yield Components Estimation

Ten plants were sampled randomly from a sampling area of 3.185 m² (10 plants × 0.35 m × 0.91 m) measuring 0.91 m row width and 0.35 m spacing between plants using the crop cut technique described by Sapkota et al. (2016). The 10 plants were chopped using pruning shears, placed in a weighing box and measured for total biomass fresh weight plus ears using a weighing scale. The maize ears representing the 10 sampled plants were collected and bagged in 10 kg paper bags and taken to the laboratory to measure the following yield components: number of bad and good ears, grains per row, rows per ear, total fresh weight of ears, dry weights of stover and ears.

3.3.3 Whole Field Yield Data Collection using a Combine Harvester

Using the sampling for harvest unit technique described by Sapkota et al. (2016), 8 out of 24 rows (only 4 plots had 16 rows) of maize plants per plot were harvested using a combine harvester, the grains were measured for weight and % moisture content per plot on-site. The total yield or productivity per plot was computed by multiplying the yield of eight rows by the total area of the plot divided by the sampled harvest plot area of the eight rows, as recommended by Castellanos-Navarrete et al. (2013).

3.3.4 Soil Physicochemical and Biological Analysis

Soil physical, chemical and biological properties were measured using standard procedures at Nvirotek Laboratories (Pty) Ltd (ISO/IEC 17025 international standard for Testing laboratories Accredited Testing Laboratory, certified SANAS and SADCAS assessors, certified AgriLASA Fertilizer Laboratory, Unit 6, Nviro Business Hub, Ifafi, 0260, South Africa). The soil samples were dried, sieved and analysed for density prior to preparation of the 1:2 water extract slurry used to measure macro-and micronutrients using the Mehlich III extraction method (FERTASA, 2016). Total P was determined using the P Bray II method using hydrochloric acid and ammonium fluoride in a 1:7.5 extraction ratio (mass) for all soils with pH (KCl) < 7 (FERTASA, 2016).

Active carbon was measured using the permanganate-oxidizable active carbon method (Weil et al., 2003). Soil respiration was estimated using the standard protocol of the Solvita CO₂-burst test following manufacturer's instructions. Solvita CO₂ test estimates the microbial activity in the soil at field capacity and it is positively or highly related to soil fertility (Haney et al., 2015; Stott, 2019). On the other hand, *in situ* soil penetration resistance readings were taken in-rows, in-between rows and tractor traffic during dry conditions at 1 cm increments to a depth of 50 cm in every plot on the same day on 23rd of May 2017 using a Geotron Hand Penetrometer Model P5 (Geotron Systems, Potchefstroom, South Africa) up to 5000 kPa. The

soil bulk density was obtained from undisturbed soil samples collected at 20 cm and 40 cm depth using a handheld core sampler of known volume, and then dried and weighed to get the dry mass, from which the bulk density was calculated.

3.3.5 Soil Pathogen DNA Quantification

3.3.5.1. Field Rhizosphere Soil DNA Extraction

Approximately 0.25 g rhizosphere soil was subjected to total DNA extraction with a DNeasy® PowerSoil® Kit (QIAGEN Group, MoBio Laboratories, Inc.) following the manufacturer's instructions and samples were stored at -20 °C for further use. This commercial isolation and purification kit for soil DNA extraction was standardized, optimized and validated for soil types found in the EFS by Dube et al. (2019). The genomic DNA quality and quantity were verified using agarose gel electrophoresis and Qubit dsDNA high sensitivity assay as described in Dube et al. (2019).

3.3.5.2 Soil Pathogen DNA Copies across 'Dummy' Treatments

Concentrations of pathogen DNA in the rhizosphere and bulk field soils were determined simultaneously with standards and negative (no-template) control on each run. *Fusarium graminearum* translation elongation factor 1 alpha (tEF1α) (size 129-130 bp) was amplified successfully using TaqMan-based qPCR hydrolysis probe assay and primers listed in Table 3.1. Specific amplification was verified by assessing the size of the PCR product by 1% agarose gel electrophoresis.

Table 3.1 *Fusarium graminearum* specific primers and probe used in this study.

Sequence name	Description	Sequence (5′→3′)	Tm (°C)	Reference
EF1G_F	Forward	TGGTCTCATTTTCCTCGATCG	54.5	Marburger et al. (2015)
EF1G_R	Reverse	AAATTACGACAAAGCCGCAA	53.4	
EF1G_P	Probe	/56-FAM/CTCGATACG/ZEN/CG CCTGTTACCCC/3IABkFQ/	61.9	
Probe: labelled with 6-FAM-IB®FQ, Tm: Melting temperature				

Conventional PCR detected *F. graminearum* in the field rhizosphere soil samples (Season 2016-2017 pre-plant and post-harvest). The species-specific primers and probes were synthesized by Integrated DNA Technologies, Inc. (<http://www.idtdna.com>). Primer and probe protocols validated and described by Marburger et al. (2015) for *F. graminearum* were used. All the qPCR protocols were programmed and run in a 96-well PikoReal 96 thermal cycler (Thermal Fisher Scientific, California, United States). The species-specific primers and probes were synthesized by Integrated DNA Technologies, Inc. (<http://www.idtdna.com>).

A total volume of 20 μ L of TaqMan-based qPCR reaction mixture contained 0.8 μ L of DNA template, 10 μ L of 2x concentrated SsoAdvanced universal probes supermix (Bio-Rad Laboratories, Hercules, CA, United States), 0.4 μ L of each primer (from 10 μ M stock), 0.4 μ L of probe (from 10 μ M stock), and 8 μ L of nuclease-free water (New England Biolabs, Inqaba Biotech). Amplification conditions consisted of 50 °C for 2 min, 95 °C for 2 min, 40 cycles of 95 °C for 10 sec and 60 °C for 20 sec.

No-template controls included sterile nuclease-free water (New England Biolabs, Inqaba Biotech) and no template DNA.

3.3.5.3. *Fusarium graminearum* Standard Curve Preparation

Genomic DNA (gDNA) of *Fusarium graminearum* Schwabe culture number CBS 110261 from Forestry and Agricultural Biotechnology Institute (FABI) (University of Pretoria CMW culture collection laboratory) was extracted using the Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (ZYMO RESEARCH, Corp.) according to manufacturer's protocol and diluted in a series of 1:10 (initial concentration 10 ng/ μ l) to estimate the amplification efficiency of real-time PCR assays. The total genomic DNA quality and quantity were verified using 1% agarose gel electrophoresis and Qubit dsDNA high sensitivity assay as described in Dube et al. (2019). Reference *F. graminearum* cultures from CBS were revived and sub-cultured in PDA. Mycelia was successfully harvested and gDNA extracted. A Qubit 2.0 fluorimeter (Thermo

Fisher Scientific, California, United States) instrument estimated 4 – 40 ng/μl quantities of DNA template using the Qubit dsDNA HS Assay kit according to the manufacturer's instructions. Multiple samples were used as replicates to screen and select the sample concentrations which gave better DNA integrity for qPCR workflow.

The 10-fold dilution series was prepared, stored at -80 °C and thawed once before use. A log-linear regression standard curve of a first point 2-fold (20 ng/μl to 10 ng/μl) and five point 10-fold dilution series (10 ng/μl to 0.001 ng/μl) were generated by plotting the logarithms of known concentrations of *F. graminearum* DNA against the C_q values. PCR efficiency and coefficient of determination (R²) values were calculated using the Piko Real software. A slope of -3.32 represents the ideal 100% amplification efficiency. The sensitivity or minimum detection limit of the assay was estimated as minimum quantity of target DNA detected when the cycle threshold was attained at 40 cycles. All reactions were run in duplicate and samples that gave positive fluorescence before no-template controls were considered positive.

3.3.6 Soil Bioassays using Four Dryland Crops as Phytometers of Soil Heterogeneity

The soil bioassays were conducted and published in Mushonga et al. (2020) to compare phytometer data across blocks as part of the conditioning phase (phase 1) biomass.

3.3.7 Spatial Data Collection

Logging of spatial sampling of soil properties and maize yield components was done using a handheld global positioning system (GPS) device (eTrex Legend® HCx, Garmin International Inc., Olathe, Kansas, USA). The distribution of soil biological and physicochemical factors, climate, fungal and bacterial diversity, soil-borne pathogen DNA and yield components data was subjected to spatial analysis to determine the localization, geographic space and temporal period of soil and plant health.

3.3.8 Data Processing and Statistical Analysis

3.3.8.1 Univariate Analysis using Analysis of Variance (ANOVA)

The data from the uniformity trial was tested using the analysis of variance (ANOVA) as if there were four treatments, each of which was represented as ‘dummy’ treatments D1 to D4, each replicated eight times. The block and ‘dummy’ treatments were taken as fixed effects and their variation partitioned according to the general linear model for RCBD (Model 3.1 ANOVA) as described in Dicks (2006). The response of the unit/plot, y_{ij} , for the i^{th} treatment in the j^{th} block (replication) is written as:

$$y_{ij} = \mu + \tau_i + \beta_j + \epsilon_{ij} \quad \text{(Model 3.1 ANOVA)}$$

where μ is the general mean, τ_i is the ‘dummy’ treatment effect, β_j is the block effect, and ϵ_{ij} is the experimental error. In this case, the effects of the ‘dummy’ treatment and block effects are assumed to be additive i.e. the combined response is the sum of their separate response without interaction between ‘dummy’ treatments and blocks. The experimental errors are assumed to be independent and the block effects are considered to be random. The measured response variables included grain yield corrected to 12.5 % moisture (kg/ha) and soil metrics. The null hypothesis was that there were no statistically significant differences in response variable of D1, D2, D3 and D4 ‘dummy’ maize crop treatment means. The yield data did not appear to be normally distributed and therefore the non-parametric Kruskal-Wallis rank sum test was performed. The ANOVA statistical analysis was done in R version 3.5.2 (R.Core.Team, 2018) assuming that; ‘dummy’ treatment effects are constant over the different blocks, block effects are also constant for all plots within a block, and that the errors follow a normal distribution with mean of zero and variance of σ^2 .

Considering the low estimates in DNA copies of *F. graminearum* in field rhizosphere soils, pre-plant soil samples were analysed using only a one-way ANOVA. Phytometer plant estimates of soil productivity under greenhouse conditions were analysed using ANOVA. Block variation was analysed using the conditioning phase plant biomass data of the greenhouse study described in Mushonga et al. (2020). Block origin (categorical) was used as

an explanatory variable for the response variable (expressed as biomass yields in g per pot) to estimate heterogeneity in soil productivity.

3.3.8.2 Multivariate Analysis of Field Experiment Data using Principal Components Analysis (PCA) and Canonical Variate Analysis (CVA) or Discriminant Analysis with Redundancy Analysis (RDA)

The search for patterns of redundancy in plots, groups of plots clustered based on their similarities, and to test among existing groups of plots was achieved using multivariate analysis soil metrics at harvest with principal components analysis (PCA) and canonical variate analysis (CVA) algorithms in R version 3.5.2 (R.Core.Team, 2018), Canoco version 5 (Lepš & Šmilauer, 2014; ter Braak & Šmilauer, 2018) and ade4 (Thioulouse et al., 2018) software packages. The canonical variate analysis (CVA) or discriminant analysis with redundancy analysis (RDA) was calculated to explore soil properties that best separate the eight blocks (replications) using Fisher's multigroup linear DA. Constrained ordination using RDA statistical test was used to calculate the statistical significance of the relation between response variables (blocks data) and the explanatory variables (soil properties data). The study sought to reveal how the eight replication blocks differed in their soil properties at the end of the maize uniformity trial in season 2017. A total of 36 soil properties were measured per sample across the eight field blocks. Soil properties were taken as discriminant functions while blocks were class centroids.

3.3.8.3 Spatial Relationships using Principal Coordinates of Neighbour Matrices (PCNM)

PCNM was performed to separate spatial variation from that due to other predictor variables. The attribute plots in ordination space were visualized in two axes and displayed the data as a; (a) symbol plot and (b) contour plot. In a symbol plot, symbols of different size and colour are plotted based on the value of the selected attribute. Contour plots display the variation of an attribute across the ordination diagram using fitted regression model. The contour plots were

based on local weighted regression (loess) - smoother regression models (Lepš & Šmilauer, 2014; ter Braak & Šmilauer, 2018).

3.4 Results

The objective of the uniformity trial was to set up the environmental context for the soil and plant health studies using soil factors as explanatory variables of the plot environment. Most soil properties showed little variation between the four dummy treatments and across eight blocks. The *p*-values represent the conditional probability that a value of the test statistic or a more extreme value represented in our results could have been due to chance alone, given that the null hypothesis is true or correct (Gibson, 2015).

3.4.1 Climate

The field experiment was planted with maize in the 2016/2017 season following a potato uniformity trial in the previous season under summer rainfall in a randomized complete block designed trial with eight replicates of four dummy treatments. The average maximum air temperature was warm at 24.8 ± 0.276 °C while the minimum was cool at 9.46 ± 0.313 °C (Figure S3.1A and S3.1C, respectively). Highest maximum temperatures above 30 °C were recorded in December 2016 to February 2017 (Figure S3.1A). After this hot period, the temperatures were below 30 °C and favourable for flowering. In the month of planting there was an increase in vapour pressure deficit but reduced thereafter into the winter of the year 2017 (Figure S3.1E). The minimum air temperature was below zero at the beginning of the winter season end of May 2017 and in winter June 2017 at the end of the growing season (Figure S3.1C) although no frost damage was experienced during the growing season. The evaporative demand (Eto) was high in November 2016 to December 2016 during this rainfall period thereby reducing the risks of drainage losses (Figure S3.1B).

About 68% of the evaporation demand occurred between November 2016 and March 2017 (Figure S3.1B). The maximum relative humidity was generally above 80% for most of the

growing season (Figure S3.1A) while the minimum relative humidity range was 82.6% (Figure S3.1C). As expected, total solar radiation (MJ/m²) was higher in summer than in winter of the growing season (Figure S3.1B). The average wind speed was light (3.37 ± 0.102 m/s) during the growing season. A moderate breeze in December 2016 and February 2017 coincided with the high rainfall during the growing season (Figure S3.1D). A total of 587 mm was measured, from planting in November 2016 to harvesting in June 2017 (Figure S3.1D). Most of the rains were received in December 2016 and February 2017 (Figure S3.1D) during the growing season. In addition, more than 90% of the rain occurred from October 2016 to March 2017 (Figure S3.1D) which was ideal for a summer crop like maize.

3.4.2 Yield Distribution and Patterns

Soil properties are dynamic and they cause soil heterogeneity across plots of the same size and shape. In field trials, soil variability minimizes precision and leads to misinterpretation of field data. Legacy effects of crop rotations were investigated in the Eastern Free State, South Africa. Measurements of yield, soil biology and soil physicochemical properties representing response variables were done at crop physiological maturity i.e. at final harvest or end of the growing season. Season 1 uniformity trial was a true “blank trial” ($p > .05$) using the same maize variety, grower and farm equipment on the same day from planting to harvest. In our study, we partitioned the statistics orthogonally using a RCBD. The RCBD layout ensured statistical independence between separate blocks and as a result the field data collected in one block was not influenced by the presence of other neighbouring blocks.

Figures 3.2 and 3.3 show box plots of maize yield parameters in different ‘dummy’ treatments and blocks, respectively. Medians of yield was approximately constant in ‘dummy’ treatments and in blocks. The grain dry yields were obviously similar (Figure 3.2a). The grain yield corrected to 12.5% moisture (kg/ha) was not significantly different ($p > .05$) among the dummy treatments using the Kruskal-Wallis rank sum test ($\chi^2 = 3.6534$, d.f = 3, $P = 0.3474$).

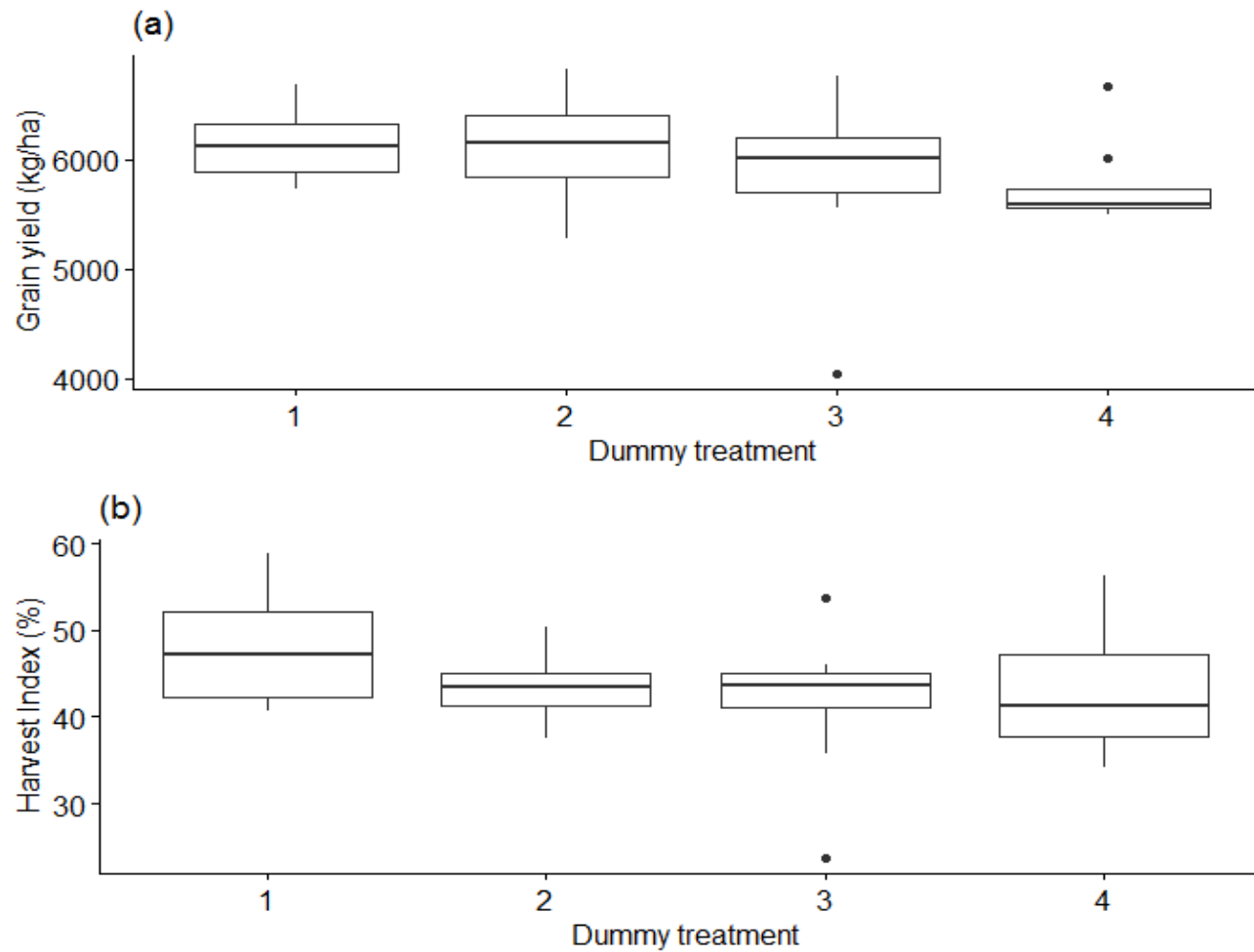


Figure 3.2 Boxplots comparing yield parameters in four 'dummy' treatments measured at harvest; (a) grain yield (kg/ha) and (b) harvest Index (%). Each 'dummy' treatment had 8 replications. Each block had 4 'dummy' treatments. Horizontal heavy bar = median, box=lower and upper quartiles, whiskers=minimum and maximum values, filled dot=outliers.

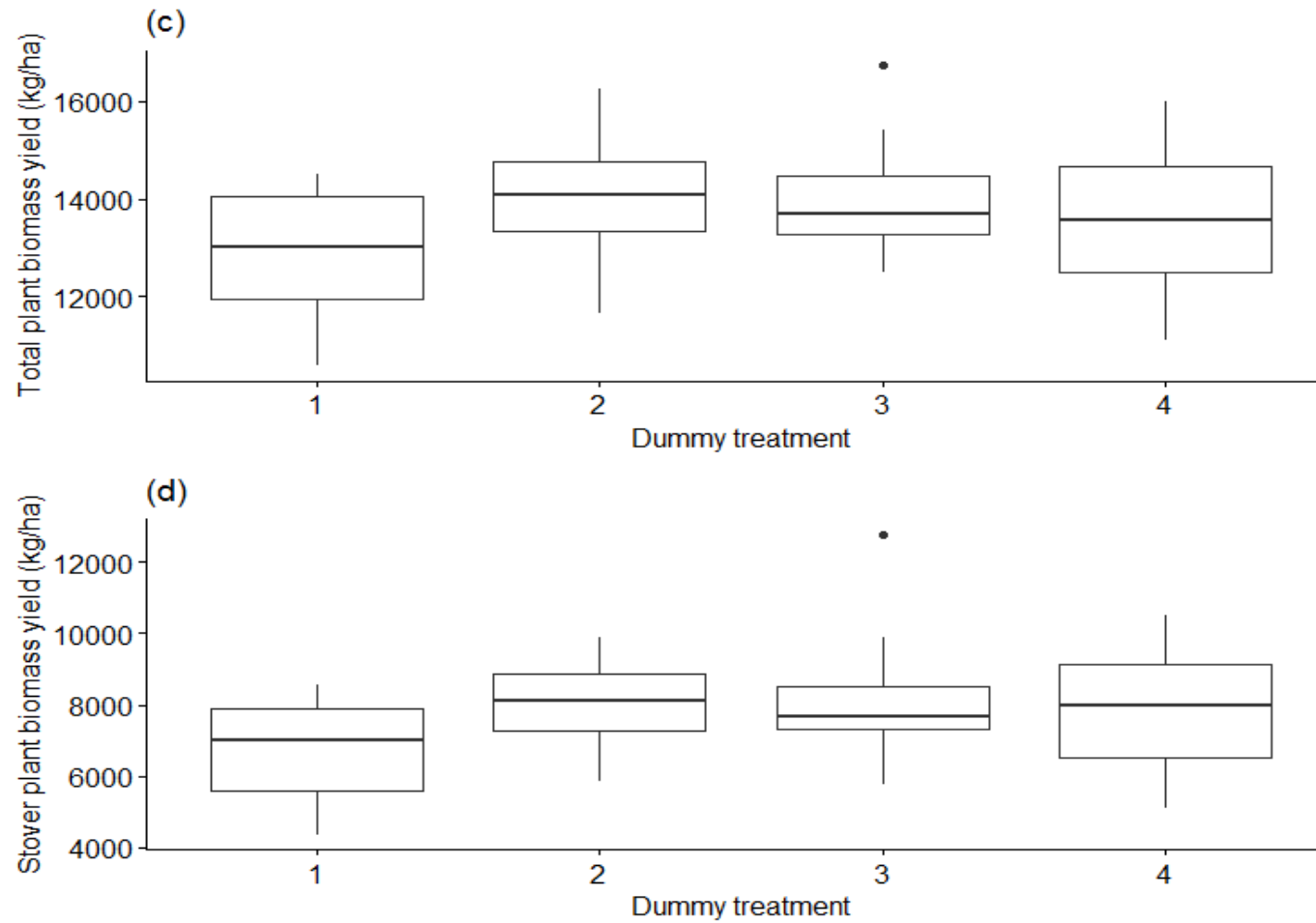


Figure 3.2 Boxplots comparing yield parameters in four 'dummy' treatments measured at harvest; (c) total plant biomass yield (kg/ha), and (d) stover plant biomass yield (kg/ha). Each 'dummy' treatment had 8 replications. Each block had 4 'dummy' treatments. Horizontal heavy bar = median, box=lower and upper quartiles, whiskers=minimum and maximum values, filled dot=outliers.

Similarly, there was not enough statistical evidence ($p > .05$) that one or more dummy treatments were different in harvest index (Figure 3.2b; Table S3.1A), total plant biomass yield (Figure 3.2c; Table S3.1B) and stover biomass yield (Figure 3.2d; Table S3.1C). Blocks 1 and 6 (S1 and S6) had the highest mean grain yield and block 5 (S5) had the lowest mean yield albeit highly variable (Figure 3.3). The block and ‘dummy’ variable effect on all yield parameters were not statistically significant ($p > .05$, Tables S3.2A to S3.2C, Figure 3.3) suggesting that blocks explain very little variability in yield parameters and there was no need to include block effects in yield modelling. The variable ‘block’ was orthogonal to ‘dummy treatment’ by having a sequential sum of squares (Seq SS) equal to the adjusted sum of squares (Adj SS) for each of the two variables i.e. dummy treatments and block in the resulting ANOVA table for total plant biomass response (Table S3.2A), stover plant biomass response (Table S3.2B) and harvest index response (Table S3.2C).

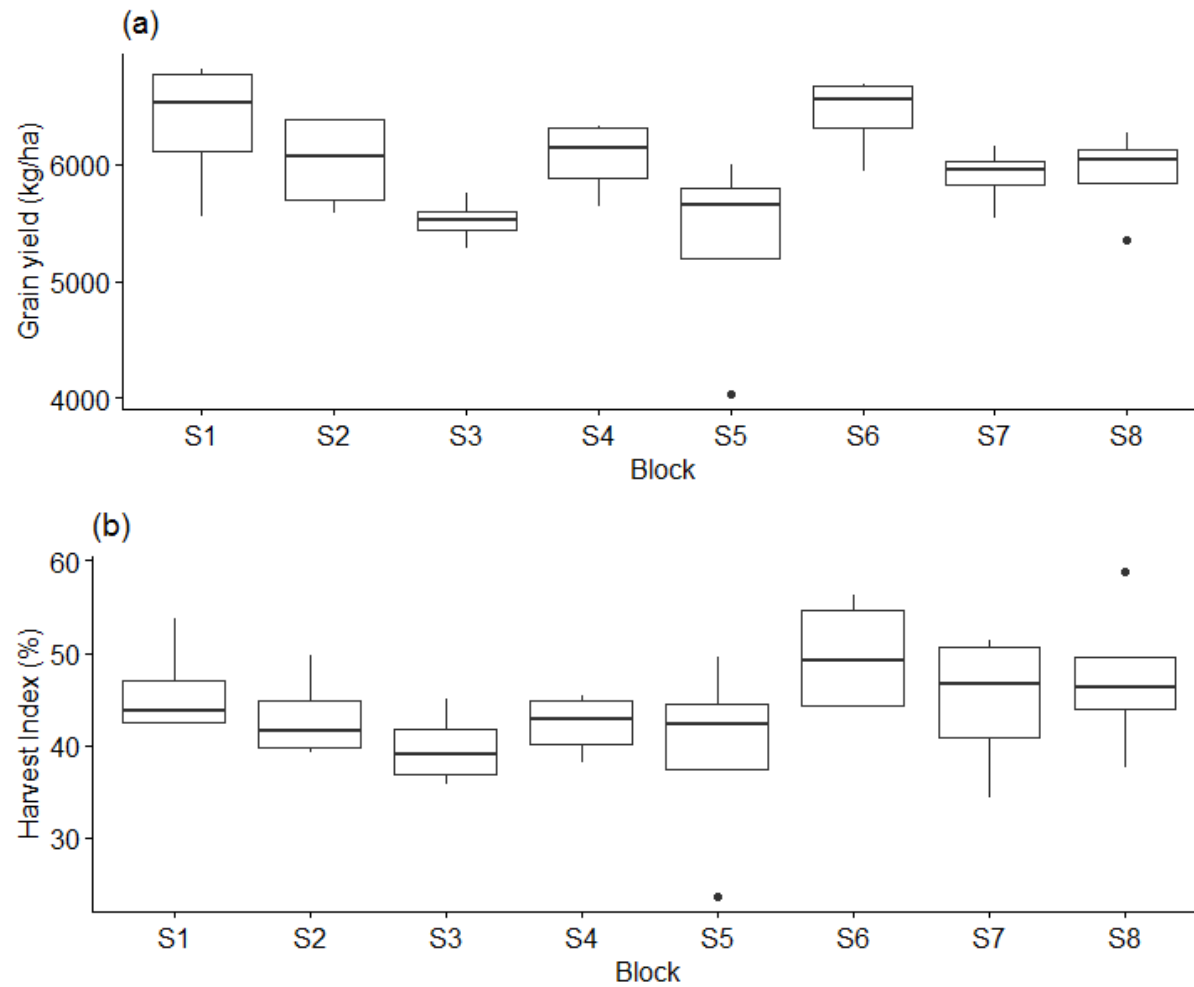


Figure 3.3 Boxplots comparing yield parameters in eight replications/blocks measured at harvest; (a) grain yield (kg/ha) and (b) harvest Index (%). S1 to S8 represents blocks Blo 1 to Blo 8 series. Each block had 4 ‘dummy’ treatments. Horizontal heavy bar = median, box=lower and upper quartiles, whiskers=minimum and maximum values, filled dot=outliers.

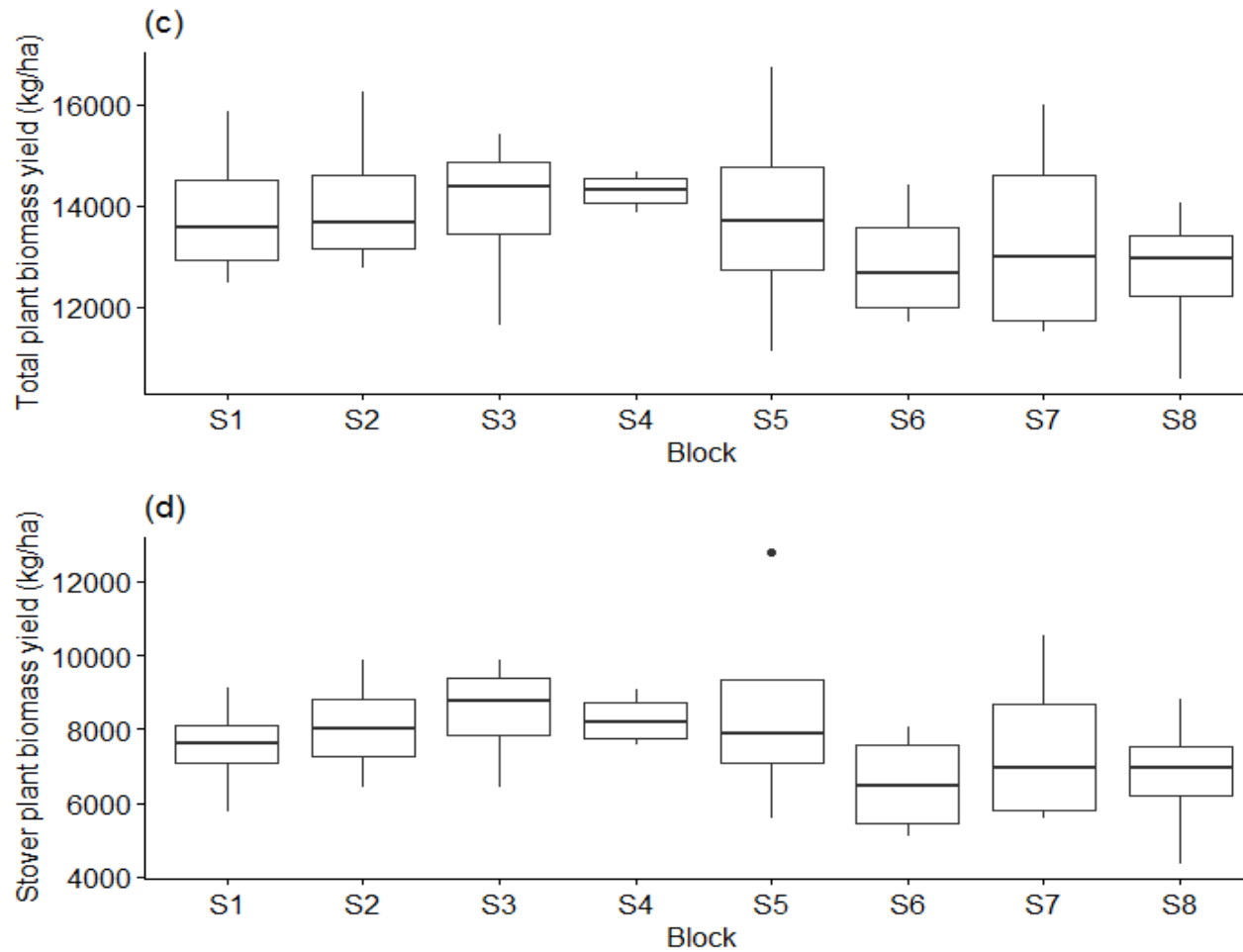


Figure 3.3 Boxplots comparing yield parameters in eight replications/blocks measured at harvest; (c) total plant biomass yield (kg/ha), and (d) stover plant biomass yield (kg/ha). S1 to S8 represents blocks Blo 1 to Blo 8 series. Each block had 4 ‘dummy’ treatments. Horizontal heavy bar = median, box=lower and upper quartiles, whiskers=minimum and maximum values, filled dot=outliers.

3.4.3 Legacies of Soil Health Properties across Dummy Treatments and Blocks

3.3.3.1 Soil Physicochemical and Biological Properties

The soil was sandy (8.94% clay, 9.80% silt, 81.30% sand) (Figure S3.2A) and acidic (pH (KCl) 4.86, pH (H₂O) 6.23) with normal %Ca (71.1%), P (Bray=55.42 mg/kg, Mehlich=48.74 mg/kg), S (7.96 mg/kg), Mg (54.78 mg/kg), Fe (66.1 mg/kg), B (0.24 mg/kg), Ca:Mg (4.14), (Ca+Mg)/K (10.7) (Figure S3.2B). The 1:2 Water extract N - NO₃, 1:2 Water extract N - NH₄, Mg:K, Ca, Na, K, %acid saturation, Mn, Cu and Zn were below the normal levels according to the FERTASA (2016) and A & L Laboratories et al. (2001) guidelines. On the other hand, the %Mg, %K, %Na, S-Value, and Al levels were above optimum levels in the soil using the Albrecht categories cited in Sait (2018).

The boxplots (Figure S3.2C) show that the dummy treatment soils had similar Solvita CO₂ respiration values averaging between 4.5 and 5 ppm. The soil respiration values of CO₂ predicted a 16.81-28.02 kg/ha nitrogen (N) release per year based on the Solvita® index color scale and predicted N contribution (Sciarappa et al., 2017; Brinton, 2016). It was obvious that there were no differences in Solvita CO₂ values across the dummy treatments (Figure S3.2C). In Tables S3.3, P Bray II averaged at 55.42 ±1.64 ppm between ideal levels of 40 ppm and 60 ppm for good crop production (A & L Laboratories et al., 2001). As expected, Al levels (274.15 ±6.36 ppm) were above the ideal < 16 ppm in acidic soils (A & L Laboratories et al., 2001). It is obvious that there were no differences in dummy treatment averages of Solvita CO₂ values across the treatment blocks 1 to 8 (Table S3.3). The highest average CO₂ levels were observed in block 8 (6.08 ±0.99 ppm) and block 7 (5.80 ±0.26 ppm) (Table S3.3). Soil strength readings were done on 23 May 2017 at crop maturity before harvest. Soil strength readings represented a measure of the mechanical resistance of soil against root penetration. The general trend from highest to lowest soil consolidation was as follows: (i) between rows; Tractor traffic 1(5312 kPa)>Tractor traffic 2>Block 1>Block 7>Block 3>Block 6>Block 2>Block 8>Block 5>Block

4 (1600 kPa), (ii) in rows; Block 2 (2250 kPa)>Block 8>Block 6>Block 4>Block 1>Block 7>Block 5>Block 3 (1310 kPa) (Figure S3.2D).

Variation in yield and soil properties across block sites are displayed using a common graphical representation in Figure 3.4.

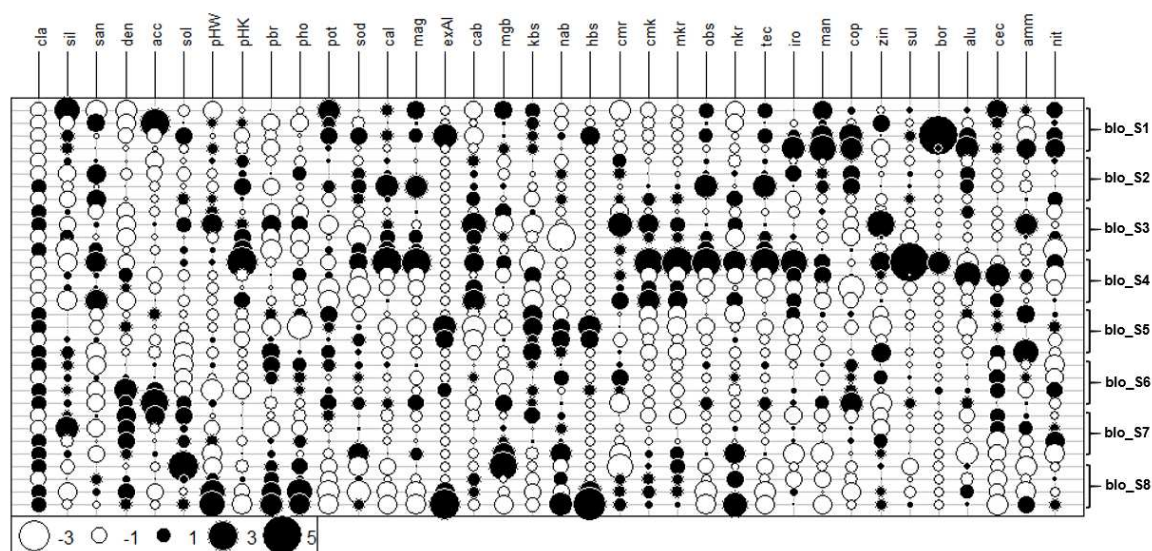


Figure 3.4 Graphical representation of soil properties. There were 36 soil properties that were measured per plot (rows) soil sample. Columns correspond to soil properties and the corresponding size of the circles from lowest (–, open) to highest (+, filled) values. Data has been scaled for graphical comparisons of variables. Rows represent 32 plots for the 8 blocks (blo_S1 to blo_S8). Each block has 4 plots arranged in chronological order.

acc - Active carbon (mg/kg)	cab - %Ca	alu - Al (mg/kg)
amm - 1:2 Water extract N - NH ₄ (mg/l)	cla - Clay (%)	bor - B (mg/kg)
cec - CEC (cmol+)/kg)	cop - Cu (mg/kg)	cal - Ca (mg/kg)
cmk - (Ca+Mg)/K (10.0-20.0)	hbs - %acid saturation	iro - Fe (mg/kg)
cmr - Ca:Mg (1.5-4.5)	kbs - %K	mag - Mg (mg/kg)
exAl - OUT H+Al (cmol+)/kg)	mgb - %Mg	man - Mn (mg/kg)
mkr - Mg:K (3.0-4.0)	nab - %Na	pHK - pH (KCl)
nit - 1:2 Water extract N - NO ₃ (mg/l)	san - Sand (%)	pho - P (mg/kg)
tec - T (cmol+)/kg) tec - T (cmol+)/kg)	sil - Silt (%)	pHW - pH (H ₂ O)
obs - S-Value (cmol+)/kg)	sul - S (mg/kg)	pot - K (mg/kg)
pbr - P Bray II (mg/kg)	zin - Zn (mg/kg)	sod - Na (mg/kg)
sol - Solvita CO ₂ respiration (ppm)	den - Density (g/cm ³)	nkr - Na:K

The linear ordination method PCA was suggested as a method of choice considering the low heterogeneity in selected soil properties. The linear ordination method PCA was suggested as

a method of choice considering the low heterogeneity in selected soil properties (Plant, 2012).

Furthermore, the PCA was the method of choice because:

- PCA is an exploratory tool that elaborate general hypothesis from the collected data and to extract and weight information in complex univariate datasets (Wander & Bolero, 1999; Sena et al., 2002; Thioulouse et al., 2018)
- We were sure of what farmers in the Potatoes SA needed and the sample collection and handling was standardized for EFS (Dube et al., 2019).
- PCA enables good comparisons of the large and complex datasets of soil health properties involving physical, chemical and biological data (Sena et al., 2002).
- PCA assists in finding trends or explaining/verifying temporal and spatial soil events (Cadet et al., 2003).
- PCA allows the identification of multicollinearity issues in the datasets (Clark et al., 1999).

This way, the ideal soil health factors including the confounding factors are identified, consequently the field experiment can be managed to obtain ecological balances at the same time avoiding chemical abuse in this particular context (Cadet et al., 2002). In Figure 3.5, the PCA biplot indicates that the two dummy treatment replicates (D3 and D4) in Block 8 (S8) are higher than D1 and D2 in the same block in %acid saturation (by $8 \times 10^7\%$ difference), P Bray II (mg/kg) (by 12% difference), P (mg/kg) (by 19% difference), salt-replaceable acidity (cmol(+)/kg) (by $7 \times 10^7\%$ difference), Density (g/cm³) (by 3% difference), %Na (by 7% difference), Clay (%) (by 11% difference) and these factors show a higher correlation amongst themselves. One dummy treatment replicate in Block 4 (D1 in S4) is different from other replicates within this block due to soil factors such as pH (KCl), S (mg/kg), Fe (mg/kg), Ca (mg/kg), Mg:K, Ca+Mg)/K, %Ca. The first two PCA axes explain 47.89% of the variance in

blocks data (Table S3.4A) indicating that the variation is ideal for discriminating between variables.

The relationship among variables in Figure 3.5 was determined by the directional cosines of the angle formed between lines drawn from the origin to each variable. Biotic variables Solvita CO₂ respiration (ppm) and active carbon (mg/kg) had no effect on each other while P (mg/kg) and pH (H₂O) were highly correlated but orthogonal to pH (KCl). Na:K and K (mg/kg) variables had opposing effects as well as the pH (H₂O) and active carbon (mg/kg) (Figure 3.5). Most blocks did not have important differences in soil properties as they were close to the centroid (Figure 3.5). The variables K (mg/kg), %K, salt-replaceable acidity(cmol(+)/kg), %acid saturation, Na:K, Ca+Mg)/K, Mg:K, %Ca, pH (KCl), Mg (mg/kg), Ca (mg/kg), S-Value (cmol(+)/kg), T (cmol(+)/kg) significantly contributed to the variation observed between plots than other soil properties (Figure 3.5, Table S3.4B and S3.4C). %Ca, Mg:K, Ca+Mg)/K and pH (KCl) seem to have more direct influence on one of the plots in Block 4 than the rest of the plots (S4 in Figure 3.5).

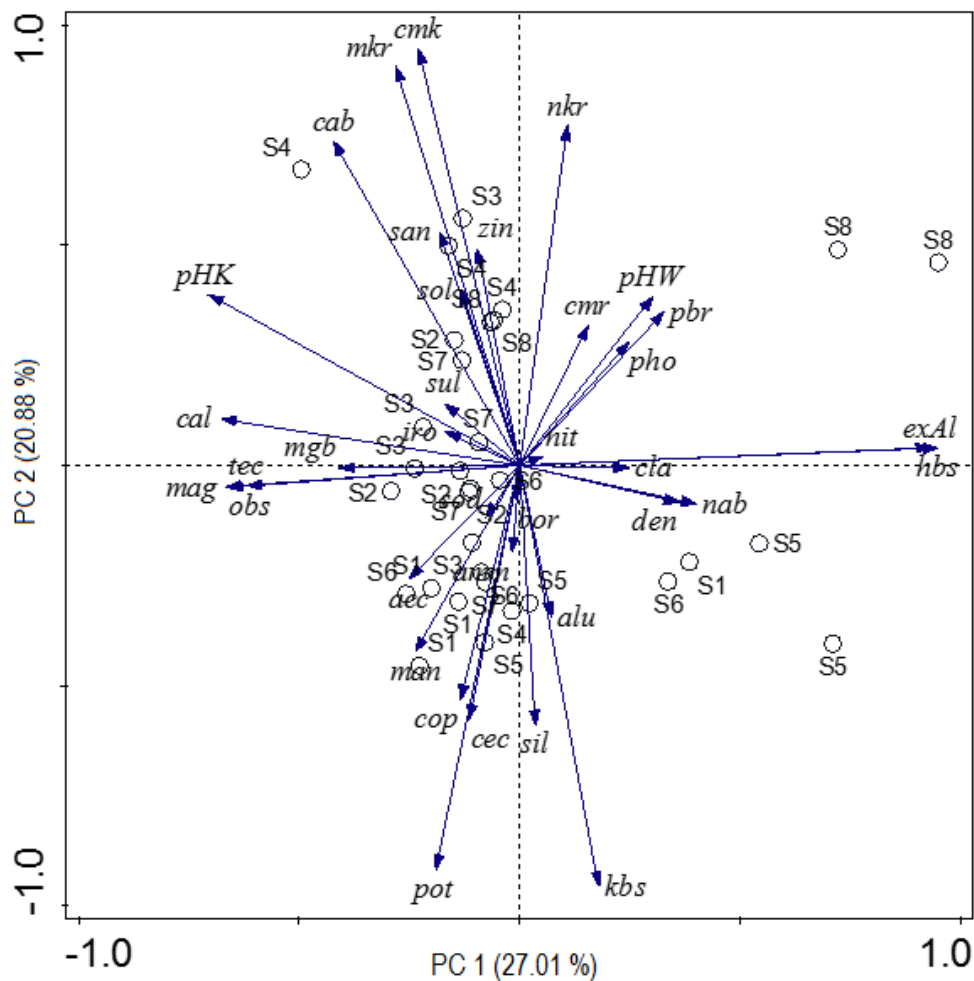


Figure 3.5 Visualization of the application of principal component analysis in soil health studies in a uniformity field trial. Blocks-soil factors biplot diagram from PCA (the first two PCA axes are plotted explaining 47.89% of the total variance) with soil properties data variables passively projected into the ordination space. Blue arrows represent soil factors direction of the change while circles for soil samples from block sites (S1 to S8).

acc - Active carbon (mg/kg)	cab - %Ca	alu - Al (mg/kg)
amm - 1:2 Water extract N - NH ₄ (mg/l)	cla - Clay (%)	bor - B (mg/kg)
cec - CEC (cmol+)/kg)	cop - Cu (mg/kg)	cal - Ca (mg/kg)
cmk - (Ca+Mg)/K (10.0-20.0)	hbs - %acid saturation	iro - Fe (mg/kg)
cmr - Ca:Mg (1.5-4.5)	kbs - %K	mag - Mg (mg/kg)
exAl - OUT H+Al (cmol+)/kg)	mgb - %Mg	man - Mn (mg/kg)
mkr - Mg:K (3.0-4.0)	nab - %Na	pHK - pH(KCl)
nit - 1:2 Water extract N - NO ₃ (mg/l)	san - Sand (%)	pho - P (mg/kg)
tec - T (cmol+)/kg)	sil - Silt (%)	pHW - pH(H ₂ O)
obs - S-Value (cmol+)/kg)	sul - S (mg/kg)	pot - K (mg/kg)
pbr - P Bray II (mg/kg)	zin - Zn (mg/kg)	sod - Na (mg/kg)
sol - Solvita CO ₂ respiration (ppm)	den - Density (g/cm ³)	nkr - Na:K

The two dummy treatment replicates from block 8 (D3 and D4) showed higher in soil pH (H₂O), P (Bray), Ca: Mg ratio and P than other two replicates (D1 and D2) within the same block. One dummy treatment replicate from block 4 (D1) was higher in eight soil factors than the other three replicates (D2, D3 and D4) from this block. The soil Mg:K ratio and (Ca+Mg)/K ratio are positively correlated with each other but negatively correlated with soil K, %K, Cu, %silt, CEC, Mn, Al, density, %Na, %clay and Mg, and soil pH (H₂O), P (Bray), Ca:Mg ratio and P are highly correlated with each other and not correlated with the soil Cu, Na, Mg, K, Mn, CEC, %silt and %K (Figure 3.5). The S5 outlier is not important since most replicates in this block were clustered in the same principal components. All block replicates and soil factors that were close to the centroid represented unimportant features (Sena et al., 2002) while those far from the origin were the influential factors in the particular principal components. (Cavigelli et al., 1995). Soil factors that were close to a plot unit (i.e. a replicate), will have high direct influence on it whereas, if soil factor (s) are distant from a plot unit, they will have a high inverse influence on it (Sena et al., 2002). However, caution must be taken to avoid preemptive conclusions based on correlations from PCA results considering that the subsequent correlations and analysis in the next seasons may rule out or confirm these relationships.

The % acid saturation, salt-replaceable acidity (cmol(+)/kg), (Ca+Mg)/K and %K concentration were the most important factors for soil composition while the least important factors were values from clay, N - NO₃ and N - NH₄ (Table S3.4B). S-Value (cmol (+)/kg) is proportional directly to (K+Ca+Mg+Na) and inversely to CEC while %acid saturation is proportional directly to Al+H and inversely to the cation exchange capacity (CEC) (FERTASA, 2016). The base saturation percentages were above ideal levels but in sandy soils with CEC < 5 these values are ignored.

The crop yield data is very useful in evaluating the productivity of long-term field trials, nevertheless, yield is not an ideal indicator in commercial agriculture because most farmers

will try anything and/or everything to get the best harvest figures. Stover and total plant biomass yield was positively correlated with each other but negatively correlated with harvest index and yield corrected for moisture (Figure 3.6). Yield corrected for moisture was highly correlated with Solvita CO₂ respiration (ppm) and %Mg (Figure 3.6).

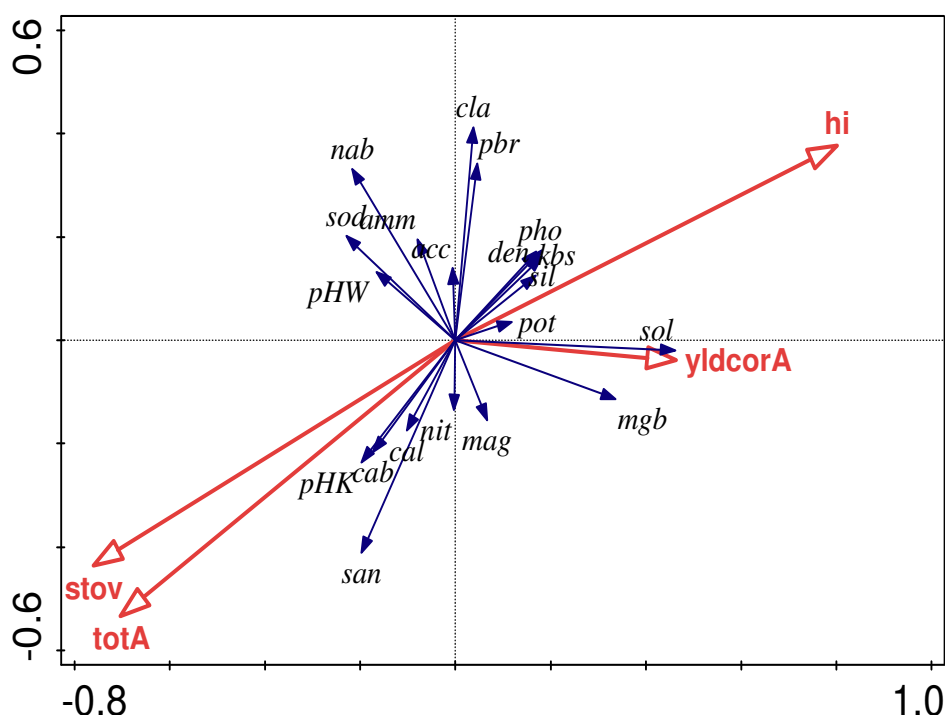


Figure 3.6 Biplot diagram using redundancy analysis (RDA) axes of the effects of the environment (predictor variables direction of the change labelled in black arrows) on the yield parameters (response variables direction of the change labelled in red arrows). Eighteen soil quality properties and four yield parameters are shown.

acc - Active carbon (mg/kg)	cab - %Ca	nab - %Na
yldcorA – Yield (kg/ha)	cla - Clay (%)	pHK - pH(KCl)
pbr - P Bray II (mg/kg)	sil - Silt (%)	cal - Ca (mg/kg)
nit - 1:2 Water extract N - NO ₃ (mg/l)	san - Sand (%)	pho - P (mg/kg)
totA – Total plant biomass yield (kg/ha)	pot - K (mg/kg)	kbs - %K
sol - Solvita CO ₂ respiration (ppm)	den - Density (g/cm ³)	mag - Mg (mg/kg)
hi - Harvest index (%)	mgb - %Mg	pHW - pH(H ₂ O)
stov- Stover yield (kg/ha)	sod - Na (mg/kg)	

The canonical variate analysis (CVA) or discriminant analysis with redundancy analysis (RDA) was calculated to explore soil properties that best separate the eight blocks (replications)

using Fisher's multigroup linear DA. The permutation test indicated that simple effects (independent effects of all explanatory variables) and conditional effects (represent variation and significance explained by a predictor after accounting for the effect of the predictors placed above it in the list) are detailed in Table S3.4A, Table S3.4B and Table S3.4C.

Similar to PCA, RDA two axes represented 47.89% of the total variation in soil properties across eight blocks (Tables S3.4A and S3.5). The % acid saturation, salt-replaceable acidity (cmol(+)/kg), Ca+Mg/K, and %K contributed the highest variability than 1:2 water extract N - NO₃ (mg/l), clay (%), 1:2 water extract N - NH₄ (mg/l) (Figure 3.6, Table S3.5).

The comparison of constrained and unconstrained ordinations (Tables S3.5 and S3.6) showed that the combined explanatory efficiency of the most important soil properties for yield and its parameters included; % acid saturation, salt-replaceable acidity (cmol(+)/kg), (Ca+Mg)/K (10.0-20.0), %K, Mg:K (3.0-4.0), K (mg/kg), S-Value (cmol(+)/kg), pH(KCl), Ca (mg/kg), Mg (mg/kg), T (cmol(+)/kg), %Ca, Na:K, S (mg/kg), Mn (mg/kg). The conditional effects of some variables decreased drastically indicating that these variables were closely correlated to % acid saturation, %K, S (mg/kg), Mn (mg/kg).

Sampling the microenvironment or the root zone maximized the detection of microvariation at the same time minimizing soil meso- and macrovariation. CVA analysis discriminated best between the eight blocks using linear combination of soil properties based on an analysis of variance (ANOVA) *F*-ratio (Jongman et al., 1995). The findings (Figure 3.7) indicate that the second axis best discriminates between the eight blocks explaining 47.89% variance (Table S3.4A). The triplot (Figure 3.7) shows that blocks 4, 6 and 8 soil samples were very different from the other blocks based on the Mahalanobis distance scale (Legendre & Legendre, 2012). Blocks 2, 3 and 7 were very close to one another suggesting relatedness of the original variables to one another, and similarities in blocks 1 and 5 were detected (Figures 3.5, 3.6, and 3.7). The triplot evidence (Figure 3.7) of soil properties projection around the centroid as the focal point

confirms that soil properties had something to do with the observed block differences, in other words, most of the measured soil properties were homogenous across blocks. Also, Tables S3.7, S3.8 and S3.9 summarize the means and discriminant functions of the variables for all data to predict membership of a particular sample.

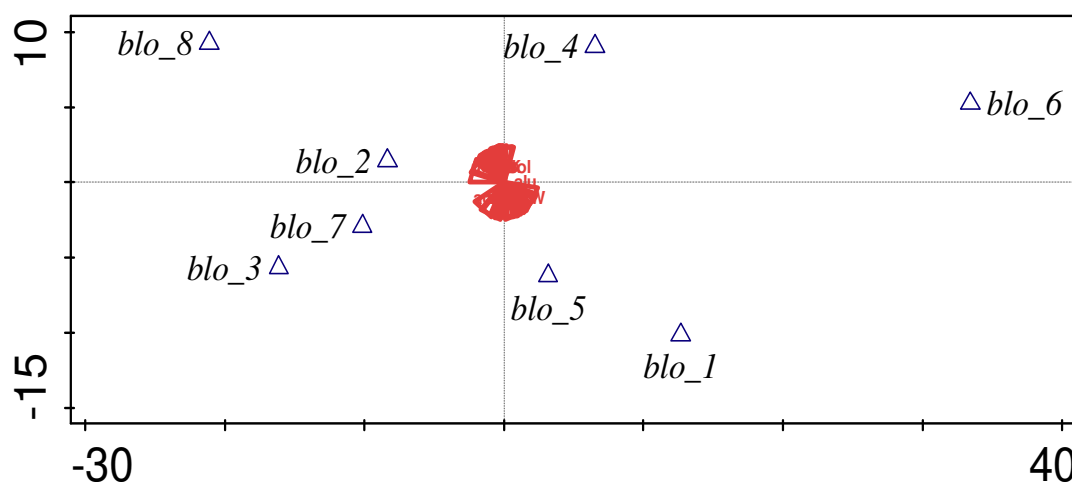


Figure 3.7 Triplot of uniformity trial data based on discriminant analysis and the classification factor site (blocks) and soil properties (red in colour) as the focal point. Canonical variate analysis in discriminant analysis is summarized in Tables S3.7-S3.9. The closer the blocks to each other the smaller the inter-block distance. The short red arrows (not clear in this triplot in Figure 3.7) for a soil property indicate that it does not differ that much within blocks.

acc - Active carbon (mg/kg)	cab - %Ca	alu - Al (mg/kg)
amm - 1:2 Water extract N - NH ₄ (mg/l)	cla - Clay (%)	bor - B (mg/kg)
cec - CEC (cmol(+)/kg)	cop - Cu (mg/kg)	cal - Ca (mg/kg)
cmk - (Ca+Mg)/K (10.0-20.0)	hbs - %acid saturation	iro - Fe (mg/kg)
cmr - Ca:Mg (1.5-4.5)	kbs - %K	mag - Mg (mg/kg)
exAl - OUT H+Al (cmol(+)/kg)	mgb - %Mg	man - Mn (mg/kg)
mkr - Mg:K (3.0-4.0)	nab - %Na	pHK - pH(KCl)
nit - 1:2 Water extract N - NO ₃ (mg/l)	san - Sand (%)	pho - P (mg/kg)
tec - T (cmol(+)/kg)	sil - Silt (%)	pHW - pH(H ₂ O)
obs - S-Value (cmol(+)/kg)	sul - S (mg/kg)	pot - K (mg/kg)
pbr - P Bray II (mg/kg)	zin - Zn (mg/kg)	sod - Na (mg/kg)
sol - Solvita CO ₂ respiration (ppm)	den - Density (g/cm ³)	nkr - Na:K

3.4.3.2 Quantification of *Fusarium graminearum* DNA Copies in Field Soils using the TaqMan Hydrolysis Probe qPCR Assay

Serial dilutions of the gDNA extracted from *F. graminearum* had a minimum detection limit of 0.005 ng/μl (Figure 3.8) based on the minimum quantity of target DNA detected at the threshold value of the qPCR machine set automatically. The average C_q values range of samples were 32 to 39.51 (Figure 3.8a), 32 to 37.07 (Figure 3.8b). C_q values ranging between 32 and 34 (Figure 3.8a), 39.2 and 39.6 (Figure 3.8b) i.e. above the average C_q values were taken as 'pathogen DNA present' but not quantifiable.

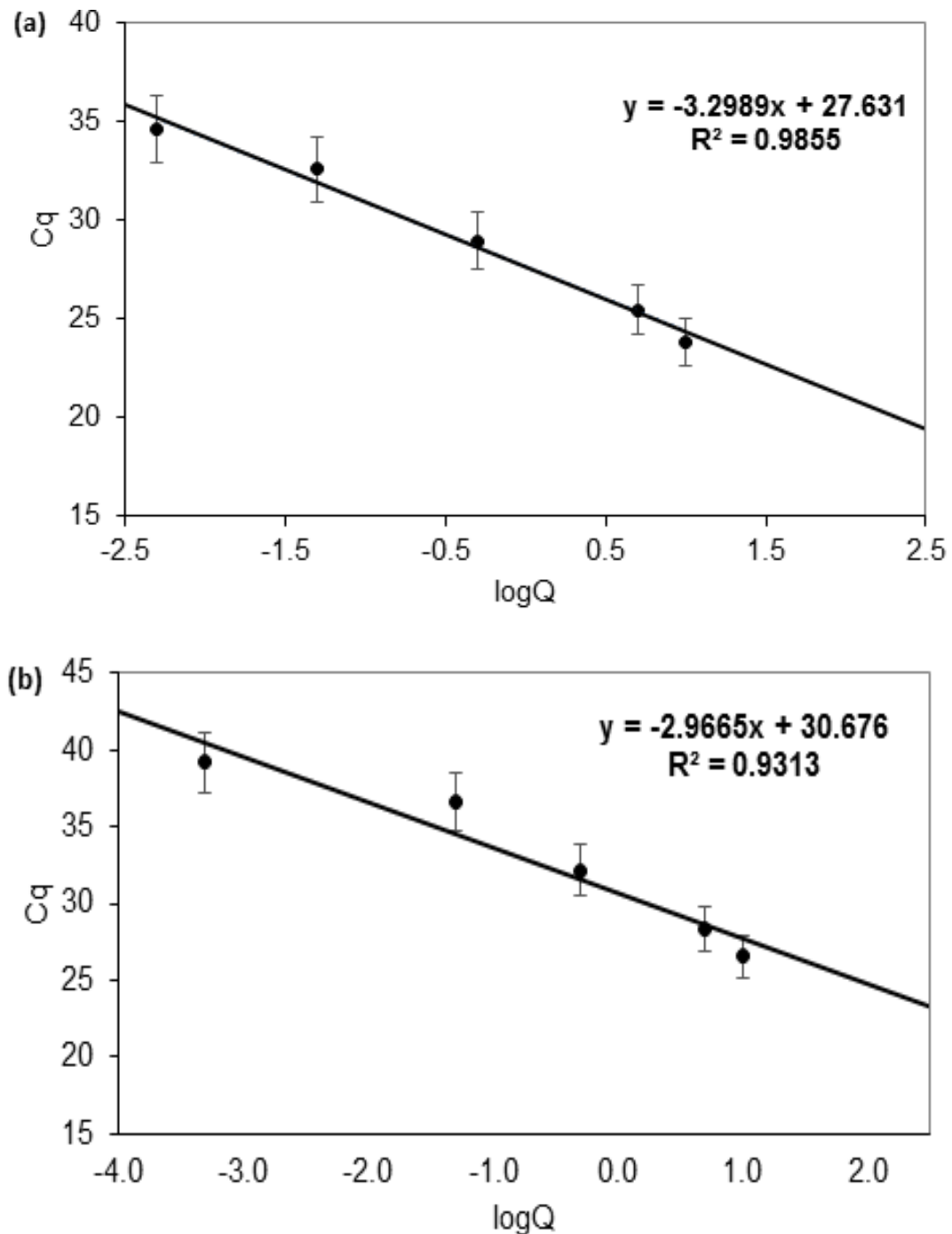


Figure 3.8 Standard curve for sensitivity and linearity of the real-time PCR quantification. (a) Standard curve of quantification cycle number (C_q) plotted versus the log of the initial template concentration ($\log Q$) in ng/ μ l. The first point is a two-fold dilution while the other four points are ten-fold serial dilutions that are linear over five logs. Data represent three replicates of each dilution. Efficiency (E) = $10^{-1/\text{slope}}$; (b): Amplification plot of same experiment showing the dilution series of DNA concentrations (10.0–0.005 ng) replicated three times. Error bars represent percentage error of the mean.

The one-way ANOVA (F-ratio = 0.885, d.f = 3, $P = 0.477$) indicate that there were no significant differences in legacies of *F. graminearum* (Figure 3.9, Table S3.10, $p > .05$). The post-harvest season 2016/2017 soil samples were not quantifiable (Table S3.10).

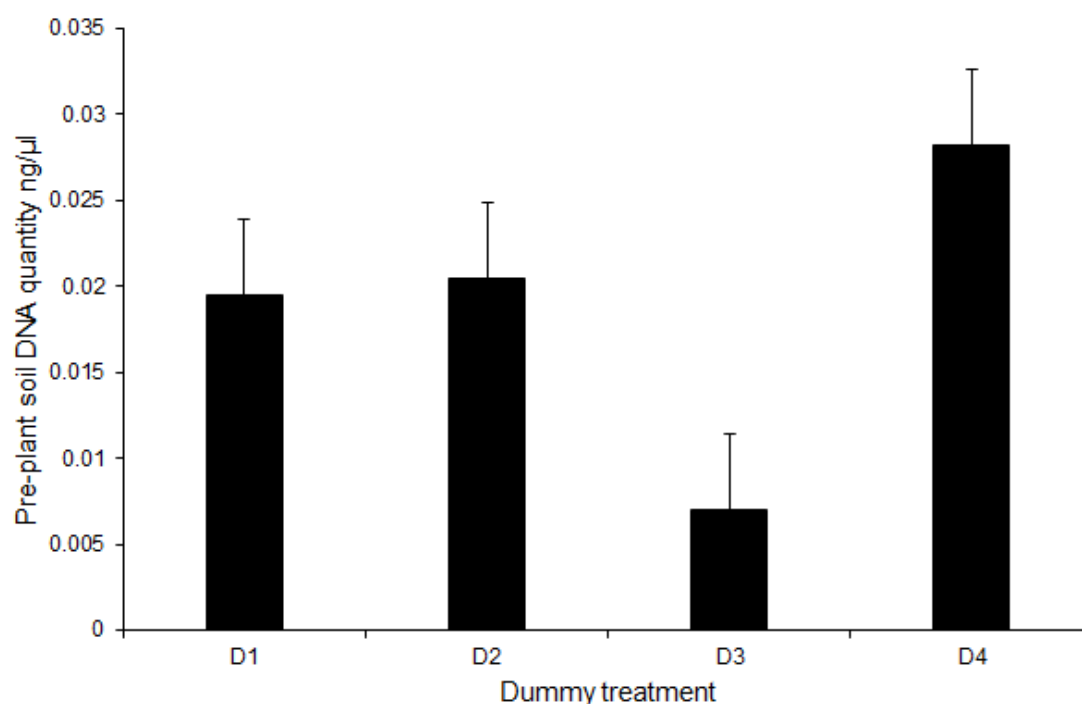


Figure 3.9 Soil legacies of *F. graminearum* pathogen DNA quantities among ‘dummy’ treatments in pre-plant season 2016/2017. In hindsight, ‘dummy’ treatments represents a history of crop rotation treatments in the succeeding seasons: After the potatoes and now maize uniformity (in bold), treatments were imposed to give the following cropping sequences as: D1: PMMT – Potatoes-**Maize**-Maize-Teff, D2: PMSBM – Potatoes-**Maize**-Sugar beans-Maize, D3: PMSOYM – Potatoes-**Maize**-Soybeans-Maize, and D4: PMSUNM – Potatoes-**Maize**-Sunflower-Maize rotations.

3.4.3.3 Soil Bioassays to estimate Soil Heterogeneity

The soil bioassays indicate that soils collected from the eight blocks were similar in yield parameter legacies across the selected plant phytometers. Boxplots (Figure 3.10) indicate that each block was homogenous including its four units. The plant mass within phytometers data were not significantly different ($p > .05$, Table S3.11a to S3.11d) across the soils sampled from the eight field blocks. This suggests that the soils from blocks 1 to 8 were uniform in yield responses under controlled conditions.

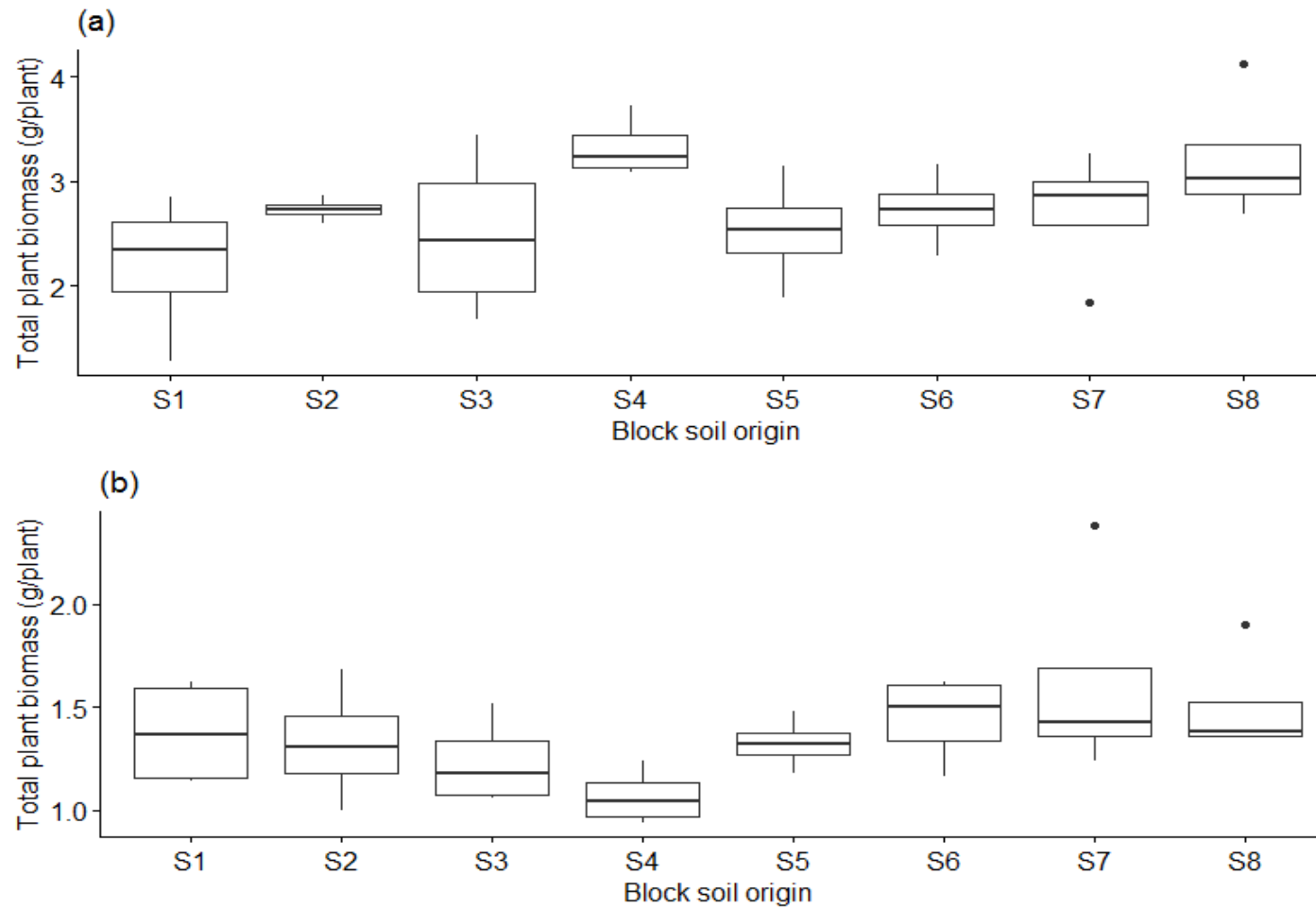


Figure 3.10 Comparing plant phytometers (a) soybean and (b) maize cultivar recommended for EFS dryland planting. Block sites were uniform in plant mass response to soil properties. Each block had 4 ‘dummy’ treatments. Horizontal heavy bar = median, box=lower and upper quartiles, whiskers=minimum and maximum values, filled dot=outliers.

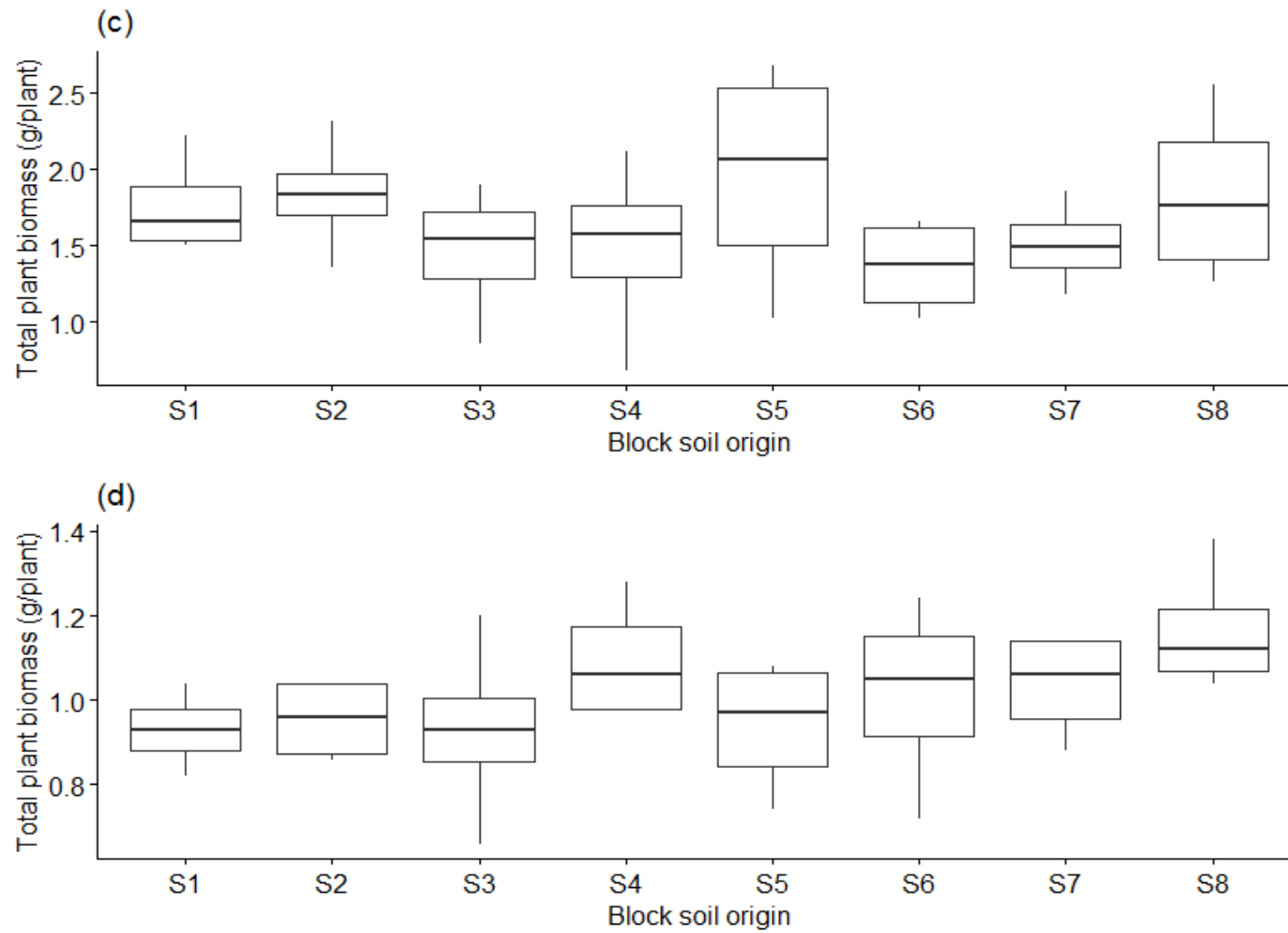


Figure 3.10 Comparing plant phytometers (c) sugar bean and (d) sunflower cultivar recommended for EFS dryland planting. Block sites were uniform in plant mass response to soil properties. Each block had 4 ‘dummy’ treatments. Horizontal heavy bar = median, box=lower and upper quartiles, whiskers=minimum and maximum values, filled dot=outliers.

3.4.3.4 Spatial Relationships using Principal Coordinates of Neighbour Matrices (PCNM)

Yield parameters were used as a focal table for variation partitioning model separating the effect of spatial variation and the space independent environmental effects. The y direction represented a more important spatial trend than the x direction, and then we compared the overall variation in yield parameter values with the variation fraction explained by environmental variables. The variation in yield that was attributed uniquely to space b amounted to 44.6 % much larger in size than the other two unique effects fractions of either environment a (23.1%) or both space and environment c (32.3 %) which were substantial in size (Table 3.2). However, the overall spatial variation was not statistically different for fractions $a + b + c$ and $a + c$ ($p > .05$, Table S3.12) but significant for fraction $b + c$ ($p < .05$, Table S3.12).

Table 3.2 Variation explained, where a represents the unique effect of environmental variables after accounting for the effect of spatial eigenfunctions, b is the unique effect of spatial eigenfunctions, partitioning-out the effect of environmental variables and the linear trend, and c is the shared portion of the variation in yield parameters that can be accounted for either by the environmental variables or by the spatial eigenfunctions. d.f, degrees of freedom.

Fraction	Variation (adjusted)	% of explained variation	% of all variation	d.f.	Mean Square
a (environment)	0.093542	23.1	9.5	2	0.06194
b (space)	0.18078	44.6	18.4	3	0.07611
c (space + environment)	0.13068	32.3	13.3	--	--
Total explained variation	0.405	100	41.1	5	0.10101
All variation	0.9852	--	100	29	--

The PCNM analysis (Figure 3.11) now called distance-based Moran's eigenvector maps (dbMEM), partitions the variation in the response data (yield parameters) into effects of spatial variation (and autocorrelation) from the effect of other explanatory or predictor variables. The loess smoother model illustrates (Figure 3.11) the constrained case scores on axis 1 of the spatial effects where individual eigenfunctions are orthogonal (mutually uncorrelated). In this analysis, 7 predictors were chosen out of the total 10 eigenfunctions with positive values to

reflect the finer scale spatial variation (Figure S3.3) representing more localized and suddenly changing patterns.

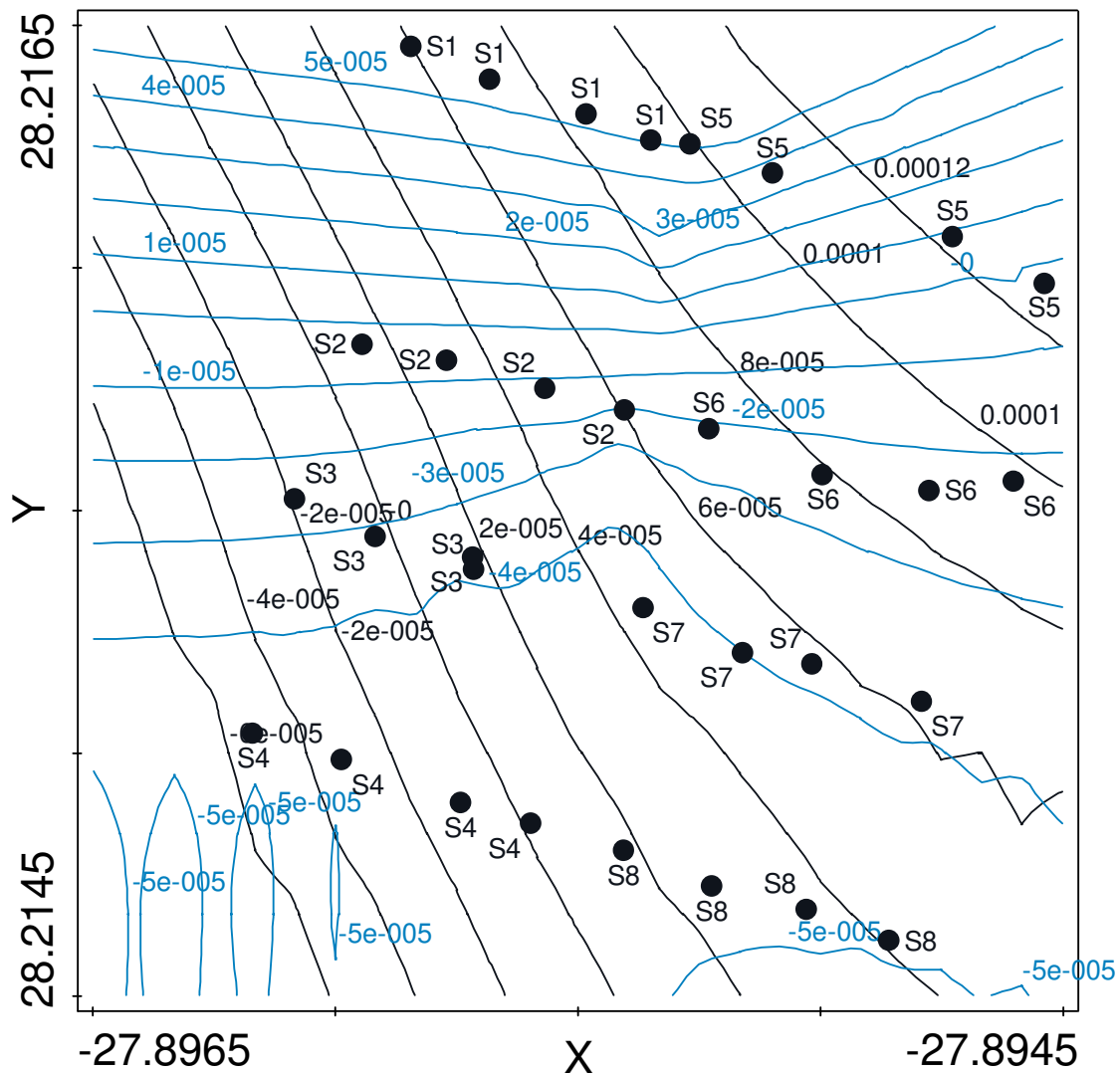


Figure 3.11 Visualization of spatial variation using contour plot using loess model. Contour map of the first axis of the yield variables in the uniformity field experiment constrained by a spatial eigenfunctions analysis. Blue contour lines represent the variation of attribute %sand (PCO.11 scores) against x and y axis spatial coordinates while the black contour lines represent the attribute Solvita CO₂ respiration in ppm (PCO.7 scores) against x and y axis spatial coordinates. There is a huge overlap between the variation explained by the space and by the %sand and Solvita soil respiration (also indicated in Table 3.2). The plot of the sampling sites is according to latitude and longitude geographic coordinates X and Y, respectively.

The variation along the first constrained axis is dominated by the PCO.7 (7 representing the PCO axis number 7) predictors correlating with yield corrected for moisture and harvest index while the second axis is correlated with the PCO.1 predictor (others) and the total plant biomass, as well as the seven predictors are correlated with this axis. The blocks with the negative eigenfunctions scores along first axis I included blocks 2, 3, 4, 6, 7 and 8 while low positive eigenfunction scores were for blocks 1 and 5 (Figure 3.11 and Figure 3.12). These results indicate that blocks 1 and 5 (S1 and S5) are spatially different from all the other blocks (S2, S3, S4, S6, S7 and S8). The plots are regularly spaced and ideal for producing a good spatial model using spatial eigenfunctional analysis (Legendre & Legendre, 2012).

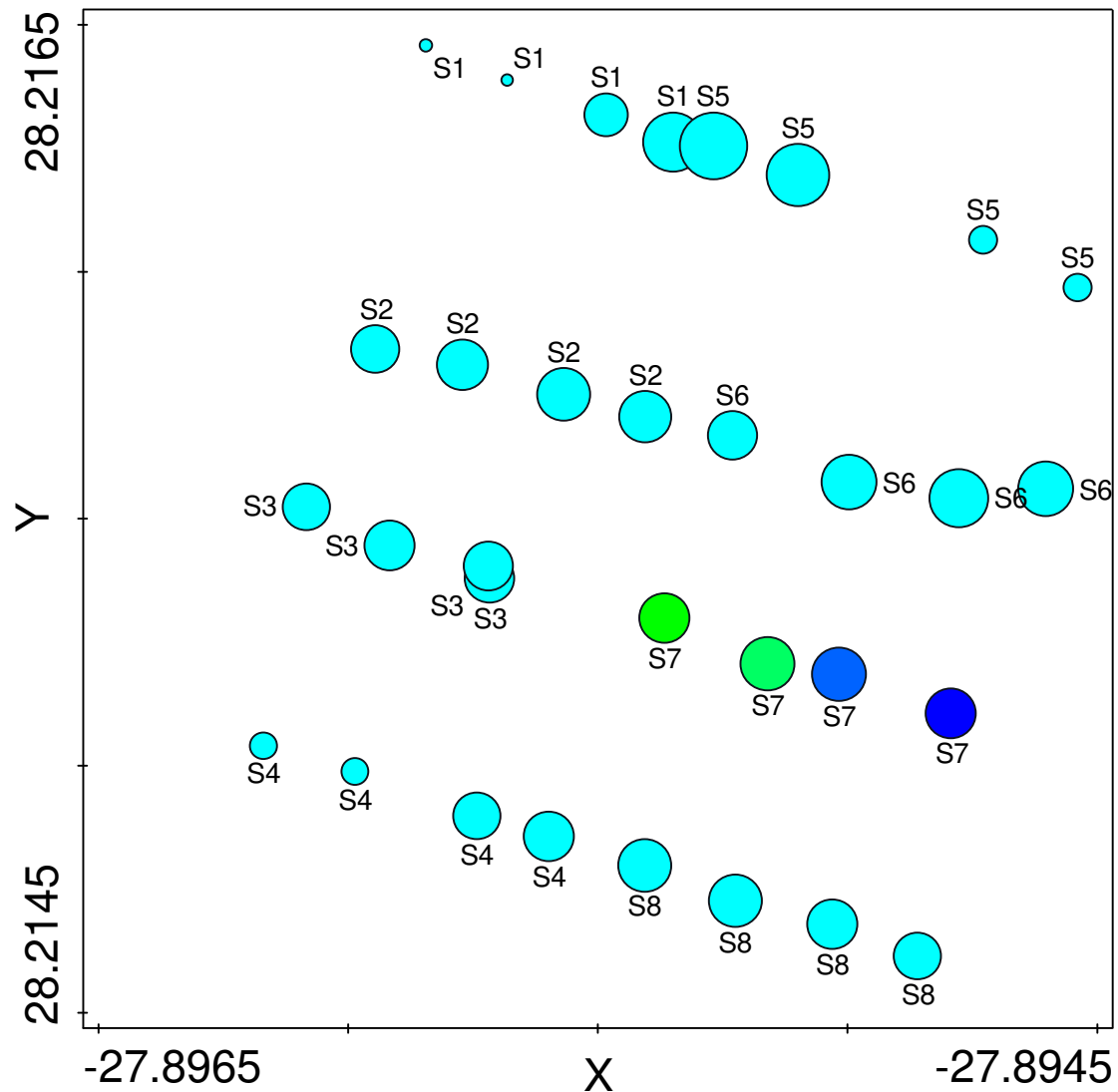


Figure 3.12 Symbol plot. Two attributes are selected, Solvita CO₂ respiration (ppm) (PCO.7) affects symbol size, while the sand (%) (PCO.11) determines the symbol fill colour (Lepš and Šmilauer, 2014). The plot of the sampling sites is according to latitude and longitude geographic coordinates X and Y, respectively.

3.5 Discussion

3.5.1 Yield Distribution

Maize (*Zea mays*) is a strategic grain in Southern Africa with a local consumption of around 825000 tonnes per year in South Africa (Prinsloo & Uys, 2015). It is a C₄ plant naturally adapted to a warm season and rain-fed agricultural systems (Lambers et al., 2008). The climate especially in terms of temperature and rainfall patterns including other abiotic factors at the

time from plant emergence to natural foliage die-off have a great impact on yields and yield components. In this study, maize was an ideal indicator of dryland environmental conditions. The impact of soil fertility factors on crop biomass growth and yields are complex and non-linear at different temporal and spatial scales (Plant, 2012). Within-block variation is a common challenge in large experiments. Climate changes and their impacts within and between seasons are difficult to analyze because climate-induced effects may not be caused by one climatic factor only, such as temperature, rainfall or humidity, but by the effect of a combination of climatic factors, which need to be characterized using time series data at the soil microclimate level using *in situ* soil probes (National Academies of Sciences, Engineering, and Medicine, 2021; Swart, 2021). The ‘mathematical association’ of the climate and the soil-plant health systems can underline the resilience and resistance of the systems and give suggestions on the impact that small or large climate changes could have on dryland production systems in the EFS region. In short, there is always a need to control this spatial variation for accurate and unbiased estimates of routine treatment effects (Grondona et al., 1996).

3.5.1 Climate Characteristics

The summer rainfall regions account for 86% of South Africa’s three main climatic rainfall regions (Smith, 2016). The summer evaporation, medium rainfall amount and distribution was ideal for maize production. The below zero temperatures at the end of the growing season probably quickened natural plant death considering that maize has a low tolerance to frost. The turbulent and high speed winds in December 2016 and February 2017 could have increased the evaporative rate and drying-out of soils (Smith, 2016). There were no climatic changes that destroyed or limited the crop production in any one of the plots, as a result, the climate did not affect the orthogonality of the uniformity trial.

The Eastern Free State is a summer rainfall area receiving between 300 and 800 mm of rainfall annually and has temperatures ranging from 20 °C to 30 °C with soils favourable for crop

production under dryland farming (Armour, 2010; Dube et al., 2016). On average, the proportion of the national maize crop produced annually in the Free State amounts to 36% (Hensley et al., 2006, Directorate Statistics & Economic Analysis, 2017a, Directorate Statistics & Economic Analysis, 2017b).

3.5.2 Soil Properties

The soil was sandy, acidic and with a clay content of less than 35% that did not require potassium fertilization (FERTASA, 2016; Smith, 2016). Although nitrogen fertilization has been implicated in worsening the problem of acidic soils in the Free State, it is essential for a higher root biomass that can sequester carbon in the rhizosphere (Zang et al., 2016).

Sandy soils are inherently low in organic matter and associated minerals such as Mg, S, K and Zn as well as the highly leachable anions such as B and Mo (A & L Laboratories et al., 2001). Minerals influence one another prior to affecting plant growth whereby high levels of one micro- or macronutrient are antagonistic to a nutrient in the same group (Sait, 2003; 2018), but this was not observed from the soil analysis results.

The soil was not acidic enough in pH (H₂O) and pH (KCl) values to perform lime testing (FERTASA, 2016). However, acidity in soil leads to a deficiency in Ca, B, Zn, Mo and P while inducing toxicity caused by Al, Mn and nickel (Fey, 2001). As expected in acidic soils, the Al levels (274.15 ± 6.36 ppm) were above the ideal < 16 ppm suggesting the potential of aluminium toxicity (A & L Laboratories et al., 2001). Plant uptake of Ca, K, Mg, Mo, and P are greatly affected in acidic soils but Sait (2003; 2018) suggests that these soil micronutrient deficiencies are by-passed via foliar nutrition spraying. Most soil chemical properties were highly correlated and discriminated between the different eight blocks, with strong positive and negative effects (Figures 3.5 and 3.6). From this distribution, meaningful conclusions about the role of chemical properties in “separating” the eight block soils can be made (Cadet et al., 2004). In general, the positive effects of soil composition on yield included Solvita CO₂

respiration, K, %Mg, Mg, P (Mehlich 3), %silt and density while the negative effects included pH, Na, %Na, active C, P (Bray), clay, sand, Ca, %Ca and nitrate-N. This means that the important nutrients that can limit yields or act as confounding factors must be managed before imposing treatments in the successive seasons. Also, there is need to consider interactions between nutrients since a lack of only one of these nutrients can influence the soil and plant health outcome of the field trial.

The soil biotic variables (Solvita CO₂ soil respiration and active carbon) had no effect on each other and were of limited use in giving new information on block variability (Cadet et al, 2004). However, it is speculative and too early in the study to conclude the suitability of using these Solvita test findings as an indicator of soil health in a dryland field (Figure 3.6). The average soil CO₂ values across blocks were very low to moderately low in both fertility and microbial activity typical of dry sandy soils with little or no organic matter (Brown, 2015; Haney et al., 2015). Solvita carbon dioxide production correlates with the relative activity of soil microorganisms and predicts potential availability of mineralized nitrogen, respectively (Sciarappa et al., 2017). Several field studies have confirmed the soil respiration measurement as an index of soil microbial activity and an emerging indicator of soil health fundamentally from bacterial and fungal biomass (Brown, 2015; Haney et al., 2015; Stott, 2019). Active carbon is positively correlated with organic matter, aggregate stability, and with measures of biological activity such as soil respiration rate (Lucas & Weil, 2012). Active carbon averaged 170.93 ± 6.76 ppm indicating a poor soil quality (Weil et al., 2003; Culman et al., 2011; Seyedbagheri, 2014; Bongiorno et al., 2019). In the long-term, soil respiration can provide an estimate of soil functionality and activity but overall soil health for plant health will involve a suite of soil physics, biology and chemistry under defined agronomic, environmental and management situations. The success of the EFS maize productivity is due to intensive fertilization (high P and K) and chemical control of weeds, pests and pathogens (FERTASA,

2016). However, it is evident from this study that the yield success is associated with soil acidity and very low soil microbial activity including inherent low SOM content in sandy soils. This paradox of high yields but poor ecosystem functions and services is due to low soil biological health (Van der Putten, 2021). The dry sandy soils in the EFS are bereft of maintaining and breaking down organic matter in the soil, however, implementing sustainable production beyond achieving high yield targets may increase SOM legacies under summer rainfall dryland systems. Significant soil health changes through the build-up of SOM content have usually only been observed after 10 to 15 years of repeated practices (Eisenhauer et al., 2010, Venter et al., 2016; Van der Waals et al., 2021). Soil type, together with other factors such as climate gradients, crop rotation diversity, and tillage management practices within and across seasons may have a huge temporal impact on shifts in health thresholds. Conducting fragmented and *ad hoc* trials in region-specific studies will not create long-term solutions for all soil types in the EFS region. Further research on the impact of various crop rotation sequences on soil and plant health are needed.

Modelling the relationship of plant health and crop yields to the risk of *F. graminearum* disease in the field could not be done due to low levels of pathogen DNA copies in sampled field soils. Other authors have highlighted similar challenges with real-time qPCR data of low soil-borne pathogen DNA quantities for modelling disease risk prediction for diseases of many crops (Marburger et al., 2015). The unquantifiable detection in post-harvest season 2016/2017 soil samples may be due to remnants of the inoculum from previous plantings of host crops such as wheat (Marburger et al., 2015) which is now a ‘dying crop’ with no financial support in the Eastern Free State (Bester, personal communication). Despite a relative decline in wheat production globally, wheat for dry grain is still the largest and widely cultivated cereal crop on an estimated 216 M ha of land globally ahead of maize cultivated on estimated 197 M ha (Erenstein et al., 2022). Maize is a host for *F. graminearum* and this pathogen is a problem in

maize (Rivas-Franco et al., 2019; Crippin et al., 2022; Gimeno et al., 2020; Cao et al., 2023; Savignac et al., 2023). Nevertheless, caution must be taken when interpreting the presence of *F. graminearum* in our findings since maize is a well-known host of this phytopathogen. Reducing soil borne diseases is part of good practice in soil and plant health management of crop diseases (Larkin, 2020).

Caution must be taken in the overall interpretation of soil and plant health results from our study because this study did not quantify confounding effects of well-known serious pests. For example, the African maize borer (*Busseola fusca* Fuller) has a huge impact on maize production systems of South Africa (Prinsloo & Uys, 2015). On one hand, the field trial soil rhizosphere and topsoil analyses were able to provide data on soil and plant health, but on the other hand, they did not give information about the contribution of other soil biota (i.e. micro-, meso-, macro- and megafauna) (Bardgett, 2009). Nevertheless, Marias et al. (2017) highlighted that the most common plant feeding nematodes in maize fields include *Meloidogyne* spp. (root-knot nematodes), *Pratylenchus sea* (lesion nematode), *Nanidorus minor* (stubby root nematode), *Helicotylenchus dihystra* and *Scutellonema brachyurus* (spiral nematodes). More information on the distribution patterns of these nematodes across blocks in our uniformity experiment is provided in Marias et al. (2017).

3.5.3 Spatial Variation of Soil Factors and Crop Yield Parameters

The individual blocks were homogenous in both soil properties and crop yield distribution suggesting that blocking minimized effects of spatial heterogeneity on measurement of the ‘dummy’ treatments (Plant, 2012). According to Forster (1937), the variation in crop yields across field blocks is a consequence of soil type or relief while variation within field blocks is often due to small local random fluctuations. In addition, variation emanates from the cumulative effects of microvariation which often mask treatment effects (Perrier & Wilding,

1986). Controlling spatial heterogeneity in agricultural field trials is crucial for the efficient and accurate estimate of treatment effects (Hoeﬂer et al., 2020).

The RCBD design was well-suited to control environmental and spatial heterogeneity effects on crop yield parameters as illustrated in Figure 3.7 (Lepš & Šmilauer, 2014). Replications of ‘dummy treatments’ were ‘latinized’ to avoid clustering, at the same time controlling for spatial trends and heterogeneity due to environmental factors. Theoretically, the RCBD was a perfect design since the ‘dummy’ treatments variation remained the same with or without blocking the design (Grafen & Hails, 2010). In other words, the blocks variable did not influence the predictive power of the ‘dummy’ treatments variable. However, Scheiner & Gurevitch (2001) cautions that ignoring block effects in controlled experiments has serious consequences for data interpretation using Fisher’s ANOVA.

In this study, the difference between the ‘dummy’ treatment effects on soil factors was approximately zero ($p > .05$). The blocks explained very little variability in yield parameters ($p > .05$) and there was no need for covariance adjustment of block effects on yield modelling in the succeeding seasons’ results. Block sites 1, 2, 3, 4, 5, 6 and 7 showed negative eigenfunctions predictors which correspond to negative spatial correlation while block 8 had positive eigenfunctions predictors which mirror positive spatial correlation patterns (Figure 3.11). This meant that for the succeeding seasons, the crop treatments can be allocated within the seven similar blocks excluding block 8 (Keuhl, 2018) or including block 8 as a covariate adjustment. Actually, only two plots in block 8 plots were unique in %acid saturation, P Bray II (mg/kg) , P (mg/kg), salt-replaceable acidity(cmol(+)/kg), density (g/cm³), %Na, clay (%) and these predictors would likely produce a good spatial model for within block variation (Lepš & Šmilauer, 2014). However, spatial variability can be controlled at the modelling level with the use of spatial corrections and other modelling strategies (Hoeﬂer et al., 2020).

These findings present the motivation and steps for describing long-term soil and plant health studies in the EFS dryland systems and elsewhere using soil properties likely to be predictors or descriptors of the environmental situation. Despite the absence of statistical differences in soil properties and plant metrics, there is a need to estimate spatial variation not only using horizontal variations but vertical variation in the soil depth profile (Robertson et al., 1999). In addition, an ideal approach to reduce within-site variation would be to recalculate crop yields according to the system of averaging the composition of main soil factors that contribute the soil heterogeneity (Cadet et al., 2003).

3.5.4 Phytometers of Soil Legacies

The yield metrics of the phytometers showed that heterogeneity in the soil environment under a greenhouse climate was minimized to control for factors that we may or may not be interested to investigate (Robertson et al., 1999; Gibson, 2015). There were no differences in dry yield for soils originating from within and among blocks (Mushonga et al., 2020) suggesting that there were no differences in soil characteristics.

The data from the double uniformity trials in seasons 2015/2016 (potatoes) and 2016/2017 (maize, in this study) indicate differences in crop treatments and associated fertilization may lead to detectable residual soil and plant health differences which may be crucial to successional planting in a dryland system (Westernman, 1990).

Sandy soils, low activity clays, and harsh dryland climatic conditions are the main reasons for nutrient loss in the topsoil to leaching, erosion and low organic status of soils. Consequently, soil biota plays a crucial role in the fertility of these soils. In the Western Cape of SA, the sustainability of winter dryland farming systems relies on conservation agriculture to promote biological activity and diversity in soils (Strauss, 2017). Fallowing and manure amendment are thought to be the most popular means (Manlay et al., 2000; Cadet et al., 2005). This is especially important, because, except for climate, clay and fine silt content of soil is the best

predictor for organic matter content of tropical soils with low-activity clay (Feller and Beare, 1997). Manlay et al. (2000) confirmed that even if %sand is high, the %clay is perhaps more important because therein lies the potential to form aggregates". From literature, the pH, N, P and K have a strong correlation to yield and any variance or imbalances in this study were expected to be corrected/controlled/managed during the subsequent growing season through various fertilization and liming programmes (FERTASA, 2016). The spatial blocking of four dummy treatments was effective because the variance was reduced as confirmed by the non-significant randomized blocks ANOVA results (Figure 3.2, Figure 3.3, Table S3.1; Table S3.2; Figure 3.10; Table S3.11; Brownie & Gumpertz, 1993). The impact of spatial differences in soil Solvita CO₂ respiration, K, %Mg, Mg, P (Mehlich 3), %silt, density, pH, Na, %Na, active C, P (Bray), %clay, %sand, Ca, %Ca and nitrate-N or their confounding effects did not increase the variance of yield and its components within dummy treatment categories (D1, D2, D3 and D4) as well as across blocks (Blo 1 to Blo 8). Also, some of these soil properties are; controllable through farming practice (see Table S9.1 in supplementary material for chapter 9 e.g. soil K, P (Mehlich 3), P (Bray) and nitrate-N), indirectly related to yield (pH, Na, %Na, %Mg, Ca, %Ca, Solvita CO₂ respiration, %silt, density, %clay, %sand) and not affected by the crop treatments in the following years of the trial. As such, there was no need to consider these soil factors for covariate adjustments or 'mathematical removal' to correct the yields of the plots in subsequent seasons when under dryland conventional tillage experiments (Keuhl, 2018). The relative efficiency of the blocking using an RCBD was equally effective in providing information as a completely randomized design (refer to chapter 4 results subsection 4.4.2) indicating that blocking increased the precision with no irregularities in yield caused by soil heterogeneity. In summary, these findings suggest that there was no need for re-allocating dummy treatments within the blocks of similar plot units to be used for subsequent experiment (Keuhl, 2018). The uniformity trial provided dummy treatment data that can be used to explain

and justify soil health indicators in the following years. There is need for long-term studies on the constancy of the plot units in their relative productivity in different seasons using other crops/cultivars, under different climatic regions of SA so that our findings can be used to establish soil health indicators or measurements at scale (Sanders, 1929).

3.6 Conclusion

The key contributions of this uniformity trial study include the possibility of using covariance adjustment in analysing repeated treatment effects between seasons, soil and crop health. Dummy treatments illustrated that spatial interspersion through blocking was effective in minimizing variance to random effects caused by natural variation in the field experiment. We also offer a suggestion to replace or adjust outlier blocks within a field trial to maximize interpretation and use of the ANOVA. As such, block effects can be excluded from all statistical testing as a fixed or covariate effect but included as a random effect that has no interaction with any of the soil health properties that vary within blocks in the randomized complete block design (RCBD). Principal component analysis and discriminant analysis gave similar results in identifying some differences in the eight blocks, albeit in very few soil properties, probably due to the many more explanatory soil properties than the soil samples. Generalization on soil health for plant health in this field experiment can be modelled after introducing treatments in successive dryland farming seasons.

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Conflict of interest

Authors have no conflict of interest to declare.

CHAPTER 4

Soil and Plant Health in the Root Zone of Four Crops under Dryland Field Conditions

4.1 Abstract

Background: Sustainable dryland production relies on promoting organic matter and associated biological activity caused by microbial diversity.

Aims: This study aimed to assess the impact crop type on soil physical, chemical, and biological properties in the root zone. The presence and/or absence of soil microbes and biological activities influence soil nutrient composition and availability in the root zone and, consequently soil and plant health. Four dryland crop species namely, *Zea mays* L., *Helianthus annuus* L., *Phaseolus vulgaris* L. and *Glycine max* L. (Merr.) were hypothesized to have specific rhizosphere microbial biomass and activities associated with the particular crop type under dryland field conditions.

Methods: An experiment with a randomized complete block design was used to test plant-soil feedback and the health of the four crop species in a dryland field previously homogenized by a *Z. mays* uniformity trial.

Results: The soils were more acidic in the subsoil ($\text{pH } 5.48 \pm 0.0345$) than in the topsoil ($\text{pH } 5.60 \pm 0.0446$) possibly due to legacies of topsoil lime amendments. The legacies in active carbon and Solvita soil respiration were strongly correlated with legume *P. vulgaris*, specifically the *P. vulgaris* while *G. max* had a poor soil fertility legacy except for the Ca:Mg ratio. *Helianthus annuus* was a high-yielding grain associated with a rich soil fertility legacy in both micro- and macronutrients. Comparisons among crop rhizospheres indicated that *H.*

annuus had a significantly higher fungal to bacterial biomass ratio and microbial enzyme activity compared to bulk soil ($p < .05$). Furthermore, the Gram positive to Gram negative PLFA ratio was relatively high in *H. annuus* (12.7 ± 6.07) and *Z. mays* (12.0 ± 5.48) when compared to bulk soil. The *H. annuus* species cultivated 1.7 times and 1.5 times more saprophytic fungal biomass markers than bulk soil and the rhizosphere of *Z. mays*, respectively ($p < .05$). The microbial enzyme activity in the *H. annuus* rhizosphere was 1.5 times higher than in bulk soil ($p < .05$) suggesting that the rhizosphere of this crop supports relatively high metabolic activities, which are closely related to healthy root and plant growth (Veen et al., 2019). Soil chemical content in P Bray 2, P, K, Mg, Zn, B, electrical conductivity and N - NH_4 were significantly ($p < .05$) different between the 20cm and 40cm soil depths. The high soil microbial respiration in season 2018 can be explained by relatively high levels of Cu, K, P Bray 2 and P legacies from season 2017, while the yield in 2018 was associated with Zn, Ca, Mg legacies of season 2017.

Conclusion: Results showed that bacteria dominated the microbial composition for all rotations, but sunflower consistently had higher fungal levels, as well as a higher ratio of fungi to bacteria than the other three dryland crops. Sunflower, therefore, seems to have the potential of increasing fungal to bacterial ratios, supporting saprophytic microflora and microbial enzyme activities in the root zone of a dryland soil environment under crop rotation management.

Key words

Zea mays L. (Maize); *Helianthus annuus* (sunflower); *Glycine max* (soybean); *Phaseolus vulgaris* (sugar bean); Phospholipid fatty acids (PLFA); dryland production system; plant-soil feedback (PSF).

4.2 Introduction

Dryland agricultural production constitutes 43% of the African continent and more than 70% of agricultural land (Souidi et al., 2020). Arid climates are associated with low soil organic matter despite the high temperatures and adequate precipitation that can support the build-up of soil organic matter (Souidi et al., 2020). A dry soil environment provides limited water resources for microbial growth and activities, but fungi are more active in dry soils than bacteria (Dixon & Tilston, 2010; Madigan et al., 2015). Soil biodiversity remains a “black-box” when testing plant-soil systems. In soil analysis, priority is given to soil quality measurements that are related to farming practices such as fertilizing and liming for improved soil and plant productivity. However, monitoring soil health in agriculture systems requires corroborative quantification of soil factors and processes that are sensitive to management practices at the plant-soil interface. Microorganisms, as biological agents in the soil and rhizosphere, participate directly or indirectly in nutrient availability, plant growth and plant health by interacting with each other, the soil environment, plants and animals (Marschner, 2007). Their diversity and abundance are a reflection of nutrient and growth conditions in the soil environment.

The rhizosphere, aggregatusphere, detritosphere, porosphere and drilosphere represent hotspots of a healthy soil (Wall et al., 2012). Plants were taken as soil engineers of soil health legacies through rhizodepositions, root-drilling, root-tunnelling, and organic litter inputs (stover and dead roots). The four dryland crops were expected to cultivate different microbial biomass, composition and activity in the rhizosphere for soil health legacies at the end of the growing season. In simple terms, microbial legacies will answer how much live microbial biomass is present in the soil. Microbial composition will highlight the taxonomic groupings of bacteria and fungi and microbial activity will estimate the rate of processes such as respiration and enzyme activity. Broadly, a healthy soil environment provides physicochemical

and biological resources and growth conditions that support plant health in succeeding seasons (Berendsen et al., 2012). However acidic and low organic matter sandy soils pose an endemic challenge to crop cultivation in the Free State of South Africa (FERTASA, 2016).

Lipid biomarkers were used to determine fungal and bacterial biomass composition in the rhizosphere of four crops under a uniform dryland cropping environment in South Africa. Phospholipid fatty acid analysis (PLFAs) is a biochemical technique for quantifying live soil biota or biomass by measuring lipid biomarkers linked to nutrient cycling, organic matter decay and carbon sequestration (Willers et al., 2015). Gram positive to Gram negative bacterial PLFA ratio decreases with improvements of soil health (Stott et al., 2019). In addition, management activities such as straw effects (Lu et al., 2015), application of fungicides (Zelles et al., 1999), liming (Frostegård et al., 1993) and other region-specific perturbations (Zelles et al., 1999, Wang et al., 2016) produce different PLFA signatures (Bossio et al., 1998). Therefore, indexing PLFA data will likely provide information on microbial biomass, microbe physiological stress, community structure, and indicators of taxonomic or functional groups associated with selected dryland crops at the rhizosphere level.

In this study, medium-maturity crops were used to minimize confounding differences common to fast- versus slow-growing plants in field experiments. *Zea mays* is a C₄ photosynthetic cereal grass while the two forbs (*P. vulgaris* and *G. max*) are N-fixing C₃ legumes and one (*H. annuus*) is a non-legume. The legumes build N legacies in the soil that drive positive plant-soil feedbacks (PSF), whereas *H. annuus* and *Z. mays* are net consumers of N that drive negative PSF (Cortois et al., 2016; Cesarano et al., 2017). The C₄ grass plant ecophysiology of *Zea mays* is known to have superior photosynthates and root exudate production relative to the C₃ leguminous (*P. vulgaris* and *G. max*) and non-leguminous (*H. annuus*) forbs (Ladygina & Hedlund, 2010). Hypothetically, grasses support soil microbial diversity better than forbs do (Ladygina & Hedlund, 2010).

Increasing the fungal to bacterial ratio in soil has been linked to litter decomposition and increasing carbon storage (Malik et al., 2016). The decomposition process is favoured over plant biomass growth by abiotic factors such as high moisture and high mean daily temperatures (FERTASA, 2016). In the present study, the operational definition of soil health represents a balanced state of soil properties that nourishes soil biodiversity and protects the soil community from harmful factors and mismanagement (Vieweger & Döring, 2015). Plant health refers to any biological or physicochemical factors occurring on a crop rhizosphere that may harm plant form, physiology and performance in a given geographical and temporal setting (Berendsen et al., 2012; Savary et al., 2016). Soil and plant health are context-dependent especially in drylands where the climate changes to extremes such as droughts and flooding (Souidi et al., 2020). Du Toit & du Preez (1995) observed that a reduction in N fertility was linked to mismanagement due to unsustainable crop cultivation under dryland conditions. Management practices such as cultivation, fertilization, and pesticide and herbicide spraying also affect microbial presence, composition, and activity (Tsiafouli et al., 2015; Veen et al., 2019). Perspectives on sustainable management of crop production in South Africa are reviewed by van der Laan et al. (2017).

In this study, the aim was to determine the concentration of microorganisms in the rhizosphere of *Zea mays* (maize), *Helianthus annuus* (sunflower), *Glycine max* (soybean) and *Phaseolus vulgaris* (sugar bean) planted in a dryland soil environment. The aim of this long-term study was to explore the “legacy effects” of crop rotations on yields, soil, and plant health under dryland farming conditions in the Eastern Free State in South Africa.

4.3 Materials and Methods

4.3.1 Field Site Description and Sample Collection

The trial was conducted in a field made up of 32 plots of similar rectangular shape (35 m × 21.91 m) and size 766.85 m² across a total area of 2.98 ha as a randomized complete block

design (RCBD) situated in the eastern Free State region of Petrus Steyn in South Africa (27° 53' 45.5" E 28° 12' 59.8", Elevation 1705 m). The soil in the field was an arenosol greyish sandy soil (81.3±1.47% sand, 9.8±1.34% silt, 8.9±1.01% clay) containing 170.9±38.25 mg/kg active carbon (Chapter 3, Figure S3.2). The eastern Free State is a summer rainfall area receiving between 300 and 800 mm of annual rain with maximum temperatures ranging from 20 °C to 30 °C typical in rain-fed summer dryland farming systems. Statistically significant homogenization of the field plots in seasons 2016 and 2017, through planting potatoes and maize uniformity trials respectively, (Chapter 3) meant that the field trial site was suitable for investigating the impact of different crop treatments and management activities on soil factors under dryland conditions at field scale.

In the 2018 growing season, the field was planted with eight replications of each crop (*Zea mays*, *Phaseolus vulgaris*, *Glycine max* and *Helianthus annuus*) on 28 November 2017 following dryland conventional tillage practice of the EFS (see Table 1.1 in Chapter 1). However, due to hailstorm damage on 28 December 2017, soybeans and sugar beans were replanted on 5 January 2018. The sunflower and maize plots were damaged, but the advanced season did not warrant replanting. All soil sampling activities were georeferenced with the aid of a handheld global positioning system device (GPS) (eTrex Legend® HCx, Garmin International Inc., Olathe, Kansas, USA). Rhizosphere soil samples were recovered from roots grubbed up in a W-shaped sampling pattern and pooled to make one sample per plot on 13 February 2018. This was done in maize at 77 days to 50% tassel (VT stage), sugar beans at 39 days to 50% flowering (V8 stage), soybeans at 39 days to 50% flowering (R3 stage) and sunflower at 77 days to 50% flowering (R4 stage). Bulk soils were collected as non-rhizosphere control soils.

Potatoes in year 1 were followed by maize on all plots in 2016/17 (Year 2/ season 2017), when the physical, chemical and microbial observations of the relevant soils commenced. Third year

rotation crops were planted in 2017/18 (season 2018) and the 2018/19 summer growing season was designated year 4 (season 2019) of the four-year rotation. In this chapter, the season 2017/18 rotation crops data will be used for analysis of the field trial (refer to Table 1.1, Chapter 1).

All soil samples were stored in sterile zip-lock plastic bags at 4–5 °C and used for the determination of active carbon content, fluorescein diacetate microbial enzyme activity and PLFA microbial biomass. On 6 June 2018, topsoil from the root zone was collected as composite samples from the topsoil 0 cm to 20 cm depth in each plot using a 5 cm diameter by 8 cm height soil core. In addition, we also collected soils from the subsoil at 20 cm to 40 cm depth and subjected them to analysis for macro-and micronutrients in order to investigate vertical variation.

4.3.2 Soil Biological and Physicochemical Analysis of Horizontal and Vertical Soil Heterogeneity

The active carbon fraction of the organic matter fraction was determined using the permanganate-oxidizable active carbon method adapted and modified from Weil et al. (2003). For each 2.5 g soil sample, 20 ml of 0.02 M potassium permanganate (prepared using 0.1 M calcium chloride buffer solution) was added and mixed at 120 rpm for 2 minutes using a rotary shaker. After 8 minutes of settling the soil suspension, 0.5 ml of the liquid fraction was pipetted into 49.5 ml of water to dilute the colour of the potassium permanganate. The dilute solutions were measured at 550 nm wavelength using a spectrophotometer (CARY Bio 100 UV-Vis, Agilent Technologies, USA).

The Fluorescein diacetate (FDA) method was used as an indicator of soil enzyme activity, as described by Schnürer & Roswell (1982). Soil samples of 0.5 g were mixed with 20 ml of the 60mM sodium phosphate buffer and 0.2 ml of the FDA solution (dissolved in acetone). The mixture was mixed by agitation at 90 rpm for 1 hour in a rotary shaker. 2 ml of soil solutions

were collected into fresh 2ml Eppendorf tubes on ice to stop the FDA reaction. The samples were centrifuged at 4630 g to remove excess debris. The supernatant fractions were measured at 490 nm using a double-beam spectrophotometer (CARY Bio 100 UV-Vis, Agilent Technologies, USA).

Phospholipids fatty acid (PLFA) extraction was done as described in Marschner (2007). Frozen field-moist soil (2 g) was mixed with a citrate buffer, chloroform, methanol, and Bligh and Dyer reagent. Separation of soil and liquid fraction was done by shaking for 2 h followed by centrifugation at 2500 g. The supernatant of each sample was evaporated under a N₂ stream, conditioned with chloroform, and run through an elution chamber. The phospholipids were collected and dried again under a N₂ stream. An internal standard methyl-non-decanoate (C19:0) was added along with methanol: toluol, hexane: chloroform, acetic acid, and deionized water. The organic phase was collected and dried under a N₂ stream. The purified phospholipids, already methanolized into fatty acid methyl esters, were analysed on a Varian 430-GC gas chromatograph (Agilent Technologies, USA) and recorded in nmol/g. The study used (a) fungal PLFA markers C18:2 ω 6c, C18:1 ω 9t, C18:1 ω 9c, C18:2 ω 6t and C18:3 ω 3c, and (b) bacterial PLFA markers C14:0, i-C15:0, a-C15:0, C15:0, i-C16:0, C16:19, C17:0, i-C17:0 and C18:17 (Frostegard & Bääth, 1996; Tavi et al., 2013; Willers et al., 2015; Schwab et al., 2017).

Soil physical, chemical, biological properties were measured using standard procedures at Nvirotek Laboratories (Pty) Ltd (ISO/IEC 17025 international standard for testing laboratories Accredited Testing Laboratory, certified SANAS and SADCAS assessors, certified AgriLASA Fertilizer Laboratory, Unit 6, Nviro Business Hub, Ifafi, 0260, South Africa). The soil samples were dried, sieved and analysed for density prior to preparation of the 1:2 water extract slurry used to measure macro-and micronutrients using the Mehlich III extraction method. Total P was determined using the P Bray 2 method. Active carbon was measured using the

permanganate-oxidizable active carbon method. Soil respiration was estimated using the standard protocol of the Solvita CO₂-burst test following manufacturer's instructions.

In addition, *in situ* soil penetration resistance readings were taken in-rows, in-between rows and between tractor tracks using a Geotron Hand Penetrometer Model P5 (Geotron Systems, Potchefstroom, South Africa) up to 5000 kPa. All resistance readings were done under dry conditions at 1 cm increments to a depth of 50 cm in every plot on 23 May 2018 and repeated on 17 September 2018. The soil bulk density was obtained from undisturbed soil samples collected at 20 cm and 40 cm depths following the procedures described earlier in section 3.3.4.

4.3.3 Soil Pathogen DNA Quantification

4.3.3.1. Field Rhizosphere Soil DNA Extraction

About 0.25 g rhizosphere soil was subjected to total DNA extraction with a DNeasy® PowerSoil® Kit (QIAGEN Group, MoBio Laboratories, Inc.) following the manufacturer's instructions and samples were stored at -20 °C for further use. The total genomic DNA quality and quantity were verified using 1% agarose gel electrophoresis and Qubit dsDNA high sensitivity assay as described in Dube et al. (2019).

4.3.3.2 Quantitative PCR Amplification

Concentrations of pathogen DNA in the rhizosphere and bulk field soils were determined simultaneously with standards and negative (no-template) control on each run. All the qPCR protocols were programmed and run in a 96-well PikoReal 96 thermal cycler (Thermal Fisher Scientific, California, United States). The species-specific primers and probes were synthesized by Integrated DNA Technologies, Inc. (<http://www.idtdna.com>). The primer and probe protocol for *F. graminearum* was validated and described by Marburger et al. (2015).

A total volume of 20 µL of TaqMan-based qPCR reaction mixture contained 0.8 µL of DNA template, 10 µL of 2x concentrated SsoAdvanced universal probes supermix (Bio-Rad Laboratories, Hercules, CA, United States), 0.4 µL of each primer (from 10 µM stock), 0.4 µL

of probe (from 10 µM stock), and 8 µL of nuclease-free water (New England Biolabs, Inqaba Biotech). Amplification conditions consisted of 50 °C for 2 min, 95 °C for 2 min, 40 cycles of 95 °C for 10 sec and 60 °C for 20 sec. No-template controls included sterile nuclease-free water (New England Biolabs, Inqaba Biotech) and no template DNA. *Fusarium graminearum* translation elongation factor 1 alpha (tEF1α) (size 129-130 bp) was amplified successfully using TaqMan-based qPCR hydrolysis probe and primers listed in Table 4.1.

Table 4.1 *Fusarium graminearum* specific primers and probe used in this study.

Sequence name	Description	Sequence (5′→ 3′)	Tm (°C)	Reference
EF1G_F	Forward	TGGTCTCATTTTCCTCGATCG	54.5	Marburger et al. (2015)
EF1G_R	Reverse	AAATTACGACAAAGCCGCAA	53.4	
EF1G_P	Probe	/56-FAM/CTCGATACG/ZEN/CG CCTGTTACCCC/3IABkFQ/	61.9	
Probe: labelled with 6-FAM-IB®FQ, Tm: Melting temperature				

Specific amplification was verified by assessing the size of the PCR product by 1% agarose gel electrophoresis. Conventional PCR detected *F. graminearum* in the field rhizosphere soil samples (Season 2018 post-harvest).

4.3.3.3 *Fusarium graminearum* Standard Curve Preparation

Genomic DNA (gDNA) of *F. graminearum* Schwabe culture number CBS 110261 from the Forestry and Agricultural Biotechnology Institute (FABI) (University of Pretoria CMW culture collection laboratory) was extracted using the Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (ZYMO RESEARCH, Corp.) according to manufacturer's protocol and diluted in a series of 1:10 (initial concentration 10 ng/µl) to estimate the amplification efficiency of real-time PCR assays. The reference CBS *Fusarium* cultures were revived and sub-cultured in PDA. Mycelia was successfully harvested and gDNA extracted. A Qubit 2.0 fluorimeter (Thermo Fisher Scientific, California, United States) instrument estimated 4–40 ng/µl quantities of DNA template using the Qubit dsDNA HS Assay kit according to the manufacturer's instructions as described in the previous section. A 10-fold dilution series of gDNA was prepared, stored at -

80 °C and thawed once before use. A log-linear regression standard curve of a first point 2-fold (20 ng/μl to 10 ng/μl) and five point 10-fold dilution series (10 ng/μl to 0.001 ng/μl) were generated by plotting the logarithms of known concentrations of *F. graminearum* pathogen DNA against the C_q values.

PCR efficiency and coefficient of determination (R²) values were calculated using the Piko Real software. A slope of -3.32 represents the ideal 100% amplification efficiency. The sensitivity or minimum detection limit of the assay was estimated as minimum quantity of target DNA detected when the cycle threshold was attained up to 40 cycles. All reactions were run in duplicate and samples that showed positive fluorescence before no-template controls were considered positive.

4.3.4 Data Processing and Statistical Analysis

Data analyses were carried out using R, SPSS and Canoco 5 as described in Mushonga et al. (2020) in Chapter 5 section 5.3.4.

4.3.4.1 Randomised Complete Block Design Efficiency and Univariate Analysis

The design relative efficiency was estimated using the ration of variance of complete randomized design to variance of RCBD design. The effects of the crop treatment and block effects are assumed to be additive i.e., the combined response is the sum of their separate response without interaction between crop treatments and blocks. The experimental errors are assumed to be independent, and the block effects are considered to be random. The measured response variables included moisture-corrected grain yield (kg/ha), soil metrics, soil bacterial and fungal PLFA marker biomass and quantities of *F. graminearum* DNA. The null hypothesis was that there were no statistically significant differences in soil and plant health responses to crop treatments.

The log-transformed plant health data were analysed in SPSS version 23 using the Tukey's honestly significant difference (Tukey HSD) test to compare plant health legacies in the

rhizosphere composition across each crop treatment. In this *post-hoc* test, an ANOVA F test was done to determine the significance of crop rotation treatment effects while the Tukey HSD *post-hoc* test was done to group the treatment means (Steel & Torrie, 1960) strictly for soil and plant health data (or transformed data) of equal sample sizes that meet the normality and homogeneity of variance assumptions. Overall, this *post-hoc* test provides answers to specific meaningful questions, which were after all the objectives of the study in comparing all pairs of crop rotation treatments (Ruxton and Beauchamp, 2008; Kozak & Powers, 2017). On the other hand, the soil health properties data were analysed using a nonparametric Kruskal–Wallis test to compare soil health legacies in the root zone across each crop treatment in R 3.5.2 software.

4.3.4.2 Redundancy Analysis

A multivariate analysis of variance (MANOVA) was not used because the numbers of response variables were greater than the number of experimental units. Instead, the partial redundancy analysis (RDA) combined with Monte Carlo permutation tests were ideal for a large number of response variables (ter Braak & Šmilauer, 2018). Blocks and crop treatments were coded as factors or categorical explanatory variables while crop yield and soil properties as compositional response variables in Canoco software (Canoco 5 version 5.11; ter Braak & Šmilauer, 2018). Redundancy analysis was performed in Canoco version 5 (Lepš & Šmilauer, 2014; ter Braak & Šmilauer, 2018) software package to search for instrumental predictor variables i.e. PLFA markers, active carbon and enzyme activity corresponding to plant functional group treatments.

4.3.4.3 Standardised PCA Soil-Plant Health Feedbacks

The soil and plant health data were normed in a similar way as the sites factor map using the `dudi.pca` package in `ade4` (Thioulouse et al., 2018) to assess the PLFA ratios, active carbon and enzyme activity which were correlated to legacies of season 2017 soil health properties.

4.3.5 Partial RDA for Soil Health Legacies for Plant Health

The scaling of scores (plots) were standardized and focused on soil health properties. The average dissimilarities between soil content legacies between the crop species effects were approximated by the Euclidean distance in Canoco version 5.11 (Lepš & Šmilauer, 2014; ter Braak & Šmilauer, 2018) software package.

4.4 Results

The objective of the crop treatment season was to understand the plant-soil, soil-plant and soil-soil feedbacks using descriptive and predictive multivariate analysis of plant rhizosphere and soil health properties in seasons 2017 and 2018. The study explored crop-microbe-soil feedbacks in the root zone under a dryland environment using soil analysis and PLFAs as indicators of living microbial biomass. Data were analysed using 'descriptive' standardized principal component analysis (PCA) and 'predictive' Redundancy Analysis (RDA). Patterns and relationships in soil-soil and plant-soil feedbacks were statistically significant ($p < .05$, Sections 4.3.3 to 4.3.6). On the other hand, there were no significant differences in vertical soil quality between treatments ($p > .05$, Section 4.3.4).

4.4.1 Climate Data

In the 2018 season, the average maximum air temperature was warm at 24.2 ± 0.32 °C while the minimum was cool at 9.24 ± 0.31 °C (Figure S4.1A and S4.1C, respectively). High maximum temperatures above 34.5 °C were recorded often between November 2017 and February 2018 (Figure 1a). After this the temperatures were below 30 °C and favourable to flowering. In the month of planting there was an increase in vapour pressure deficit above 7.5 kPa (Figure S4.1E). The minimum air temperature was below zero at the beginning of the winter season end of May 2018 and in winter June 2018 at the end of the growing season (Figure S4.1C) although no frost damage was experienced during the growing season. There was a hailstorm on December 28, 2017, accompanied by 27.3 mm rain (Figure S4.1D). The evaporative demand (Eto) was high during the rainfall period from November 2017 to February

2018 thereby reducing the risks of drainage losses (Figure S4.1B). The maximum relative humidity was generally above 92.9% for most of the growing season (Figure S4.1A) while the minimum relative humidity was around 35.4% (Figure S4.1C).

As observed from the 2017 growing season, total solar radiation (MJ/m^2) was higher in summer than in winter of the growing season (Figure S4.1B). The mean wind speed was 3.33 ± 0.098 m/s during the growing season (Figure S4.1D). A total of 591 mm rainfall was received during the growing season from planting in November 2017 to harvesting in June 2018 (Figure S4.1D) although there was less precipitation in the early stages of the growing season. Most rain fell during February 2018 and April 2018 (Figure S4.1D), which is ideal for summer crops such as maize, sugar bean, soybean and sunflower.

4.4.2 Relative Efficiency of Randomized Complete Block Design (RCBD) Experiment

In this field study, the grain yield corrected for moisture was an ideal indicator or response variable of productivity because all soil, crop and disease management practices were quantified in the baseline and treatment seasons. In the yield data (Figure 4.1), there were no outliers, skewness, or non-constant variance across blocks with different crop treatment legacies. Also, there were no interaction effects between blocks and crop treatments. It was evident from the interaction plot that there was no justification for the Tukey non-additivity test to confirm the absence of interactions.

The interaction plot (Figure 4.1) suggested that the “Crop treatments $\times$ Block” interaction effect was less likely to be significant in a mixed model for the data. The design relative efficiency = $0.9958471 = [\text{variance (CRD)} / \text{variance (RCBD)}]$ was approximately 1 meaning that the information of the CRD and CRBD are equal, hence, the designs would require the same number of replications/blocks to have the same variance of treatment means. The yield data was independent and normal with homogeneous variance as illustrated in the diagnostic’s residual plots (Figure S4.1A and Figure S4.2B).

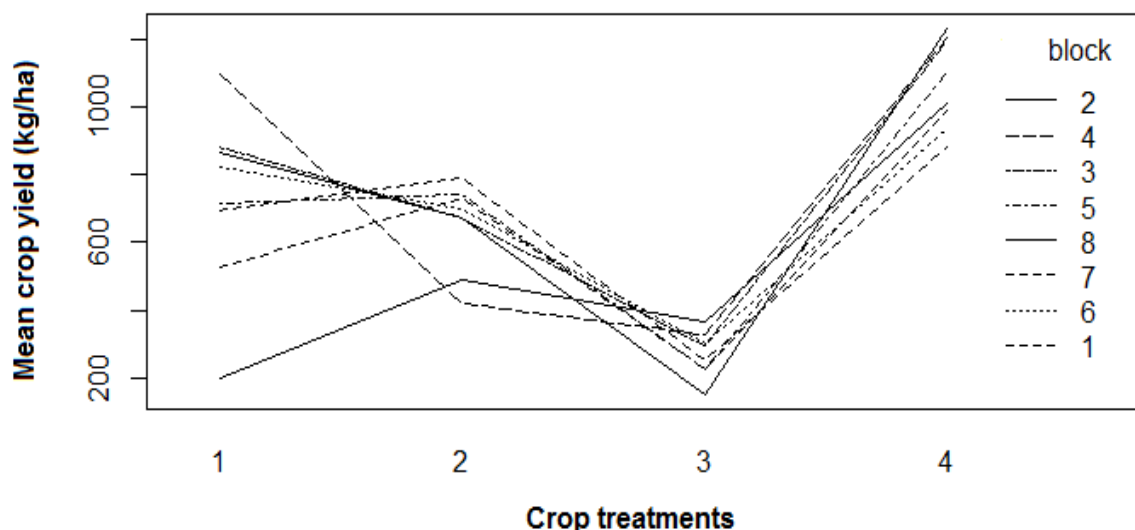


Figure 4.1 Block and four dryland crops data combined additively to predict yield corrected to 12.5% moisture (kg/ha). Each crop was planted once within each block. Average yield corrected to 12.5% moisture (kg/ha), blocks 1 to 8, crop treatments history (1: potatoes-maize-maize), (2: potatoes-maize-sugar beans), (3: potatoes-maize-soybeans) and (4: potatoes-maize-sunflower).

4.4.3 Partial Redundancy Analysis (RDA) and Monte Carlo Permutation Tests on Crop Effects on Soil Health

The objective was to investigate the effect of plant type on the soil and plant health legacies of season 2018. The block effects were taken as a covariate while the crop treatment effects were used as an explanatory variable in a partial constrained analysis. Partial RDA explained the amount of variation left after accounting for covariate (block) effects.

Biplot in Figure 4.2 indicates that *G. max* (pmsoy) and *Z. mays* (pmm) are the most different crop treatments with plots based on the separation by the first and second constrained axes from others. Grain yield is important to evaluate the productivity of long-term management trials and it was considered in the analysis. Grain yield in season 2018 was high in *H. annuus* (pmsun) and significantly correlated with legacies in soil Na, P, P Bray 2, S, Ca, and Mg (Figure 4.2). The legacies in biological properties i.e., active C and soil microbial respiration were

strongly correlated with the *P. vulgaris* (pmsb) cultivation (Figure 4.2). *Glycine max* had a poor soil fertility legacy except for the Ca:Mg ratio. *Helianthus annuus* was the highest yielding grain amid rich soil fertility legacies in P, S, Cu and K in a similar way to *P. vulgaris* (Figure 4.2).

The partial RDA ordination of soil health data are shown (Figure 4.2) by arrows while the triangles represent the centroids of crop treatments. The permutation test results on all axes were $P = 0.001$ and *pseudo-F* ratio = 6.2 while for the first axis it was $P = 0.001$ and *pseudo-F* ratio = 5.3, indicating that crop treatments had a heterogeneous legacy effect on soil health content within blocks, but not across blocks (Figure 4.2 and Figure 4.3). The Euclidean distance of the crop (*Z. mays*, *P. vulgaris*, *G. max*, and *H. annuus*) and blocks predicted the plot membership of the class as shown in Figure 4.3.

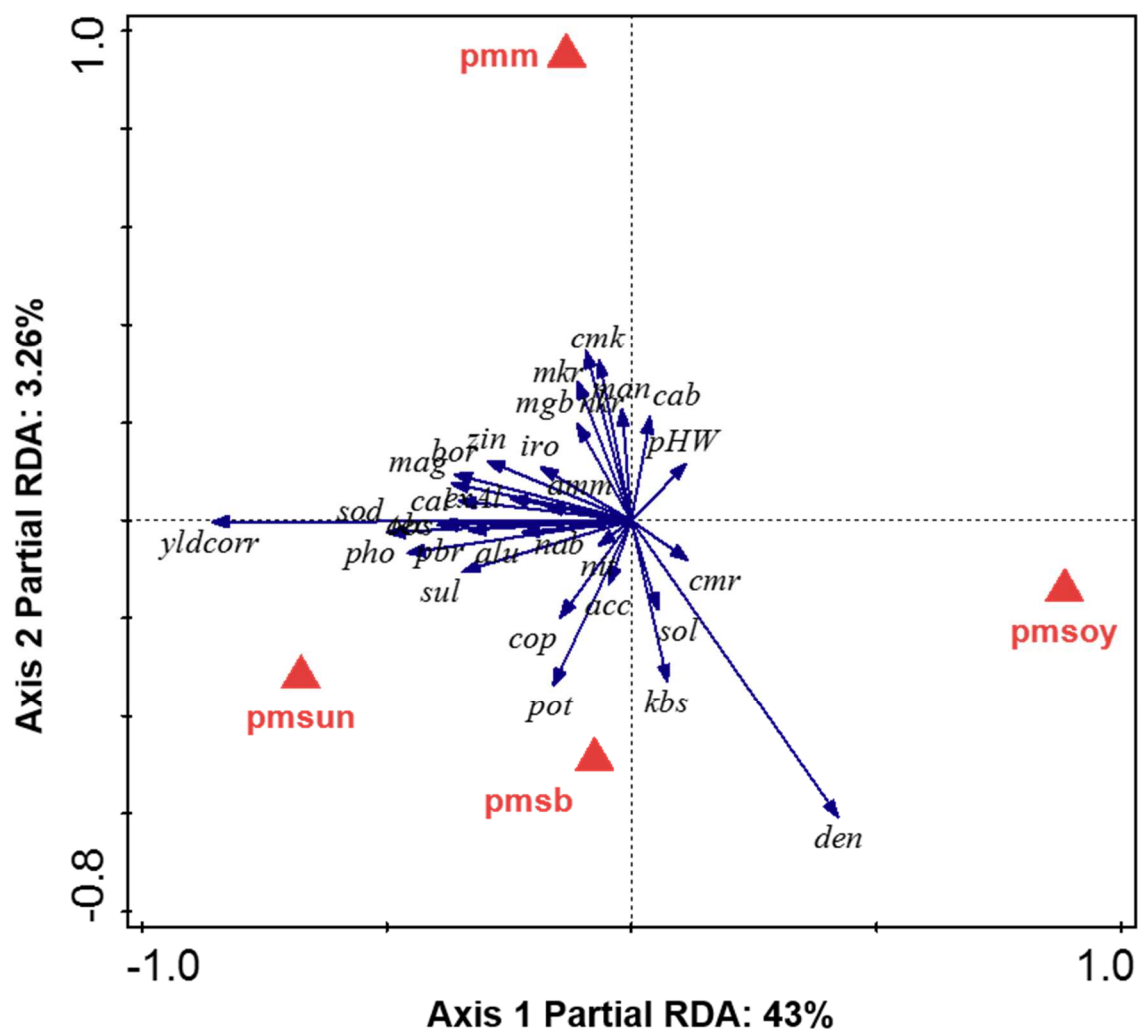


Figure 4.2 The ordination biplot diagram of the computed partial RDA to illustrate the effect of crop treatments (explanatory variables) on the 2018 season soil health factors after eliminating the block effects as a covariate. The red symbol (triangle) represents crop treatments; **pmm** – *Zea mays*, **pmsb** – *Phaseolus vulgaris*, **pmsoy** – *Glycine max*, **pmsun** – *Helianthus annuus*. The blue arrows represent the soil health legacies where:

acc - Active carbon (mg/kg)	cab - %Ca	alu - Al (mg/kg)
amm - 1:2 Water extract N - NH ₄ (mg/l)	den - Density (g/cm ³)	bor - B (mg/kg)
cec - CEC (cmol(+)/kg)	cop - Cu (mg/kg)	cal - Ca (mg/kg)
cmk - (Ca+Mg)/K	hbs - % acid saturation	iro - Fe (mg/kg)
cmr - Ca:Mg	kbs - %K	mag - Mg (mg/kg)
sod - Na (mg/kg)	mgb - %Mg	man - Mn (mg/kg)
sol - Solvita CO ₂ respiration (ppm)	nab - %Na	zin - Zn (mg/kg)
nit - 1:2 Water extract N - NO ₃ (mg/l)	nkr - Na:K	pho - P (mg/kg)
obs - S-Value (cmol(+)/kg)	mkr - Mg:K	pHW - pH (H ₂ O)
pbr - P Bray 2 (mg/kg)	sul - S (mg/kg)	pot - K (mg/kg)
exAl - salt-replaceable acidity (cmol(+)/kg)		

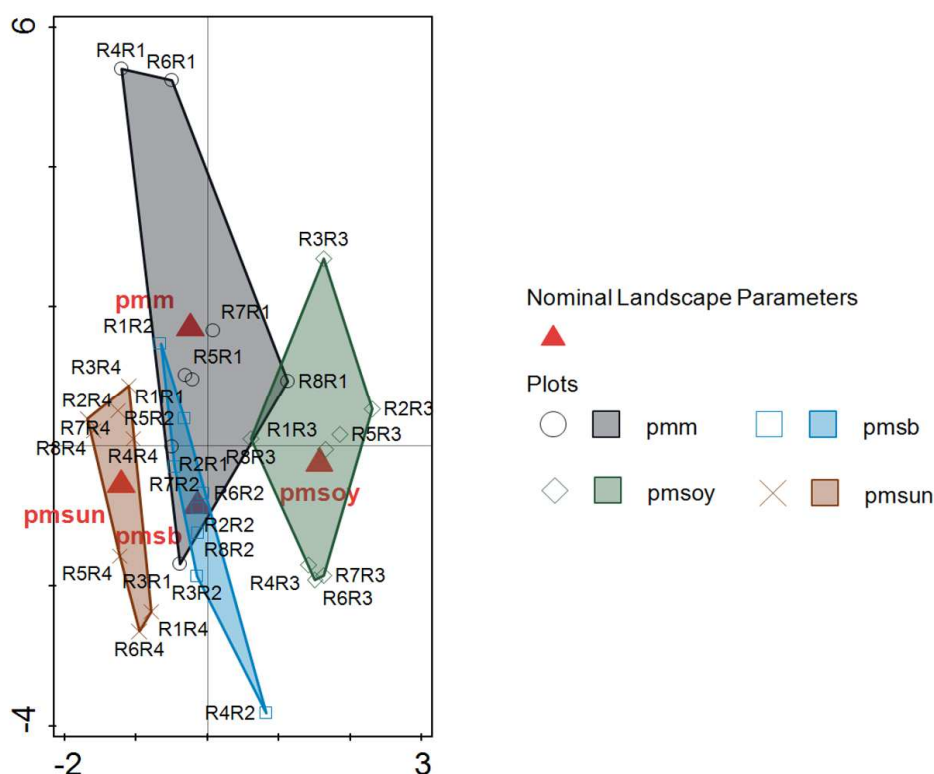


Figure 4.3 The partial RDA dissimilarity between the soil health variable composition of that landscape parameter plot and average soil health variable composition of the individual classes. The plot symbol represents crop treatments; pmm – *Zea mays*, pmsb – *Phaseolus vulgaris*, pmsoy – *Glycine max*, pmsun – *Helianthus annuus*). The distance of a plot symbol (pmm, pmsb, pmsoy and pmsun) predicts the plot membership of to the landscape parameter classes (red triangles). Score scaling is focused on soil health variable scores (standardized) and not focused on the Euclidean distances between plots. R1R1 to R8R4 represent 32 ‘field plots’.

The plot site factor map shows (Figure 4.4, right) the field plots that were allocated 'dummy' treatments in season 2016/17 and four crop treatments in season 2017/18. The soil health factor map (Figure 4.4, left) shows very clearly the positive and negative correlations in soil health properties between two seasons. The PCA maps in Figure 4.4 show that the first axis corresponded to a seasonal gradient in soil health parameters, in other words, the first axis separated soil fertility composition based on seasons. However, many labels were superimposed resulting in mixed spatial and temporal structures within seasons (Figure 4.4). Biological content in active carbon and soil microbial respiration were higher in season 2018 than in season 2017 (Figure 4.4).

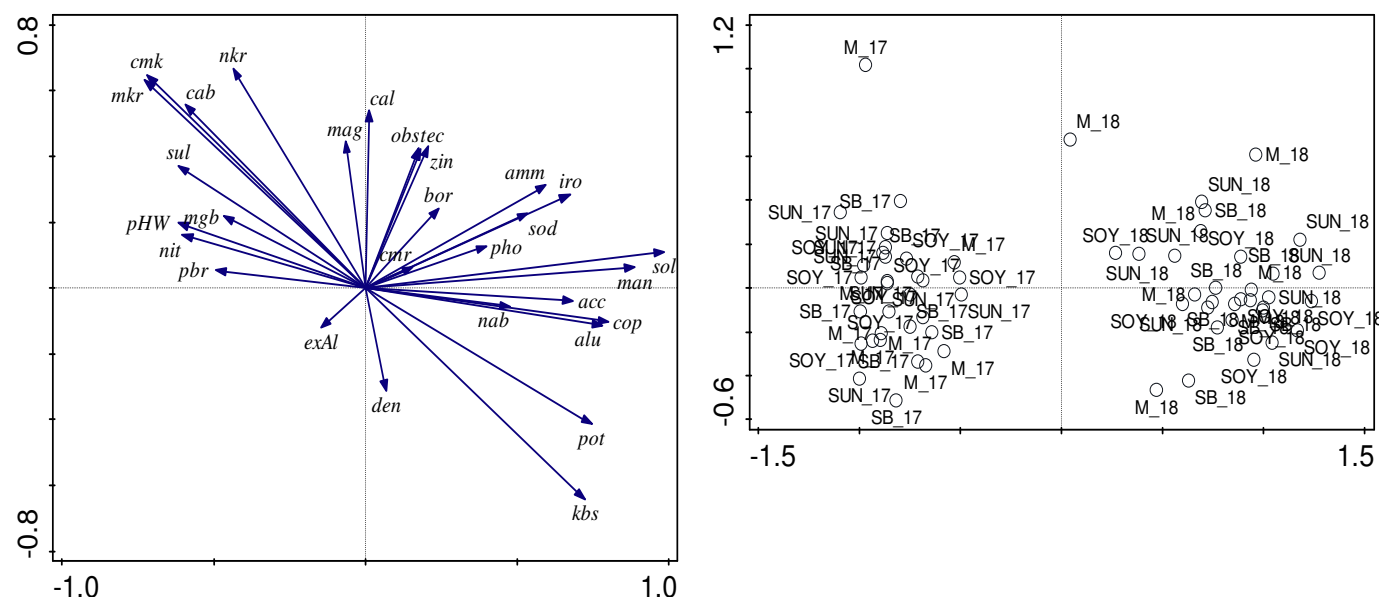


Figure 4.4 Biplot diagram separating season 2017 and 2018 based on soil health data scores from principal component analysis with axes PC1 and PC2 explaining 70.47% and 7.22% of total variability, respectively. This figure shows biplots of the soil health factor map (left) and plot site factor map (right) between the two seasons 2016/17 and 2017/18. These two biplots are interpreted together for association between soil health compositional legacies and plot sites. The left PCA factor map represents patterns of soil health factors while the right PCA factor map represents variation across field plots in 2017 (M_17, SB_17, SOY_17, SUN_17) and 2018 (M_18, SB_18, SOY_18, SUN_18) planted with *Z. mays* (M), *P. vulgaris* (SB), *G. max* (SOY) and *H. annuus* (SUN). The blue arrows represent the soil health legacies where:

acc - Active carbon (mg/kg)	cab - %Ca	man - Mn (mg/kg)	alu - Al (mg/kg)	cmk - (Ca+Mg)/K	nkr - Na:K
amm - 1:2 Water extract N - NH ₄ (mg/l)	kbs - %K	den - Density (g/cm ³)	bor - B (mg/kg)	mkr - Mg:K	cmr - Ca:Mg
sol - Solvita CO ₂ respiration (ppm)	nab - %Na	mag - Mg (mg/kg)	cal - Ca (mg/kg)	sod - Na (mg/kg)	zin - Zn (mg/kg)
nit - 1:2 Water extract N - NO ₃ (mg/l)	mgb - %Mg	hbs - % acid saturation	iro - Fe (mg/kg)	pho - P (mg/kg)	
obs - S-Value (cmol(+)/kg)	cop - Cu (mg/kg)	cec - CEC (cmol(+)/kg)	pot - K (mg/kg)	sul - S (mg/kg)	
exAl - salt-replaceable acidity (cmol(+)/kg)	pHW - pH (H ₂ O)	pbr - P Bray 2 (mg/kg)			

Chemical content in K, Al, Mn, Cu, P, Na, % K, % Na, Fe, $\text{NH}_4\text{-N}$, Zn and B were also higher in season 2018 than in season 2017. Contrary, Na:K, Mg:K, (Ca+Mg)/K ratios, $\text{pH}(\text{H}_2\text{O})$, $\text{NO}_3\text{-N}$, P Bray 2, % Mg, % Ca, S, and acid cations (H^+ and Al^{3+} ions) were higher in season 2017 than the next season (Figure 4.4).

4.4.4 Redundancy Analysis and Monte Carlo Permutation Tests on Temporal Variation in Rhizosphere Microbiology according to Crop Type

4.4.4.1 Crop Rhizosphere Soil Microbial Biomass and Activity

The microbial communities in the rhizosphere of four dryland crop species were generally like bulk soil but statistically different in fungal- and Gram positive bacterial PLFA markers as well as in soil enzyme activity as detailed in Figure 4.5, and Tables S4.1A to S4.1D. The cultivation of *H. annuus* resulted in 1.7 x and 1.5 x more saprophytic fungal biomass markers than bulk soil ($p < .05$) and rhizosphere of *Z. mays* ($p > .05$), respectively (Figure 4.5, Table S4.1A).

In addition, significant differences ($p < .05$, Figure 4.5, Table S4.1B) were also detected in fungal to bacterial PLFA ratio and microbial enzyme activity in FDA hydrolysed ($\mu\text{g}/\text{mg}$ soil) ($p < .05$, Figure 4.5 and Table S4.1D). The microbial enzyme activity in FDA hydrolysed, was 1.5 x higher in *H. annuus* rhizosphere soil than in bulk non-rhizosphere control soil. The Gram-positive value for PLFA data was 2.5 x greater in *H. annuus* than in bulk soil. The highest fungal to bacterial PLFA marker ratio was in *H. annuus* (average 0.24 ± 0.0253), while the Gram-positive to Gram-negative PLFA ratio was relatively high on average in the rhizospheres of *H. annuus* (12.7 ± 6.07) and *Z. mays* (12.0 ± 5.48). Figure 4.5 illustrates the descriptive statistics of soil microbial composition, biomass, and activity.

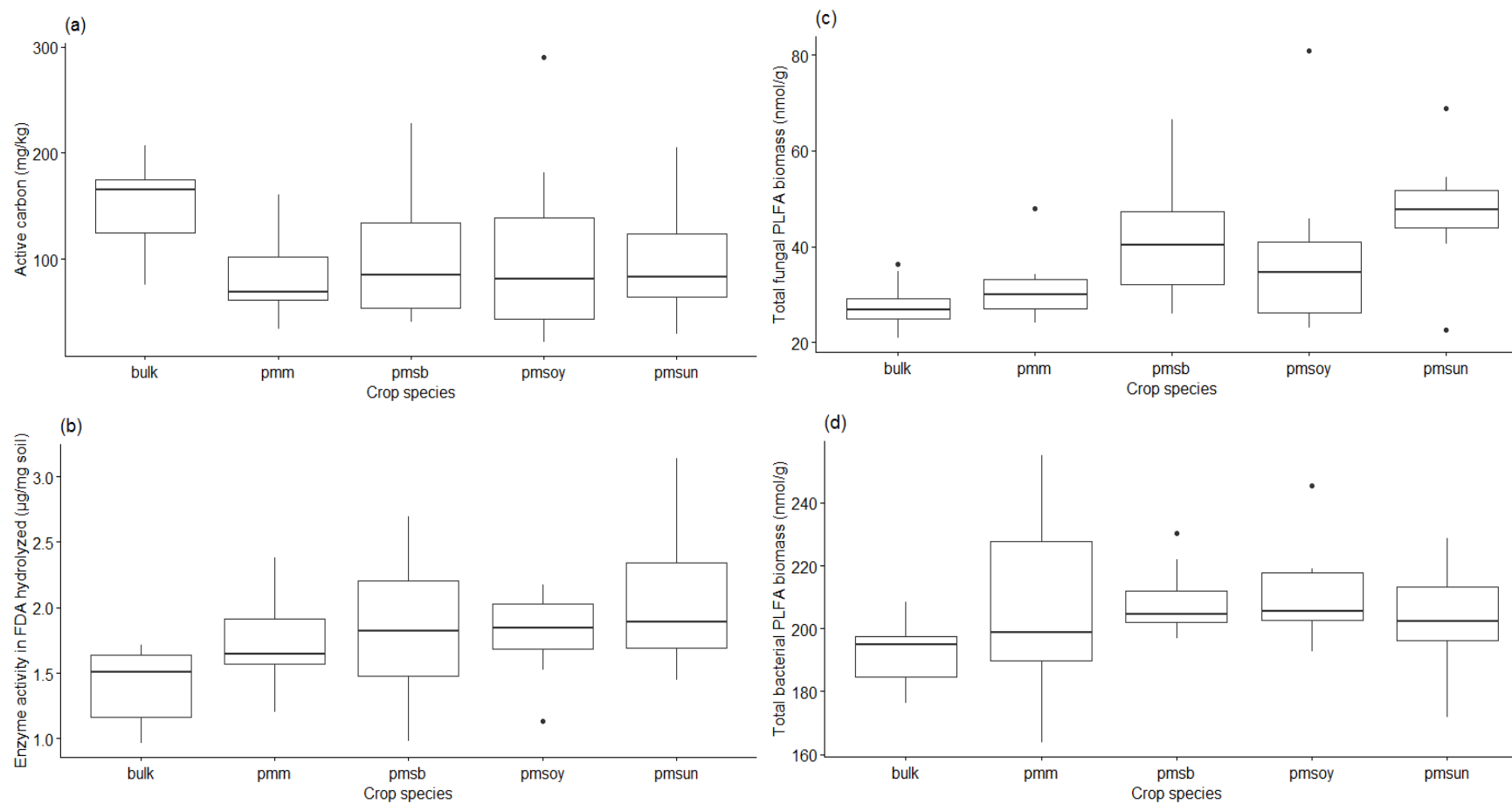


Figure 4.5 Legacies in rhizosphere soil (a) active carbon (mg/kg) and (b) FDA hydrolysed (μg/mg soil), (c) total fungi PLFA, and (d) total bacterial PLFA. bulk – bulk soil, pmm – *Zea mays*, pmsb – *Phaseolus vulgaris*, pmsoy – *Glycine max*, pmsun – *Helianthus annuus*. Each treatment had 8 replications. Each block had 4 treatments. Horizontal heavy bar = median, box=lower and upper quartiles, whiskers=minimum and maximum values, filled dot=outliers.

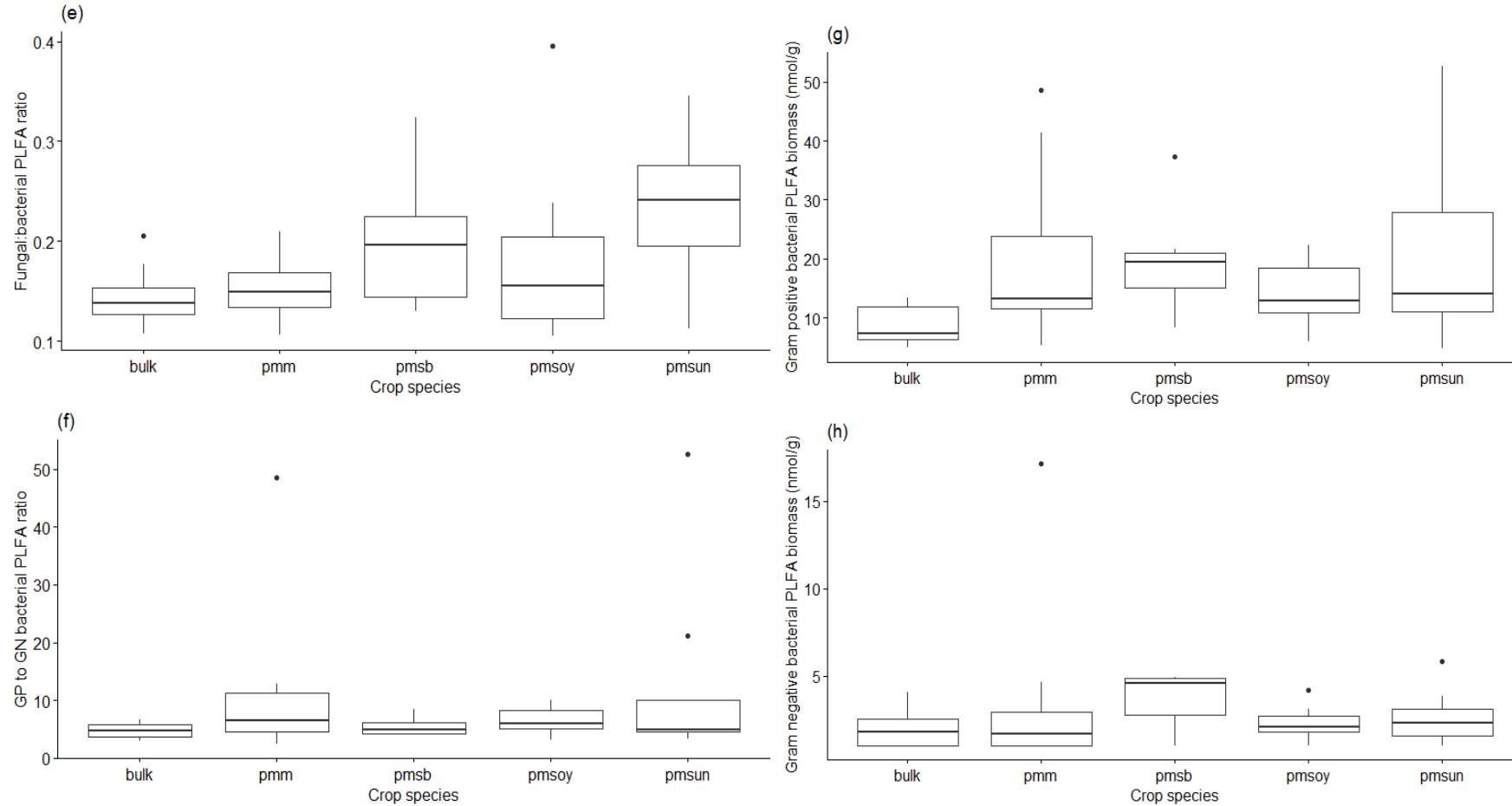


Figure 4.5 Legacies in rhizosphere soil (e) fungal:bacterial PLFA ratio, (f) Gram-positive (Gp) to Gram-negative (Gn) PLFA ratio, (g) Gram-positive bacterial PLFA and (h) Gram-negative bacterial PLFA markers. bulk – bulk soil, pmm – *Zea mays*, pmsb – *Phaseolus vulgaris*, pmsoy – *Glycine max*, pmsun – *Helianthus annuus*. Each treatment had 8 replications. Each block had 4 treatments. Horizontal heavy bar = median, box=lower and upper quartiles, whiskers=minimum and maximum values, filled dot=outliers.

The biomass of fungal marker C18:1 ω 9t was 2.5 x higher in *Z. mays* rhizosphere soil than in the bulk soil and other crop species (Table 4.2). The concentration of fungal marker C18:1 ω 9c was 1.5 x higher in *P. vulgaris* rhizosphere soil than in *Z. mays* rhizosphere soil and the bulk soil (Table 4.2). *Helianthus annuus* cultivated a significant 3 x increase of the fungal marker C18:2 ω 6c compared to bulk soil and approximately twice that of the other crop species ($p < .05$, Table 4.2). Table 4.2 summarizes the distribution of microbial composition in the rhizosphere of different crop treatments.

Table 4.2 The average concentration or composition (nmol/g) of three fungal and four bacterial PLFA biomarkers in rhizosphere and bulk soil samples. SEM represents the $\pm$ standard error of the mean (N=8). ^a Significant differences have p -values $< .05$ using ANOVA (C18:1 ω 9c, C18:2 ω 6c, α -C15:0) and Kruskal Wallis (C18:1 ω 9t, i-C15:0, i-C16:0, C16:1 ω 7c) tests.

Crop	Fungal markers			Bacterial markers			
	C18:1 ω 9t	C18:1 ω 9c	C18:2 ω 6c	i-C15:0	α -C15:0	i-C16:0	C16:1 ω 7c
Mean							
<i>Z. mays</i>	0.713	19.7	11.1	12.6	5.13	2.57	3.81
<i>P. vulgaris</i>	0.353	30.9	9.81	8.97	9.91	0.547	3.77
<i>G. max</i>	0.345	27.9	10.4	7.33	6.33	0.324	2.31
<i>H. annuus</i>	0.342	22.4	23.4	15.2	3.8	3.42	2.62
Bulk	0.278	20.3	7.13	7.06	1.28	0.428	2.08
SEM							
<i>Z. mays</i>	0.272	1.21	1.65	3.48	1.44	1.71	1.96
<i>P. vulgaris</i>	0.231	4.46	2	0.678	3.01	0.368	0.52
<i>G. max</i>	0.234	6.19	1.89	0.358	1.51	0.324	0.346
<i>H. annuus</i>	0.242	1.23	3.31	4.81	0.976	2.24	0.57
Bulk	0.184	1.45	0.645	0.563	0.519	0.291	0.441
<i>p</i>-value	0.702	0.128	$< 0.001^a$	0.192	0.017 ^a	0.736	0.163

The biomass of bacterial marker i-C15:0 was 2 x higher in *H. annuus* rhizosphere soil than in the bulk soil and for *G. max* (Table 4.2). The bacterial marker α -C15:0 was significantly enriched 3 x and 9 x in the *P. vulgaris* rhizosphere soil compared to *H. annuus* rhizosphere soil and bulk soil, respectively ($p < .05$, Table 4.2). *Helianthus annuus* cultivated an 8 x increase of the bacterial marker i-C16:0 compared to bulk soil and rhizosphere of *G. max* and *P. vulgaris*. The bacterial marker C16:1 ω 7c was relatively uniform in all crop species rhizosphere soils and in bulk soil (Table 4.2).

The permutation test results on all axes were $P = 0.46$ and *pseudo-F* ratio = 0.6, while for the first axis it was $P = 0.478$ and *pseudo-F* ratio = 1.0, indicating that crop treatments had a legacy effect on plant health content although they were not significantly different within blocks ($p > .05$, Figure 4.6 and Figure 4.7). The biplot in Figure 4.6 displays plant health variables for rhizosphere soil samples from *Zea mays*, *Phaseolus vulgaris*, *Glycine max* and *Helianthus annuus* collected in season 2018 (Figure 4.6, Figure 4.7 and Figure S4.3).

The scatter pies in Figure 4.7 show the crop rhizosphere differences in microbial community composition. This data indicated that *H. annuus* rhizosphere supported a greater community of total fungal PLFA markers, fungal to bacterial PLFA ratio, C18:2 ω 6c fungal PLFA marker and i-C16:0 Gram positive bacterial PLFA marker (Figure 4.7). Norris et al. (2023) argues that the presence of C18:2 ω 6c fungal PLFA marker in agricultural systems is unlikely to indicate the presence of mycorrhizal network, instead it may represent other other fungal functional groups such as organic matter decomposers.

Zea mays rhizosphere was superior to other classes in terms of the C18:1 ω 9t PLFA marker. This PLFA marker is probably valuable for identifying phenotypic changes in response to crop rotation across multiple members (Norris et al., 2023). In this study, the PLFA markers and necessary datasets were not time series data or large enough to link them together for the desired agronomic, environmental, soil and plant health outcomes. The integration of PLFAs

into predicting various goals such as higher crop yields and the decisiveness farmers need are still a challenge for most if not all soil health scoring systems, because there is not enough training datasets to simulate, interpolate and extrapolate PLFAs together with crop yields, soil and plant health (National Academies of Sciences, Engineering, and Medicine, 2021). There is little understanding on resilience of soil PLFAs to disturbance and climate changes under various dryland production situations.

The investigation of these situations in real-time can provide valuable information into mechanisms leading to the identification of good and or bad PLFA markers and the likely effects of climate change on soil PLFAs; ultimately enabling farmers to decisively manage their fields in ecological balances that avoid chemical abuse (Cadet et al., 2002; Saiz et al., 2012). Active carbon was highly correlated with Gram-positive bacterial PLFA markers while soil enzyme activity was closely related to both Gram-positive bacterial markers and total fungal markers. However, the RDA outputs (Figure 4.6 and Figure 4.7) should be interpreted with caution considering that the p -value of the permutation test was not statistically significant ($p > .05$). Robust interpretations can be made when more data on PLFAs and yield are collected on the same field in the following years.

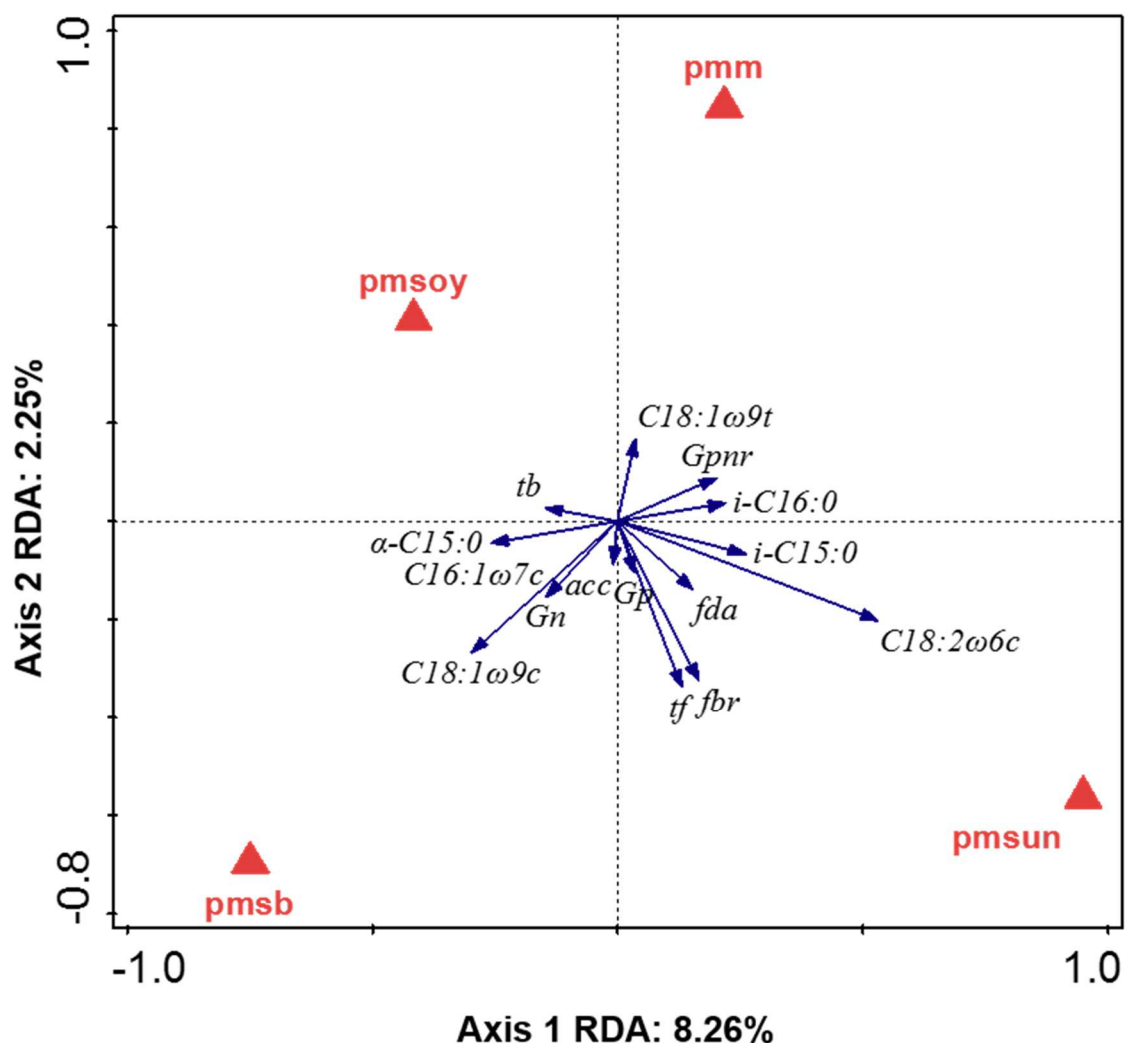


Figure 4.6 Ordination plot from redundancy analysis (RDA) of rhizosphere and the relative abundance of the seven individual PLFAs that represent Gram-positive bacteria (Gp), Gram-negative bacteria (Gn), Gp to Gn ratio (Gpnr), total bacteria (tb), total fungi (tf), fungal: bacterial ratio (fbr) of PLFA markers (Table 4.2) across plant functional group treatments (red triangles). acc- Active carbon (mg/kg), fda - enzyme activity in FDA hydrolyzed ($\mu\text{g}/\text{mg}$ soil). The red symbols (triangle) represent crop treatments; **pmm** – *Zea mays*, **pmsb** – *Phaseolus vulgaris*, **pmsoy** – *Glycine max*, **pmsun** – *Helianthus annuus*. The blue arrows represent the plant health properties, red triangles indicate the projection of the different crop treatments according to the two first RDA axes.

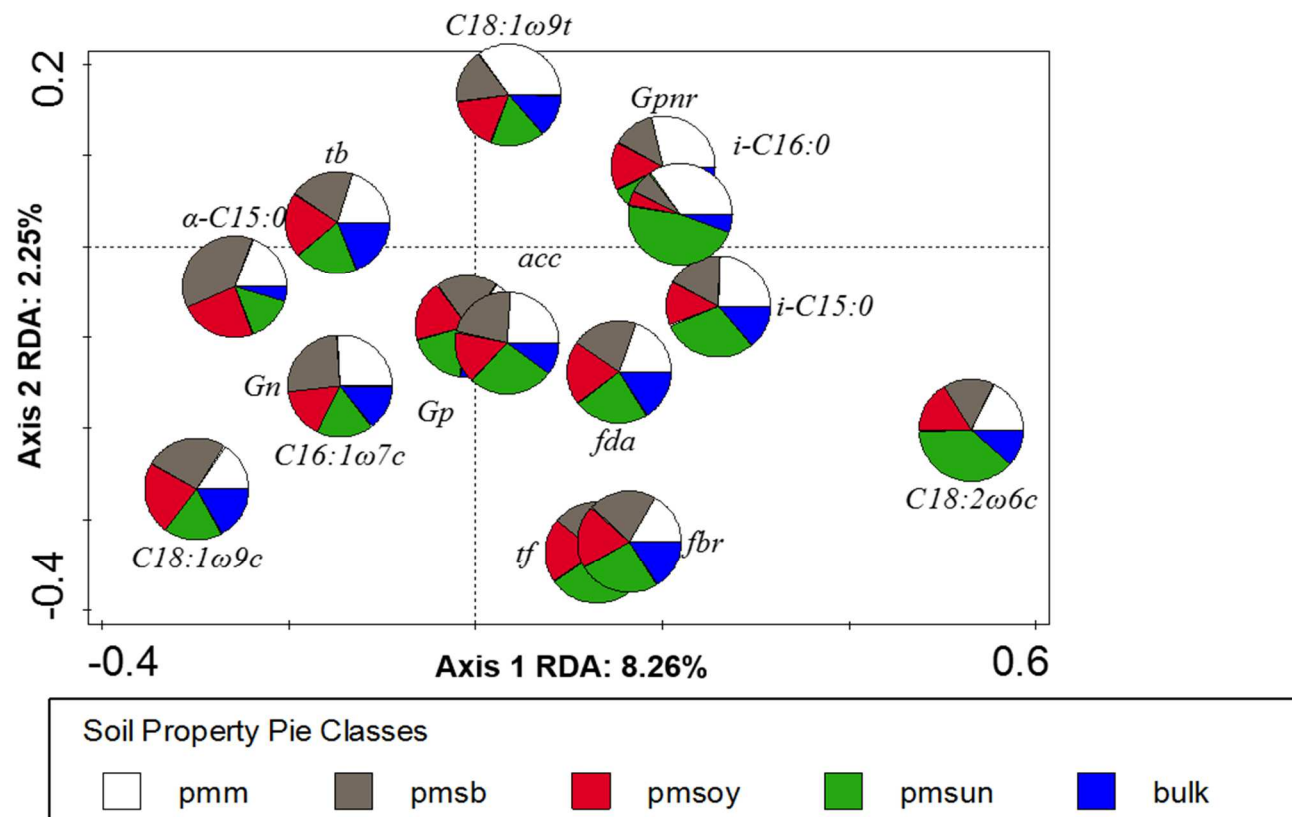


Figure 4.7 Pie plots for relative composition in active carbon, soil microbial enzyme activity, concentration and biomass ratios of fungal and bacterial biomarkers representing cases of the plant health data. Cases are visualized and displayed within an ordination space of the first two RDA axes. Gram-positive bacteria (Gp), Gram-negative bacteria (Gn), Gp to Gn ratio (Gpnr), total bacteria (tb), total fungi (tf), fungal: bacterial ratio (fbr) of PLFA markers across plant functional group treatments (filled boxes. acc- Active carbon (mg/kg), fda - enzyme activity in FDA hydrolyzed ($\mu\text{g}/\text{mg}$ soil). The pie charts represent crop treatments; pmm – *Zea mays*, pmsb – *Phaseolus vulgaris*, pmsoy – *Glycine max*, pmsun – *Helianthus annuus*.

4.4.5 Partial Redundancy Analysis and Monte Carlo Permutation Tests on Vertical Soil Health Variation

Univariate analysis of variance (ANOVA) indicated that there was enough evidence to conclude that soil chemical content in P Bray 2, P, K, Mg, Zn, B, electrical conductivity and N - NH₄ were significantly different at the two soil depths (topsoil 20cm and subsoil 40cm) for the eight crop treatment levels (Figure 4.8, Table S4.2 and Table S4.3) at crop physiological maturity. In terms of soil biological content, the soil microbial respiration was significantly different between the eight crop treatments at the two soil depths ($p < .05$). On one hand, Figure 4.8 (a) to (h) shows selected soil properties that were significantly different in composition between the topsoil and subsoil. On the other hand, in Figure 4.9, the partial RDA analysis biplot indicates the variable impact of crop cultivation on legacies of soil health properties compositions at crop physiological maturity sampled from topsoil 20cm and subsoil 40cm soil depths.

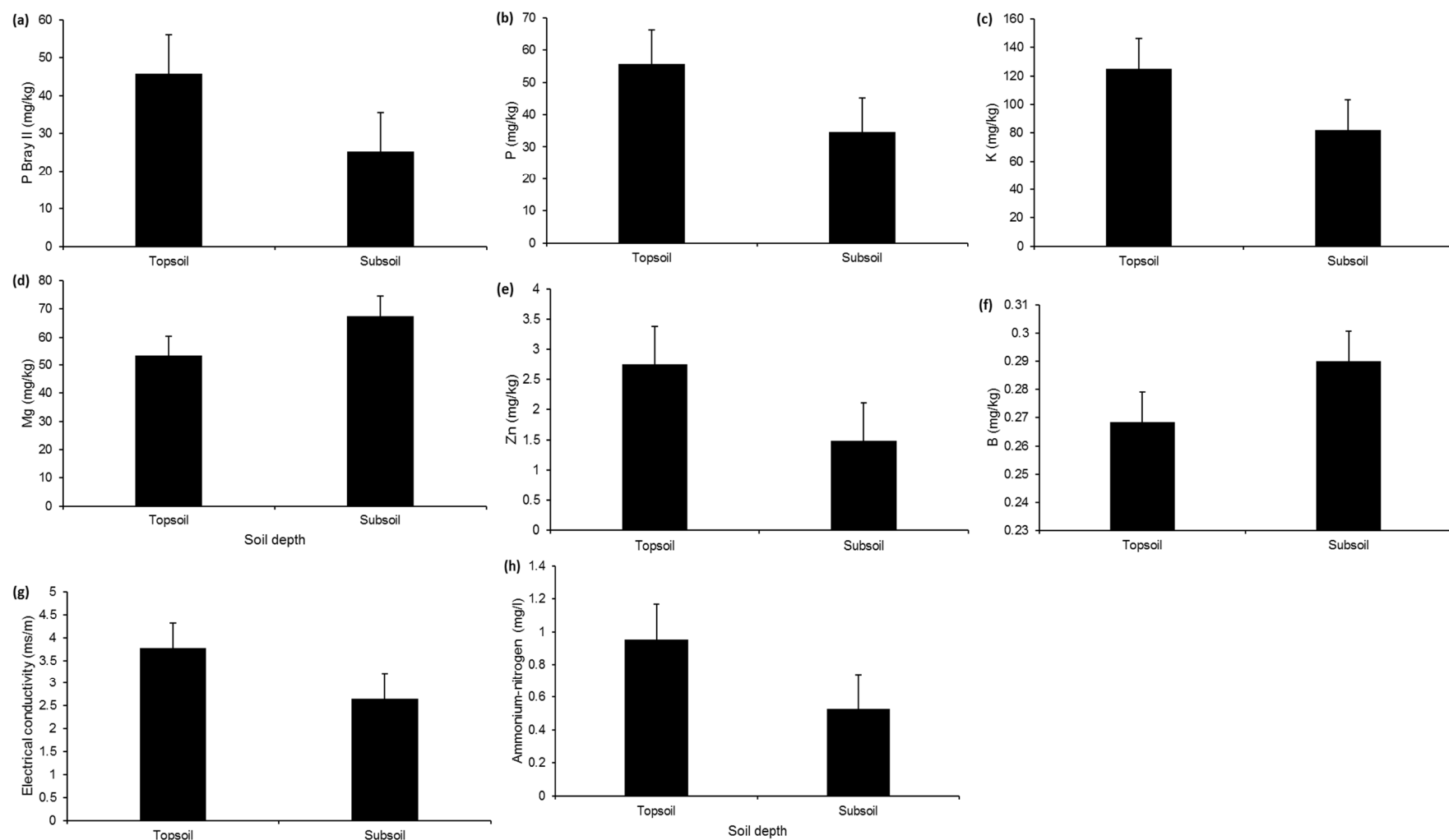


Figure 4.8 The differences between the topsoil (0-20cm depth) and subsoil (20-40cm depth) properties. All the selected soil properties (a) to (h) were statistically different at the two depths ($p < .05$). Vertical error bars represent mean $\pm$ standard error ($n = 32$).

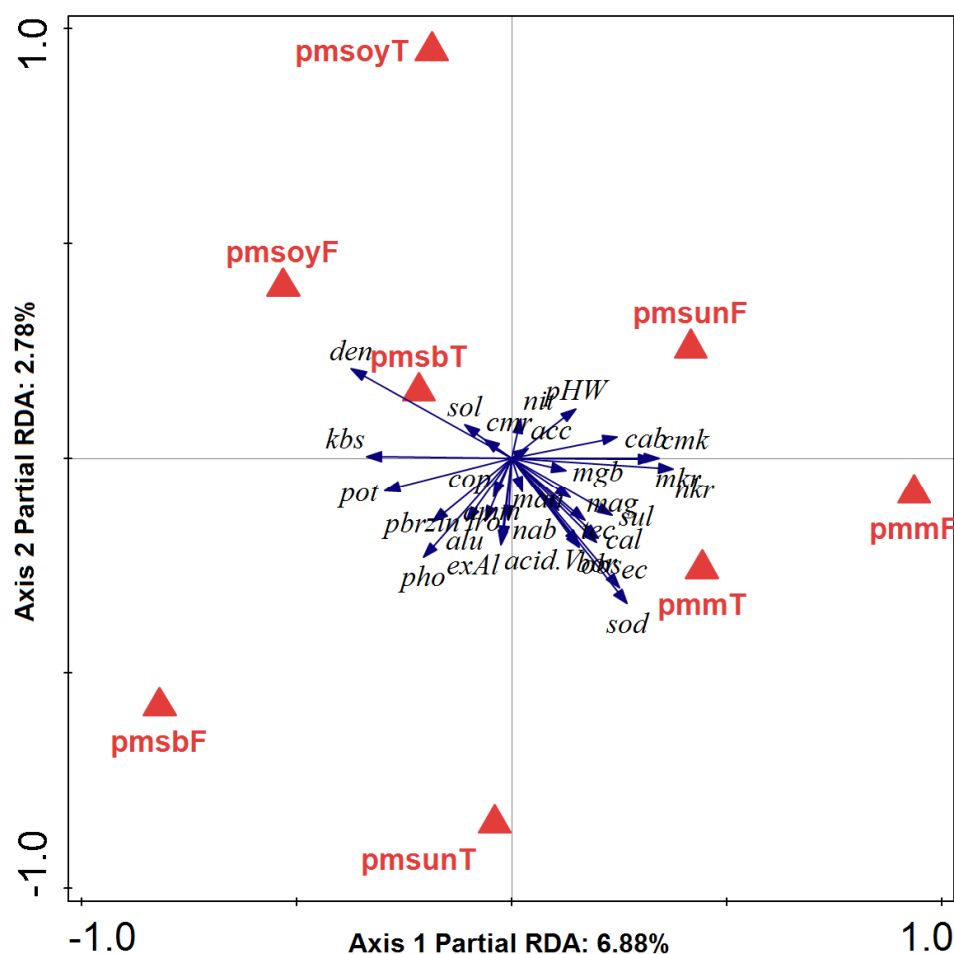


Figure 4.9 The partial redundancy analysis (RDA) biplot displaying the variation in legacies of soil health properties explained by soil depth (topsoil 20cm-T and subsoil 40cm-F), after accounting for the effects of covariates (soil depth and blocks). Symbols of soil depth classes are represented as individual symbols (red triangles) of groups (classes) of plots; pmm – *Zea mays*, pmsb – *Phaseolus vulgaris*, pmsoy – *Glycine max*, pmsun – *Helianthus annuus*, T – 0 to 20cm soil depth and F – 20cm to 40cm soil depth. The blue arrows represent the soil health legacies where:

acc - Active carbon (mg/kg)	cab - %Ca	alu - Al (mg/kg)
amn - 1:2 Water extract N - NH ₄ (mg/l)	den - Density (g/cm ³)	bor - B (mg/kg)
cec - CEC (cmol(+)/kg)	cop - Cu (mg/kg)	cal - Ca (mg/kg)
cmk - (Ca+Mg)/K	cmr - Ca:Mg	iro - Fe (mg/kg)
hbs - % acid saturation	kbs - %K	mag - Mg (mg/kg)
exAl - salt-replaceable acidity (cmol(+)/kg)	mgb - %Mg	man - Mn (mg/kg)
sol - Solvita CO ₂ respiration (ppm)	nab - %Na	zin - Zn (mg/kg)
nit - 1:2 Water extract N - NO ₃ (mg/l)	nkr - Na:K	pho - P (mg/kg)
obs - S-Value (cmol(+)/kg)	mkr - Mg:K	pHW - pH (H ₂ O)
pbr - P Bray 2 (mg/kg)	sul - S (mg/kg)	pot - K (mg/kg)
ec – electrical conductivity (ms/m)	sod - Na (mg/kg)	

Glycine max and *H. annuus* soil health legacies at topsoil 20cm depth were highly dissimilar while the topsoil of *P. vulgaris* and *Z. mays* were least dissimilar (Figures 4.9 and Figure S4.4). An increase in soil pH, active carbon, and NO₃-N was observed in the *H. annuus* soils from topsoil 20 cm to subsoil 40 cm depth. The topsoil (20 cm depth) legacies of *P. vulgaris* were higher in soil microbial respiration and density while *Z. mays* and *H. annuus* legacies were highest in most micro- and macronutrients (Figure 4.9 and Figure S4.4).

The permutation test results on all axes were $P = 0.068$ and *pseudo-F* ratio = 1.4 while for the first axis it was $P = 0.055$ and *pseudo-F* ratio = 0.7, indicating that crop treatments had a non-significant legacy effect on vertical soil health properties within the two depths, but not across plots (Figure 4.9). However, the partial RDA outputs (Figure 4.9 and Figure S4.4) should be interpreted with caution considering that the *p*-value of the permutation test was not statistically significant ($p > .05$). Loadings of soil health properties close to the origin of the coordinate system, for example, Mg, Cu, %Na and active C represent unimportant features (Sena et al., 2002). On the other hand, the loadings farther away from the centroid, for example, density, K, Na, P and %K are more influential in a PC (Cavigelli et al., 1995).

The correlated soil health properties are close (positive correlation, such as between electrical conductivity and Na) or opposite (negative correlation, between Na and density) to each other while the uncorrelated factors (Na and K) are orthogonal to each other (Sena et al., 2002). Few soil health variables were close to crop treatment symbols, such as Na was very close to topsoil samples from *Z. mays* (Figure 4.9, pmm), suggests that this crop treatment legacy will have high direct effects on the particular soil health property. Conversely, if soil health variables were distant from a crop treatment symbol, it will have high inverse effects on it (Sena et al., 2002).

4.4.6 Temporal Variation Testing of Plant-Soil Health Feedback Legacies using Principal Component Analysis (PCA), Redundancy Analysis (RDA) and Monte Carlo Permutation Tests

Plant–soil health feedbacks in the first two seasons (2017 and 2018) represented interactions such as soil-plant (Section 4.3.6.1), plant-soil (4.3.6.2) and soil-soil (Section 4.3.6.3). The assumption was that plant performance is linked to soil legacies established earlier on by the same or other plant species (Kulmatiski & Kardol, 2008).

4.4.6.1 Soil to Plant Health Feedbacks (*Plant health₂₀₁₈ = Soil health₂₀₁₇ linear relationships*)

Principal component analysis of plant-soil feedbacks at a field scale was done to compare the flow-on impacts and feedbacks between season 2017 soil health legacies and season 2018 plant health response as shown in Figure 4.10.

The fungal: bacterial ratio was closely associated with season 2017 legacies in clay, P Bray 2 and density across cultivated sites 9 (*Z. mays*), 15 (*G. max*), 20 (*H. annuus*), 22 (*P. vulgaris*), 25 (*Z. mays*) and 28 (*H. annuus*) (Figure 4.10). Gram-positive to Gram-negative bacteria ratio was highly correlated with season 2017 legacies in active carbon, K and Cu across cultivated sites in block 1 namely 1 (*Z. mays*), 3 (*G. max*), 4 (*H. annuus*) and one in block 6 namely site 24 (*H. annuus*) (Figure 4.10). Active carbon was highly correlated with clay, Fe, pH, nitrates, B and Mg across cultivated sites 2 (*P. vulgaris*), 6 (*P. vulgaris*), 7 (*G. max*) and 13 (*Z. mays*). Soil microbial enzyme activity in FDA hydrolysed was highly correlated with season 2017 legacies in Zn, sand, pH (water) and Solvita CO₂ respiration across sites 8 (*H. annuus*), 10 (*P. vulgaris*), 11 (*G. max*), 16 (*H. annuus*), 17 (*Z. mays*) and 30 (*P. vulgaris*) (Figure 4.10).

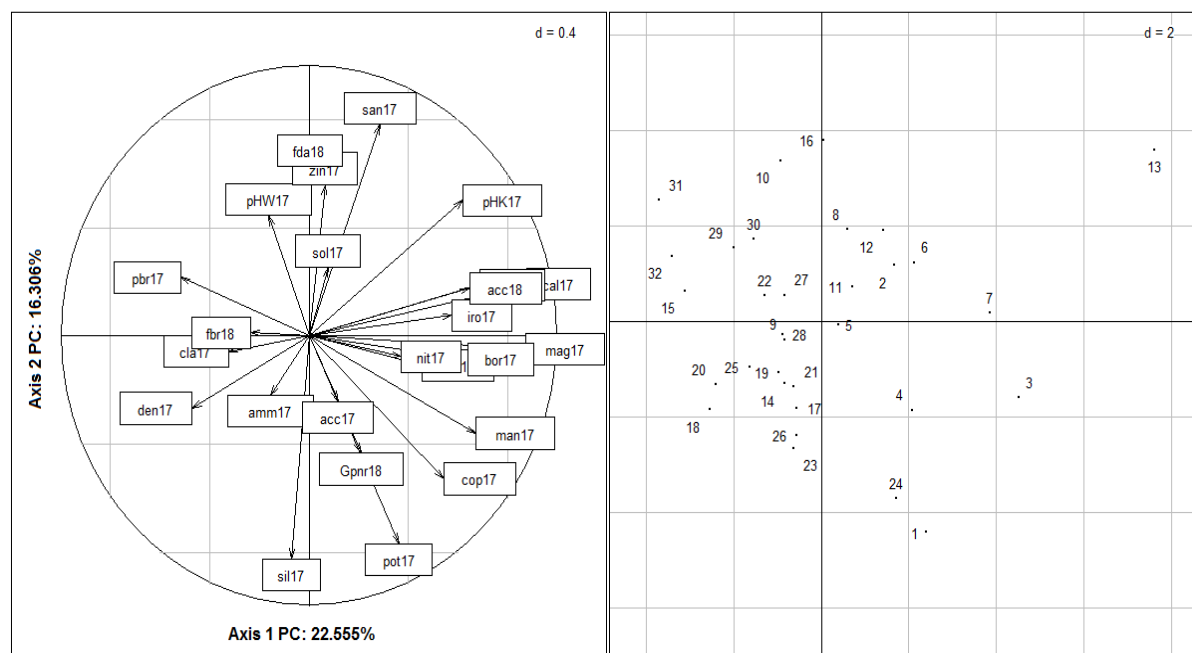


Figure 4.10 Soil health legacies for plant health legacies using standardised Principal Component analysis (PCA) using the correlation matrix PCA. On the left PCA factor map is the correlation circle map and on the right is the factor map of sites. The whole numbers from 1 to 32 (right graph) represent the plot sites cultivated with *Z. mays* (1, 5, 9, 13, 17, 21, 25, 29), *P. vulgaris* (2, 6, 10, 14, 18, 22, 26, 30), *G. max* (3, 7, 11, 15, 19, 23, 27, 31) and *H. annuus* (4, 8, 12, 16, 20, 24, 28, 32) in season 2018. The last two digits (17 or 18) in a soil property represent season 2017 or season 2018 legacies, respectively. Four plant health content include Gram-positive bacteria (Gp) to Gram-negative bacteria (Gn) ratio (Gpnr18), fungal: bacterial ratio (fbr18) of PLFA markers, acc18- Active carbon (mg/kg), and fda18 - enzyme activity in FDA hydrolysed ($\mu\text{g}/\text{mg}$ soil). The acronyms for the 21 soil health properties are represented as:

acc - Active carbon (mg/kg)	pHW - pH (H_2O)	man - Mn (mg/kg)
amm - 1:2 Water extract N - NH_4 (mg/l)	den - Density (g/cm^3)	bor - B (mg/kg)
sol - Solvita CO_2 respiration (ppm)	cop - Cu (mg/kg)	cal - Ca (mg/kg)
nit - 1:2 Water extract N - NO_3 (mg/l)	pot - K (mg/kg)	iro - Fe (mg/kg)
pbr - P Bray 2 (mg/kg)	zin - Zn (mg/kg)	mag - Mg (mg/kg)
sod - Na (mg/kg)	pHK - pH (KCl)	

The correlation circle showed a high nutrient density associated with active carbon while there was a low soil fertility associated with microbiological biomass and activity in season 2018.

4.4.6.2 Plant to Soil Health Feedbacks (*Soil health2018 = Plant health2018 linear relationships*)

Figure 4.11 displays a Redundancy Analysis comparing the flow-on impacts and feedbacks between plant and soil health content in the same season 2018 at a field scale. Fungal: bacterial PLFA ratio and total fungal PLFA biomass were highly correlated with the soil microbial Solvita CO₂ respiration, nitrates, Cu, Al, K and %K (Figure 4.11 and Figure S4.5). Active carbon and soil microbial enzyme activity were highly correlated with % Ca, pH (water), Zn, Ca:Mg, (Ca+Mg)/K, Na:K and Mg:K (Figure 4.11 and Figure S4.5). The permutation test results on all axes were $P = 0.049$ and *pseudo-F* ratio = 1.4, indicating that the crop effect on soil health content was statistically significant on all axes ($p < .05$, Figure 4.11) but not for individual PLFA markers ($p > .05$, Figure S4.6).

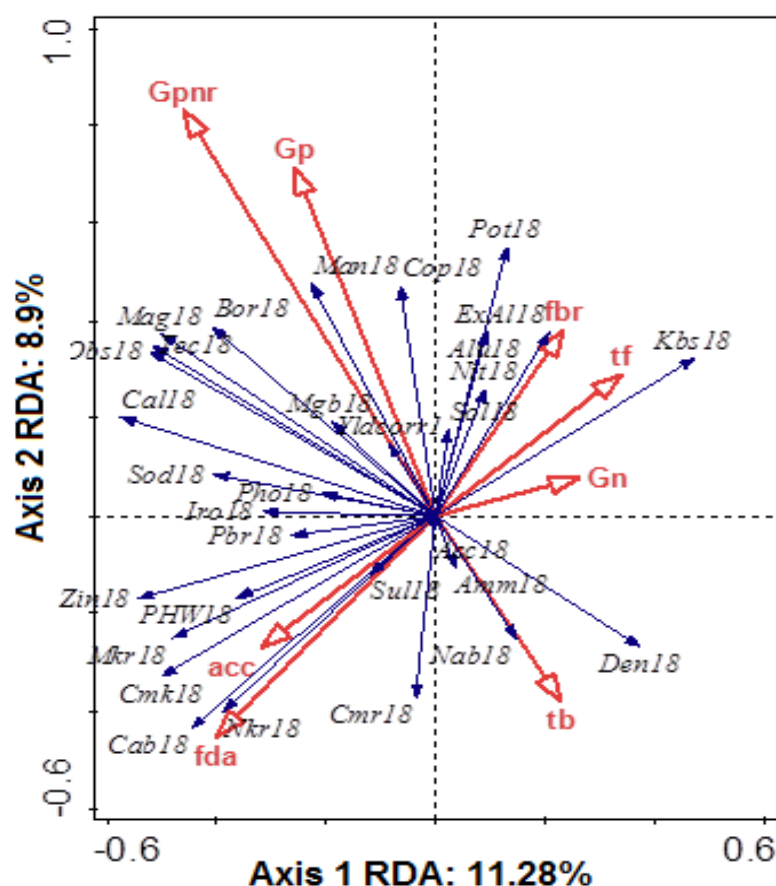


Figure 4.11 Redundancy Analysis (RDA) of data for bacterial and fungal PLFA biomass and ratios (red arrows – explanatory variables) effects on season 2018 soil health legacies (blue arrows – response variables). Four plant health properties include; Gram-positive bacteria (Gp) to Gram-negative bacteria (Gn) ratio (Gpnr), fungal: bacterial ratio (fbr) of PLFA markers, total fungal PLFA (tf), total bacterial PLFA (tb), acc - Active carbon (mg/kg), and FDA - enzyme activity in FDA hydrolysed ($\mu\text{g}/\text{mg}$ soil). The blue arrows represent the soil health legacies for season 2018 where:

Acc18 - Active carbon (mg/kg)	Cab18 - %Ca	Alu18 - Al (mg/kg)
Sod18 - Na (mg/kg)	Pho18 - P (mg/kg)	Mag18 - Mg (mg/kg)
Cec18 - CEC (cmol+)/kg)	Mgb18 - %Mg	Cal18 - Ca (mg/kg)
Cmk18 - (Ca+Mg)/K	Cmr18 - Ca:Mg	Iro18 - Fe (mg/kg)
Den18 – Density (g/cm ³)	Kbs18 - %K	Bor18 - B (mg/kg)
Ec18 – electrical conductivity (ms/m)	Cop18 - Cu (mg/kg)	Man18 - Mn (mg/kg)
Sol18 - Solvita CO ₂ respiration (ppm)	Nab18 - %Na	Zin18 - Zn (mg/kg)
Nit18 - 1:2 Water extract N - NO ₃ (mg/l)	Nkr18 - Na:K	Hbs18 - %Acid.V
Obs18 - S-Value (cmol+)/kg)	Mkr18 - Mg:K	pHW18 - pH (H ₂ O)
Pbr18 - P Bray 2 (mg/kg)	Pot18 - K (mg/kg)	Sul18 - S (mg/kg)
ExAl18 - salt-replaceable acidity (cmol+)/kg)	Amm18 - 1:2 Water extract N - NH ₄ (mg/l)	

4.4.6.3 Plant to Soil Health Feedbacks (*Soil health*2018 ~ *Soil health*2017 linear relationships)

The simulated test results on the first axis ($pseudo-F=0.2$) and on all axes ($pseudo-F=1.8$) were statistically significant, $P=0.036$ and $P=0.001$, respectively, indicating that the interpretation for the RDA outputs of 18 selected soil properties in Figure 4.12 differs for the RDA on all measured soil variables in Figure S4.7. The high soil microbial respiration in season 2018 can be explained by relatively high levels of Cu, K, P Bray 2 and P legacies from season 2017. The yield in 2018 was highly associated with Zn, Ca, Mg legacies of season 2017 (Figure 4.12). Active carbon in both seasons was projected very near to the coordinate origin indicating no correlation (Figure 4.12).

Most of the other soil health content in between season 2017 and season 2018 were significantly correlated (Figure 4.12, Figure S4.7). Figure 4.12 and Figure S4.7 summarizes the redundancy analysis of plant-soil feedbacks at a field scale comparing the flow-on impacts and feedbacks between soil health legacies of the previous season (season 2017) and the next (season 2018).

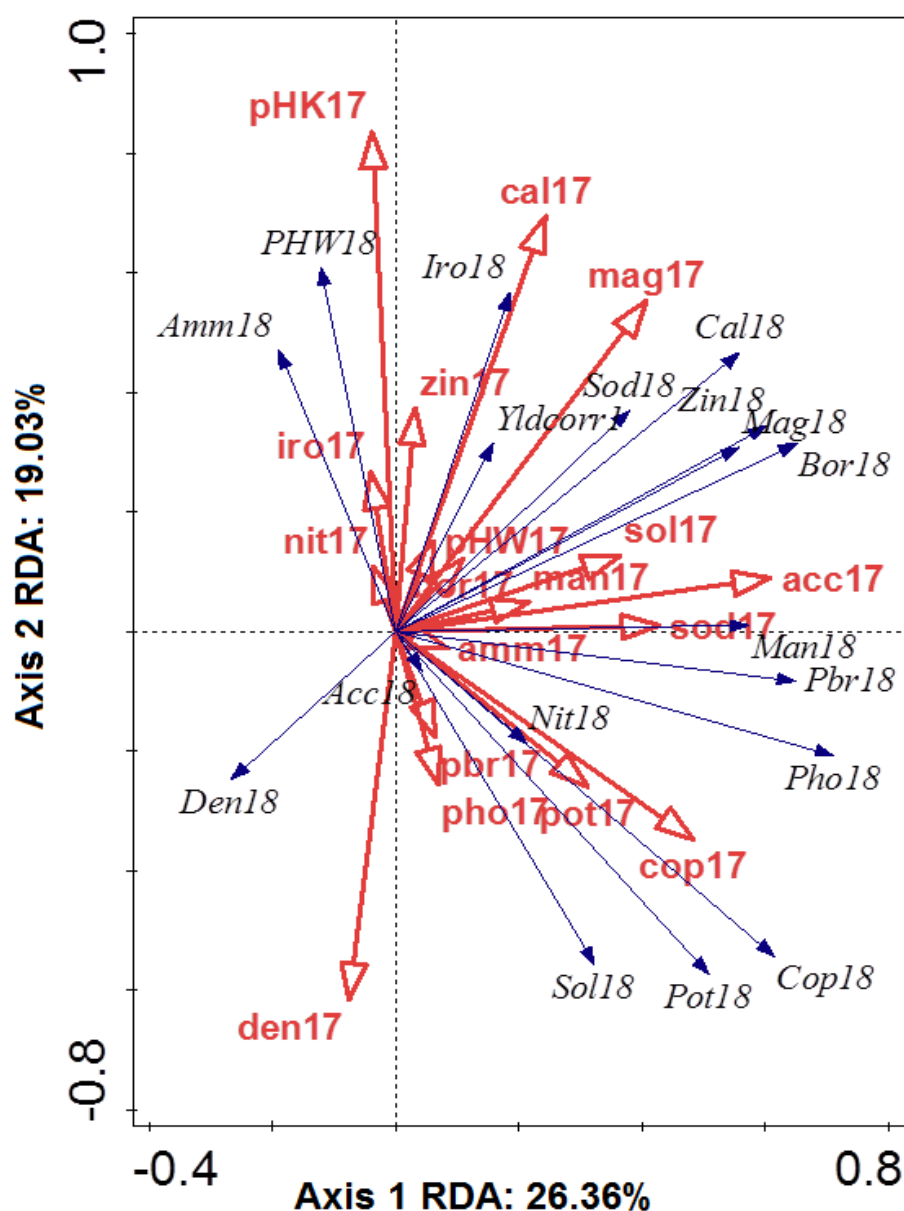


Figure 4.12 Soil to soil health feedback properties composition for seasons 2017 and 2018 using Redundancy Analysis (RDA) of selected 18 soil health variables. The last two digits (17 or 18) in a soil property represent season 2017 (predictor variables) or season 2018 (response variables) legacies, respectively. The 18 soil health properties are represented as:

acc - Active carbon (mg/kg)	pHW - pH (H ₂ O)	man - Mn (mg/kg)
amm - 1:2 Water extract N - NH ₄ (mg/l)	den - Density (g/cm ³)	bor - B (mg/kg)
sol - Solvita CO ₂ respiration (ppm)	cop - Cu (mg/kg)	cal - Ca (mg/kg)
nit - 1:2 Water extract N - NO ₃ (mg/l)	pot - K (mg/kg)	iro - Fe (mg/kg)
pbr - P Bray 2 (mg/kg)	zin - Zn (mg/kg)	mag - Mg (mg/kg)
sod - Na (mg/kg)	pho - P (mg/kg)	pHK - pH (KCl)

4.4.7 Quantification of *Fusarium graminearum* DNA in Field Soils using qPCR

Serial dilutions of the *F. graminearum* gDNA extracted from soil had a minimum detection limit of 0.005 ng/μl (Figure 4.13) based on the minimum quantity of target DNA detected at the threshold value of the qPCR machine set automatically. The average C_q values of samples were 32 to 39.51 (Figure 4.13a), 32 to 37.07 (Figure 4.13b). C_q values ranging between 32 and 34 (Figure 4.13a), 39.2 and 39.6 (Figure 4.13b) i.e. above the average C_q values were taken as ‘pathogen DNA present’ but not quantifiable.

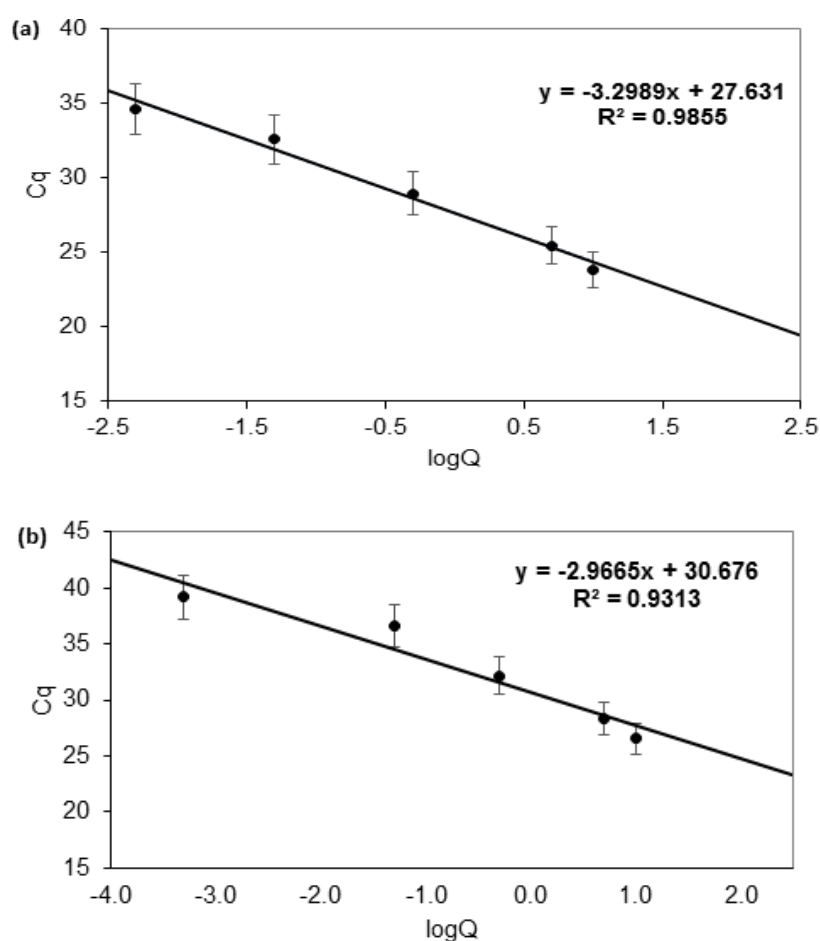


Figure 4.13 Standard curve for sensitivity and linearity of the real-time PCR quantification. (a) Standard curve of quantification cycle number (C_q) plotted versus the log of the initial template concentration ($\log Q$) in ng/μl. The first point is a two-fold dilution while the other four points are ten-fold serial dilutions that are linear over five logs. Data represent three replicates of each dilution. Efficiency (E) = $10^{-1/\text{slope}}$; (b): Amplification plot of same experiment showing the dilution series of DNA concentrations (10.0–0.005 ng) replicated three times. Error bars represent percentage error of the mean.

The qPCR data showed that there were no differences in legacies of *F. graminearum* pathogen DNA quantities after harvest among crop rotation plots in Season 2018. The post-harvest soil samples were not quantifiable except two plots of the soybean and sunflower rhizospheres had low copies of *F. graminearum* pathogen DNA at 0.051 ng/μl and 0.004 ng/μl, respectively.

4.4.9 Soil Strength Legacies

Average *in-situ* penetration resistance measurements per crop rotation at the end of the growing season are presented graphically in Figure 4.14.

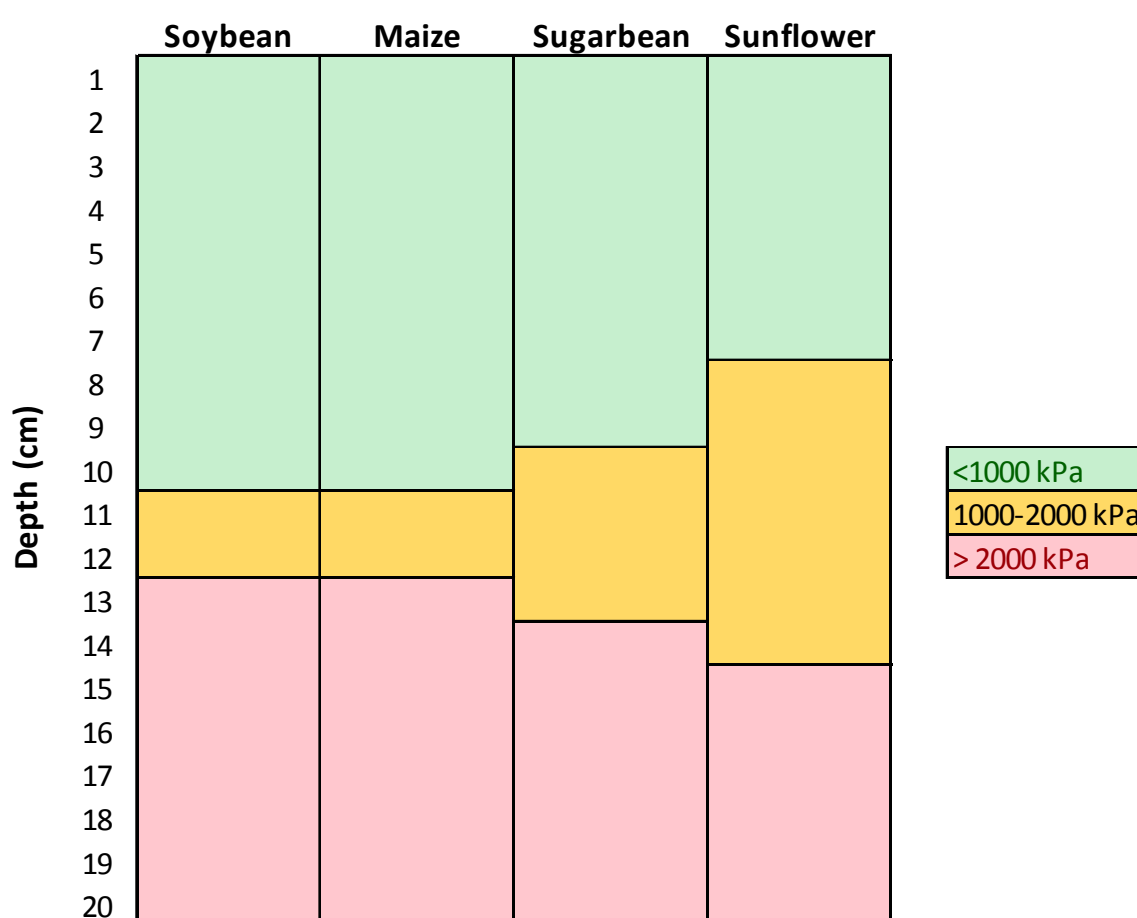


Figure 4.14 Crop effects on soil strength (kPa, penetrometer resistance) at the end of season 2018. The different colours represent different soil strength ranges, as indicated by the legend. Values represent averages across replications per crop treatment (maize-*Z. mays*, sugar bean-*P. vulgaris*, soybean-*G. max*, and sunflower-*H. annuus*).

The penetrometer data showed that soil resistance increased more rapidly with increasing depth for sunflower (*H. annuus*) followed by sugar beans (*P. vulgaris*).

4.5 Discussion

4.5.1 Rotation Crop Legacies on Plant and Soil Health

The rhizosphere supports a relatively high concentration of biologically active microbial biomass that is particularly beneficial to dry soils (Zhu et al., 2012) for pathogen suppression, microbial-genetic and -metabolic diversity required for optimum plant productivity (Wander et al., 1995). Soil biological composition and activity measurements are correlated with mineralization or crop yields (Palm et al., 2001; Six et al., 2002; Blanchart et al., 2004). For practical reasons, the high taxonomic resolution of the soil microbiome was not investigated in this study, instead the PLFA microbial composition and biomass including FDA enzyme activity and Solvita soil respiration results strongly suggest that the differences between the two seasons on a sandy soil in EFS was possibly not a result of the small climatic differences but was probably associated with; the crop type (Berendsen et al., 2012; Randall & Smith, 2019; Mushonga et al., 2020; Kumar et al., 2021), the absence of pesticides/herbicides/xenobiotics (Sena et al., 2002), clay content (Norris et al., 2023) and edaphic parameters (Dube et al., 2019) including chemical fertilizers (Sena et al., 2002). Compared with the soil chemical composition and climate characteristics, the soil microbial respiration and active C reflected the soil health legacies that were related to primary plant production (rhizodeposition effects), soil microbial (fungal and bacterial) activity and fragmented litter inputs returned to the soil in this particular context (Ganry et al., 2001; Palm et al., 2001; Six et al., 2002; Blanchart et al., 2004; Saiz et al., 2012). In other words, the differences in active C was probably a consequence of soil legacies from the aboveground and belowground plant materials (Cadet et al., 2002). Better stover or litter biomass inputs returned

to the soil at the end of season 2016/17 logically contributed more organic matter to the topsoil in season 2017/18.

The ratio of fungal to bacterial PLFA markers is an estimate of the relative abundance of fungi and bacteria in the microbial community (Bardgett, 2009). The relatively high total fungal biomass, fungal to bacterial ratio, Gram-positive to Gram-negative ratio and microbial enzyme activity observed in *H. annuus* rhizosphere soil could have been responsible for the high grain yield of this crop. *Helianthus annuus* probably secretes rhizodeposits that have species-specific effects on recruiting the microbial biomass patterns in a similar but different magnitude reported for cultivated soils versus undisturbed bulk soil in literature (Wall et al., 2012; Lal, 2020). The changes in microbial biomass in the soil is accompanied by an increase in microbial composition, diversity, and activity as well as the control of some soilborne fungal pathogens (Vančura & Kunc, 1989; Bossio et al., 1998).

The variability in microbial biomass in the rhizosphere of selected crop species can be attributed plant species and in part to other rhizosphere food web organisms such as fungivorous and bacterivorous nematodes and entomophilic insect-nematode relationships that were reported for this field experiment by Marias et al. (2019). These nematode data should be correlated with the observed soil and plant health properties in future studies. Pofu et al. (2019) suggests that certain *Z. mays* and *G. max* cultivars suppress root-knot nematode *Meloidogyne* species in potato crop rotation systems.

High bacterial biomass was recorded in *P. vulgaris* and *G. max* plots, possibly due to the cultivation of compatible rhizobia *Rhizobium leguminosarum* and *Bradyrhizobium japonicum/Rhizobium fredii* from previous historical or natural field inoculum (Bardgett, 2009), respectively. However, the high bacterial biomass was associated with poor soil legacies in both the NO_3^- and NH_4^- -N for legumes at post-harvest in season 2018. This could have been due to soil acidity effects on acid-sensitive rhizobia species present in seed or field soils

(Vančura & Kunc, 1989). In general, free-living mutualistic and symbiotic N-fixing bacteria build-up soil N under low N fertilizer rotation systems (Dixon & Tilston, 2010).

In a microbiome study of crop rhizospheres, Dube et al. (2019) found that cultivated soils under crop rotations in the eastern Free State were rich in bacterial genes associated with plant fitness and plant growth promotion, while bulk soil was superior in genes related to organic matter degradation. Nutrient-solubilizing bacteria are favoured under nutrient-poor conditions, while hormone-producing bacteria are favoured under nutrient-rich conditions (Costa et al., 2014).

The rhizospheres of *Zea mays* and *H. annuus* had a similar legacy of high Gram-positive to Gram-negative ratio values. Similarly, Buyer et al. (1999) did not detect differences in bacteria and Gram-positive groupings in *Z. mays*, *G. max* and *H. annuus*. *Phaseolus vulgaris* was superior to other crops in active carbon and soil microbial respiration while *G. max* was highest in Ca:Mg ratio, but poorest in the overall soil fertility composition legacies. The non-leguminous forb, *H. annuus* and leguminous forb (*P. vulgaris*) cultivated relatively similar soil fertility legacies that were high in Ca, P, K, S, Cu and Mg. *Helianthus annuus* tended to result in slightly higher levels of N (NO_3 and NH_4), P and K remaining in the root zone. The higher NPK content in the *H. annuus* rotation was probably due to a superior fertilizer application rate at the beginning of the season and the fact that the crop suffered severely from hail damage, resulting in less nutrient uptake over the season (Steyn et al., 2018).

There were significant differences in vertical variation between the 20cm and 40cm soil depths for soil chemical content in P Bray 2, P, K, Mg, Zn, B, electrical conductivity, and $\text{NH}_4\text{--N}$. Regarding soil nutrient status after harvest, only small differences between rotations were observed (Steyn et al., 2018). The top-and subsoils were acidic; however, pH was higher in the upper than lower horizons possibly due to historical topsoil residues of lime (Weil & Brady, 2017). As expected, the topsoil horizon was high in microbial activity expressed in Solvita CO_2 production whereas subsoil horizon is rich in minerals leached such as $\text{NO}_3\text{--N}$ from the soil

surface but low in microbial activity (Madigan et al., 2015). In short, microbiological composition and activities drive mineralization processes more than humus formation in dryland soils.

4.5.2 Seasonal Legacies of Soil Health Properties composition

The soil microbial respiration was 7 x higher while the active carbon was 1.4 x higher in season 2018 than in season 2017. Many biological processes such as soil microbial respiration, decomposition and N mineralization are greatly controlled by temperature and moisture (Robertson et al., 1999). The imbalance between the humification and mineralization processes under limited water resources and high temperatures can be addressed through optimum recycling rates of fresh crop residue inputs using the field inoculum of saprophytes or decomposers (Souidi et al., 2020). However, in the eastern Free State the winter temperatures in June-July are always cool and this leads to lowered soil microbial activity and natural decomposition of stover or crop residue.

Legumes lower the C:N ratio of crop residues and lead to mineralization of organic matter at the expense of humification thereby reducing levels of organic matter in soil (Lal, 2020). The best option would be to rotate legumes with grass species which promote high C:N ratios (Dixon & Tilston, 2010).

Most of the chemical concentrations were higher in season 2018 than the previous season likely due to replanting in some *G. max* and *P. vulgaris* plots damaged by hailstorm (Steyn et al., 2018). However, in season 2018 the soils had a lower pH, less NO₃-N and higher NH₄-N than in season 2017. Season 2018 was generally a year of low yield compared to season 2017. Haarhoff et al. (2020) stated that rainfall patterns in the eastern Free State are very variable between growing seasons and can affect yields significantly. Low yields in crops such as potatoes in the same region were reportedly due to lack of water availability at planting in season 2018 (Vester et al., 2019).

4.5.3 Plant-Soil Health Feedbacks

4.5.3.1 Plant2018=Soil2017 linear relationships

Rhizosphere fungal to bacterial biomass ratio in season 2018 were associated with season 2017 legacies superior in soil P, clay content and density, while rhizosphere Gram-positive to Gram-negative bacterial ratio correlated best with a history of high soil active carbon, K and Cu levels. Other studies have also reported that bacterial communities are closely related to soil chemical properties (Cao et al., 2021). The Gram-positive to Gram-negative bacterial ratio is an estimate of energy limitation in natural ecosystems because the Gram-negative bacteria associate with simple C compounds whereas Gram-positive bacteria associate with more complex C forms (Fannin et al., 2019). The rhizosphere microbial enzyme activity in season 2018 was positively associated with high soil microbial respiration, sand composition, Zn and pH legacies of season 2017.

4.5.3.2 Soil2018=Plant2018 linear relationships

In season 2018, the crop species cultivated a legacy of soil fungal to bacterial biomass ratio, soil microbial respiration and total fungal biomass closely associated with high legacies in soil NO₃-N, Cu, Al, K and %K. Penetration resistance measurements suggested that *H. annuus* and *P. vulgaris* soils were more compacted than the other two crop species (*Z. mays* and *G. max*). The deep-rooting *H. annuus* species create a dry soil profile despite breaking hard soil pans and opening the soil to facilitate the distribution of soil organic matter through the soil profile and allows the sequestration and protection of carbon in the deeper horizons of the soil from degradation (Souidi et al., 2020). In simple terms, the porosphere of *H. annuus* reduced the soil bulk density more than other crops.

4.5.3.3 Soil2018=Soil2017 linear relationships

The individual grain yields per crop type of season 2018 was closely related to soil legacies in Zn, Ca and Mg legacies from season 2017. In South Africa, FERTASA (2016) guidelines

recommend ideal levels of Zn (2-4 mg/kg), Ca (200-300 mg/kg) and Mg (80-300 mg/kg) for the cultivation of most grain crops. Penetrometer data showed that soil resistance increased more rapidly with increasing depth for *H. annuus*, followed by *P. vulgaris*. The higher resistance for *H. annuus* may be related to a drier soil profile after *H. annuus*, which is known to be a drought tolerant crop with ability to extract more water from the soil than most other summer crops (Steyn et al., 2018).

4.5.4 Crop Health and Soil Borne Pathogen Legacies

It appears that cultivating *Z. mays*, *P. vulgaris*, *G. max*, *H. annuus* and ploughing-down their litter residues was effective in reducing soil inoculum quantities of *F. graminearum*. This contradicts reports that this fungus is pathogenic to *G. max*, and several cereals (Pioli et al., 2004; Goswani & Kistler, 2004; Okello et al., 2019). Long-term mono-cropping enhances soil borne pathogen levels (Lal, 2020). The detection of *F. graminearum* in soil samples from the previous season (2016) may have been a result of residual pathogen inoculum which survived the potato uniformity trial and agronomic history of host crops such as wheat and maize (Marburger et al., 2015; Dube et al., 2019). The paradox of using molecular methods is that high DNA copies of the soil borne pathogens are sometimes associated with healthy rhizospheres and high yields. In other words, molecular qPCR detects microbes but does not give details on what they are doing metabolically in the soil (Dixon & Tilston, 2010). Disease activity during plant-pathogen interactions can, however, be confirmed with data from *in-vitro* antagonism assays and soil baiting assays using the same soil samples.

Although *H. annuus* consistently had comparably higher fungal levels, as well as the highest ratio of fungi to bacteria than other crop species it remains unclear whether these effects maintain soil productivity and controls weeds, pests and pathogens. Future crop rotations need to evaluate the positive and negative impact of increasing the fungal composition relative to that of bacteria (Dixon & Tilston, 2010) on soil and crop health.

4.6 Conclusion

The results have shown that differences in soil chemical, physical and biological responses due to different crop rotation systems exist and it is expected that more differences will become evident as the study continues. The superior microbial biomass of fungal and bacterial PLFA markers in *H. annuus* including highest soil enzyme activity strongly suggests that these biological properties were responsible for the observed high grain yields relative to other crops and potential resilience in soil and plant health. The findings suggest that *H. annuus* cultivates a high microbial biomass and activity in the root zone of a dryland soil. The legacy effects of the four crop species studied were a very effective buffer against the production of *F. graminearum* inoculum. Crop rotation with these crop species will be crucial for microbial succession in soils under rain-fed farming conditions. There is a need to characterize the beneficial, neutral, and harmful bacterial and fungal PLFA markers and microbial diversity so that ideal or optimum ecological balances are achieved to reduce costly integrated pest and fertilizer management programs under rain-fed conditions. The corroboration of beta-diversity data of PLFAs with NGS alpha-diversity data allows revelation of key rhizobiomes and their associated impact on soil-plant interactions important to crop productivity and health.

4.7 Acknowledgements

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Conflict of interest

Authors have no conflict of interest to declare.

CHAPTER 5

Plant-Soil Feedback Responses of Four Dryland Crop Species under Greenhouse Conditions

5.1 Abstract

Background: Plant-soil feedbacks (PSFs) give a mechanistic understanding on how soil properties established by previous plant species go on to influence the performance of the same or different species in monoculture, intercropping or crop rotation systems.

Aims: The study sought to determine how the performance and growth of dryland crops, could be influenced by plant-soil feedback legacies under controlled conditions. The inclusion of legumes can improve soil fertility and crop nutrition to consequent crops, which in turn can homogenize nutrient imbalances and minimize restricting factors such as toxic amounts of nutrients on succeeding plant mineral nutrition and growth. We hypothesized that different dryland crops such as *Zea mays* L., *Helianthus annuus* L., *Phaseolus vulgaris* L. and *Glycine max* L. (Merr.) under controlled conditions will have soil legacies that are related to the crop type.

Methods: We used a two-phase experiment to test plant performance in soils previously cultivated with the same or different plant species under greenhouse conditions.

Results: The positive plant growth for all species in their own soil microbiota suggests that mutualists had a greater impact on plant performance than pathogens. The consistent positive soil-feedback results of *P. vulgaris* were strongly associated with their own beneficial soil microbiota, meaning that the conditioning phase legacy of mutualists and decomposers were more significant than pathogens under monoculture. Despite successful nodulation in sterilized

and inoculated soils, *G. max* unexpectedly showed neutral and insignificant positive plant feedbacks, respectively. *Helianthus annuus* was superior to other crop species in creating active carbon stocks and an enzymatically active soil for the next crop. Microbial biomass results suggest that raising fungal relative to bacterial biomass can be achieved by increasing the frequency of *H. annuus* in rotation sequences. However, more studies are necessary to evaluate whether these elevated ratios promote or depress plant performance under field conditions.

Conclusion: This study showed that relative to other dryland crops, *H. annuus* seems to have the potential of increasing fungal to bacterial ratios, raising legacies in active carbon stocks and soil microbial activity that may be crucial to successional planting in dryland systems.

Key words: Crop rotation; monoculture; plant performance; soil conditioning; soil legacies.

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5.2 Introduction

Plant-soil feedback (PSF) represents plant performance linked to soil legacies established earlier-on by the same or other plant species (Kulmatiski & Kardol, 2008). A positive feedback occurs when subsequent plant growth increases, whereas in a negative feedback, growth is retarded. The direction (sign) and strength of PSFs are determined by the net effects of the associations between mutualists, pathogens and plants (Mariotte et al., 2018). Soil conditions cultivated by ‘self’ or ‘own’ plant species are known as conspecific or ‘home’ soils, while those cultivated by ‘other’ or ‘foreign’ plant species are referred to as heterospecific or ‘away’ soils (Forero et al., 2019). In this study, we hypothesized that the phytometer species represent plant growth in the feedback/test phase as a result of changes and balance of soil organisms such as pathogens and mutualists, which cause a decrease and an increase in successive plant growth,

respectively (Brinkman et al., 2010). The objective of this study was to determine how plant biomass responds to temporal changes in selected soil properties (biological, chemical and physical) induced in succession planting over time using a mechanistic PSF experimental approach, as proposed by Dias et al. (2014). We used medium-maturity plant species to minimize confounding differences common to fast- versus slow-growing plants in feedback experiments (De Long et al., 2019). We expected that a cereal grass (*Zea mays* L., nitrogen-fixing leguminous forbs (*Phaseolus vulgaris* L. and *Glycine max* (Merr.)) and a non-leguminous forb (*Helianthus annuus* L.) will have soil legacies that are linked to the plant type and functional groups. The legumes build nitrogen legacies in the soil that drive positive PSF, whereas *H. annuus* and *Z. mays* are net consumers of nitrogen that drive negative PSF (Cortois et al., 2016; Cesarano et al., 2017).

The nitrogen requirement of *Z. mays* is approximately twice that of *H. annuus*, *P. vulgaris* and *G. max* in dryland agroecosystems (FERTASA, 2016). Moreover, the root architecture and low cation exchange capacity of monocots such as *Z. mays* promote a soil legacy poor in monovalent cations such as sodium and ammonium ions, whereas dicot legumes (*P. vulgaris* and *G. max*) impoverish the soil of divalent cations such as calcium and magnesium ions (Fageria, 2013). The less dense and extensive tap root system of *H. annuus* allows it to efficiently draw out solutes and water from the soil. Nevertheless, these species are strategic crops under dryland production systems and are cultivated in rotation systems to maximize profitability, improve crop yields, and break pest- and disease cycles under rain-fed conditions (Nel, 2005; Montgomery et al., 2017).

Bardgett and McAlister (1999) demonstrated that unfertilized (nutrient-poor) grassland soils supported a higher fungal to bacterial biomass ratio than disturbed and fertile (nutrient-rich) grassland soils. The soil environment under the influence of plant roots, that is the rhizosphere, is a microenvironment which supports more soil microbial growth and maintenance than loose

field soil or bulk soil (Bardgett, 2009). The influence of root activity on soil microbial communities and nutrient legacies varies in terms of the quality and quantity of the exudates they produce, which subsequently promotes nutrient availability, sustains mutualists and reduces stress (Ryan et al., 2009; Canarini et al., 2019). Other abiotic changes such as soil pH and the accumulation of allelochemicals have an impact on microbial composition and biomass (Cesarano et al., 2017). Moreover, drought conditions also disrupt plant-soil interactions and Fry et al. (2018) found that drought led to neutral PSFs on two grassland forbs.

The knowledge of feedback mechanisms is crucial to successional planting in dryland cropping systems such as crop rotations or monoculture because of the differences in plant species and genotypes. *Zea mays* thrives best in grassland biomes of South Africa, but *H. annuus* performs better than *Z. mays* on alkaline sandy soils (Smith, 2016). Understanding plant-soil feedbacks provides insight into the flow-on impact of historical soil properties on the performance of the succeeding plant species (Kos et al., 2015). In this study, soil legacies for microbial biomass were estimated using phospholipid fatty acid analysis (PLFA), while for active carbon and soil enzyme activity, the permanganate-oxidizable active carbon method and the fluorescein diacetate (FDA) method were used, respectively. Phospholipid fatty acid analysis (PLFAs) is a biochemical technique that gives fungal to bacterial biomass ratio and active biomass of microbes (Frostegard & Bååth, 1996; Ladygina & Hedlund, 2010; Tavi et al., 2013). The patterns and swinging ratios of these lipid biomarkers in soils have been linked to nutrient cycling, organic matter decay and carbon sequestration (Willers et al., 2015).

Active carbon is readily available as carbon and energy source for soil microbial community food webs. It is very sensitive to management effects and more closely linked to biologically mediated soil properties such as respiration, microbial biomass and aggregation than measurements of soil organic carbon (Weil et al., 2003; Preusser et al., 2019). FDA analysis quantifies the microbial activity based on enzyme activity linked to microorganisms present in

the soil (Schumacher et al., 2015). Soil protease activity increases under limited nitrogen (Bardgett & Wardle, 2012) and microbes become strong sinks of nitrogen (Bardgett et al., 2003). Brinkman et al. (2010) argues that greenhouse plant-soil feedbacks differ in scale and soil biota composition from natural field experiments due to the inherent differences in geology, biota and climatic conditions including the spectrum and duration of root-influence on the soil properties.

Anaerobic soil conditions caused by high soil moisture or flooding under greenhouse conditions will likely decrease the abundance of the decomposer fungal biomass (Bossio & Scow, 1998). Nevertheless, we proposed a greenhouse experiment using soil analysis, soil sterilization and subsequent addition of inoculum (soil biota originating from the root zone of specific plant species) to reveal the mechanisms responsible for subsequent plant performance (De Long et al., 2019). Confounding biotic and abiotic effects on plant performance in PSFs for the greenhouse experiments were minimized by using independently replicated natural-field soils with a long history of *Z. mays* monoculture, pots of similar size, and procedural soil controls (Brinkman et al., 2010; Bardgett & Wardle, 2012).

5.3 Materials and Methods

5.3.1 Experimental Setup

The greenhouse experiments were conducted at the University of Pretoria (Wheatboard Greenhouse and Labs, S 25° 45' 21" E 28° 13' 51", South Africa) from 7 March 2018 to 10 December 2018. The topsoil was collected from a monoculture field planted uniformly with *Z. mays* (also called a crop uniformity trial in biometry) at 0 – 20 cm depth using a soil auger in-rows avoiding field borders, unusually eroded areas, wet or dry areas and fertilizer bands. The field layout is a randomized complete block design (RCBD) divided into eight replications separated by 10m buffer zones strips in the North-South direction (Eastern Free State, Petrus Steyn in South Africa, GPS: S27° 53' 45.5" E28° 12' 59.8", Elevation 1705 m). Before using

this field soil for the plant-soil feedback experiments, soil analysis showed that most of the selected soil properties of this arenosols3 greyish sandy soil (FAO, 2005) were ideal to support plant growth (Table 5.1 as well as in Chapter 3, Figure S3.2).

A total of ≈ 80 kg of this soil (properties in Table 5.1) was sampled from four treatment plots within each replication following the W-sampling strategy, air-dried in a hothouse for a week at 40 °C, mixed and sieved using a 5 mm mesh to homogenize and adjust the soil texture or particle size (van de Voorde et al., 2011; Soman et al., 2017) by removing coarse fragments prior to starting the greenhouse feedback experiments (Figure 5.1). This reduced confounding spatial effects linked to ecological gradients in soil properties, although other studies have shown that soil structure has no effect in PSF experiments (Bergmann et al., 2016). About 50 kg of soil from each replication was pasteurized in an electrode steam boiler (Model M40, 60 Amps, 40 KW full load at 380 V, 60 kg per hour evaporation from and at 100 °C, 550 kPa, Marshal-Fowler, Randfontein, South Africa) to destroy pathogens, while the remaining 30 kg of soil was left unsterilized (natural field soil).

Table 5.1 Biological and physicochemical properties of top-soil collected at 0 – 20 cm depth from a *Zea mays* uniformity trial. The field is located in a grassland biome of the Eastern Free State province of South Africa. N = 32 composite samples. SE = standard error.

Soil factor	Mean $\pm$ SE
Clay (%)	8.94 $\pm$ 0.18
Silt (%)	9.80 $\pm$ 0.24
Sand (%)	81.26 $\pm$ 0.26
Density (g/cm ³)	1.27 $\pm$ 0.01
pH(H ₂ O)	6.23 $\pm$ 0.10
pH(KCl)	4.86 $\pm$ 0.07
Phosphate Mehlich III (mg/kg)	48.74 $\pm$ 1.46
Phosphate Bray II (mg/kg)	55.41 $\pm$ 1.64
Potassium (mg/kg)	87.07 $\pm$ 3.25
Cation exchange capacity (cmol(+)/kg)	2.27 $\pm$ 0.05
Ammonium nitrogen (mg/l)	0.41 $\pm$ 0.003
Nitrate nitrogen (mg/l)	1.54 $\pm$ 0.05
Active carbon (mg/kg)	170.93 $\pm$ 6.76
Solventa CO ₂ respiration (ppm)	4.78 $\pm$ 0.23

The soil was transferred into pots (size 15 cm diameter and 16 cm depth) and stored until planting. Climate control in the greenhouse environment was adjusted to 25 °C maximum air temperature, 7 °C minimum air temperature, 93% maximum relative humidity, and 27% minimum relative humidity. This was typical of the Eastern Free State (EFS)-Petrus Steyn weather according to the Sonop weather station daily data from 23/10/2014 to 02/05/2017. Weather data including air temperature, relative humidity, and solar radiation was collected during the trial period using an Onset HOBO® data logger U12 (MacArthur Blvd, Bourne MA, USA).

The plant-soil feedback experiment used the two-phase approach (Brinkmann et al., 2010); 10-week conditioning phase and 10-week feedback phase (Figure 5.1).

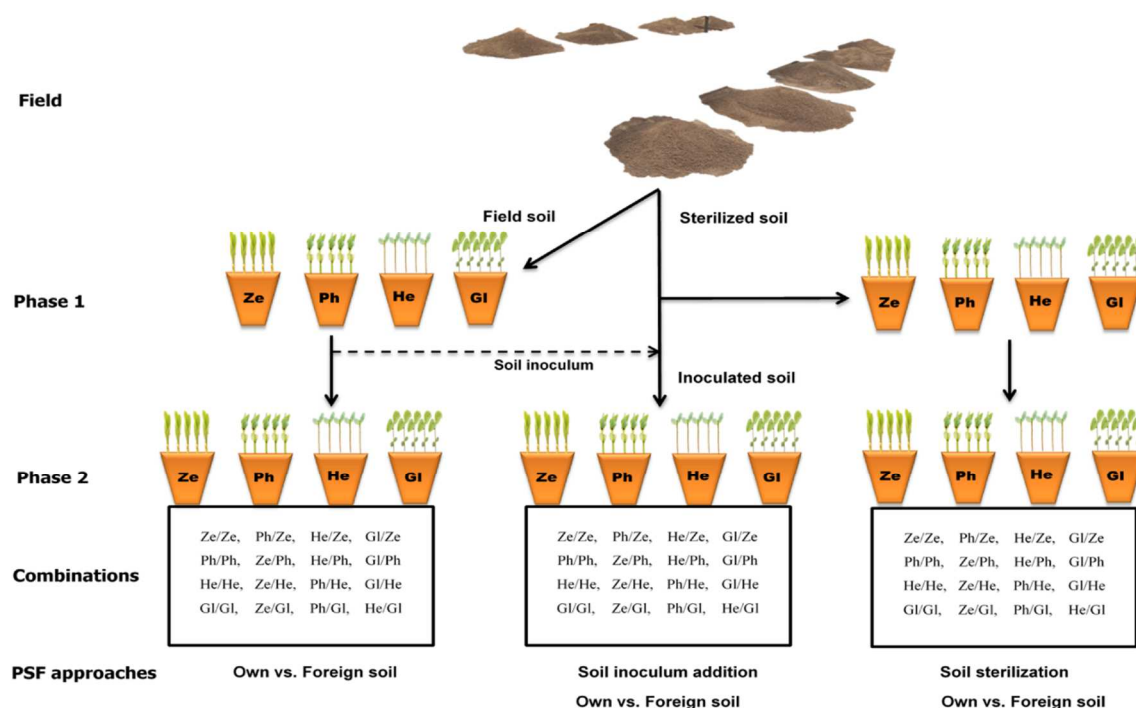


Figure 5.1 Conceptual diagram (modified from Dias et al., 2014) of the PSF experimental design. In the greenhouse, Phase 1 (conditioning phase) involved cultivating *Z. mays* (Ze), *P. vulgaris* (Ph), *H. annuus* (He) and *G. max* (Gl) in live field soil and sterilized soil. In Phase 2 (feedback phase), the phase 1-conditioned soils were planted with the same four plant species to give all possible soil combinations of conspecific and heterospecific PSFs. Dashed lines represent soil-water extract of the inoculum. Drawing courtesy of Mr. Fungai Mushonga.

The ‘unconditioned’ control soil representing the natural field/bare soil was potted and stored at the same greenhouse conditions as all pots. These control pots with nothing planted in them throughout the experiment were used to obtain soil inoculum not fed with rhizodeposits. In this way, the impact of the four plant species on soil legacies, which in turn influenced growth of phytometer species, could be differentiated for plant-soil feedbacks.

Conditioning Phase (Phase-1)

An early medium-maturity cereal grass (*Z. mays*), two medium-maturity and indeterminate nitrogen-fixing leguminous forbs (*P. vulgaris* and *G. max*) and a medium late-maturity forb (*H. annuus*) were used to study the plant-soil feedbacks due to their good performance in dryland production systems of South Africa. After sowing, plants were watered with reverse osmosis purified water (Pacific AFT 20, 24V, 80W, Thermo Scientific, Niederelbert, Germany) to soil pot capacity on a daily basis in the first week until emergence and thereafter, according to plant need. The inherent challenges of attaining and controlling uniform soil water content in pots (Kramer & Boyer, 1995) was complicated by cultivating the four different plant species (which differ in rates of transpiration) in the same environment. Nevertheless, watering was done according to plant needs at the onset of leaf and plant drooping, and drying of the upper soil in both control and planted pots. The aim was to water plants in a way that minimized drought effects on PSFs (Fry et al., 2018).

The conditioning phase lasted for 10 weeks during which time the four plant species were cultivated in unsterilized (natural) field soil and sterilized field soil with a target plant population of five plants of the same species per pot. In 10 weeks, this planting regime was ideal for conditioning the soil and measuring of plant production metrics for the three functional groups (van de Voorde et al., 2011). Cleaned pots containing 1.3 L soil were set up in a complete randomized block design in a greenhouse (light: 10 – 12 hr., dark: 12 – 14 hr.), and watered before planting with 150 ml purified water. Seeding was done simultaneously at

the beginning of each conditioning phase. *Glycine max* seeds were placed with the granular soil-inoculant (*Bradyrhizobium japonicum* WB74 strain) in the drilled holes at approximately 5 cm soil depth. The *G. max* seed was inoculated with 0.4 grams of 1×10^9 colony forming units soil-inoculant per gram of seeds at the time of planting using the strain and procedure described and validated for successful nodulation by van der Hoven (2018). The experimental layout (Figure 1) for the first phase had two levels of soil treatments (natural field soil and sterile soil) to grow four plant species (*Z. mays*, *H. annuus*, *P. vulgaris* and *G. max*) replicated 32 times, adding up to 256 pots.

After 10 weeks, plants were harvested and measured for shoot, root and total dry biomass per plant. Soil samples were collected and analyzed for legacies in active carbon (mg/kg), enzymatic activity in FDA hydrolyzed ($\mu\text{g}/\text{mg}$ soil), and fungal-to-bacterial biomass ratio.

Feedback Phase (Phase-2)

The feedback phase was conducted using two experimental approaches; inoculum addition and sterilization (Figure 1). All the replicates of soil conditioned in phase-1 were air-dried in pots and stored for 1 month at 12 – 26 °C *in situ*. This was followed by preparing inoculum from phase-1 conditioned soils for set-up of the inoculum addition treatment experiment using a modification of the method described by Bergmann et al. (2016). The soil inoculum represented a microbial filtrate or soil-water extract/fraction of soil biota originating from the root zone of plant species at the end of the conditioning phase. Soil-water extract of the inoculum was prepared by mixing composite 2.5 L natural field soil (pooled from 32 replicates) conditioned by *Z. mays*, for example, with 5.0 L deionized water, stirring vigorously for 2 minutes, allowing to settle for 1.5 minutes and decanting through a 250 μm sieve. The resulting microbial filtrate was stored at 4 °C and used within 2 days to inoculate sterilized field soil for the inoculated soil treatment experiment. These soil-water extraction steps were repeated for composite soil samples conditioned by the other three species at week 2 and 4 after seedling establishment.

Microbial filtrate of 250 ml donor inoculum was added after seedling establishment at week 2 and 4. *Glycine max* seeds were planted with granular soil-inoculant as described in the conditioning phase.

All plant species were allowed to grow for 10 weeks with a target plant population of five plants of the same species per pot. This experiment had 16 plant feedback combinations $\times$ 8 replications ($n = 128$ pots) representing the inoculum-added feedback experiment. Following the inoculum addition experiment, the remaining phase-1 conditioned field soil was replanted to make up 16 feedback combinations representing the non-sterilized treatment of the sterilization experiment. Alternatively, the control phase-1 conditioned sterilized field soil was seeded to set up 16 feedback combinations that were replicated 8 times ($n = 128$ pots) in the sterilization experiment. Overall, the second phase experimental design had three levels of soil treatments (natural field soil, sterile soil and inoculated soil) each with 16 plant feedback combinations replicated 8 times adding up to 384 pots.

After the 10 weeks of phase-2, the plants were harvested and measured for shoot, root and total dry biomass per plant, while conspecific and heterospecific soil samples were analyzed for legacies in active carbon (mg/kg), enzyme activity in FDA hydrolyzed ($\mu\text{g}/\text{mg}$ soil), ammonium nitrogen, nitrate nitrogen, nitrate-to-ammonium nitrogen ratio, total nitrogen and fungal-to-bacterial biomass ratio. *Helianthus annuus* created a unique legacy of soil properties in the preliminary results at the end of the condition phase. In light of this, further soil fungal and bacterial biomass analysis was done exclusively for selected soils conditioned by *H. annuus* in phase-1.

5.3.2 Plant Biomass Measurements

Harvesting was done after flowering (for *Z. mays* and *H. annuus*) and at physiological maturity (for *G. max* and *P. vulgaris*) after 10 weeks of plant growth. Biomass metrics of total biomass, above ground (shoot) and below ground (root) biomass were measured and recorded as dry

biomass in g per plant. Drying was done at 62 °C in a forced-air oven for 48 hours until constant weight was achieved.

5.3.3 Soil Analyses

The soil active carbon, fluorescein diacetate (FDA), phospholipid fatty acid (PLFA) methods were performed as described in Mushonga et al. (2020) specifically in Chapter 5 section 5.3.3. Soil respiration was estimated using the standard protocol of the Solvita CO₂-burst test following manufacturer's instructions.

5.3.4 Statistics

Data analyses were carried out using R 3.5.2 (R Core Team, 2018), SPSS version 23 (IBM Corp. Released 2015) and Canoco 5 version 5.11 (ter Braak & Šmilauer, 2018). Time series of climate characteristics were plotted using the 'ggplot2' and 'tseries' packages in R 3.5.2. Boxplots were drawn in SPSS version 23 for all recorded data to detect outliers in the dataset.

Measure of Correlation between Phase-1 and Phase-2 Biomass

Correlation coefficients were calculated to determine the relationships between phase-1 and phase-2 biomass using SPSS version 23. When the data were not normally distributed, the Spearman non-parametric correlation coefficient was used.

Test for Total Biomass and Biomass Ratio differences between Four Plant Species using the Kruskal-Wallis and Independent t-test Analyses, respectively

The raw biomass data were sub-set by soil type and analyzed using a non-parametric Kruskal-Wallis test to compare biomass per plant across each soil treatment. The root-to-shoot and root mass ratios were calculated and used in determining whether there were significant differences between sterilized and field soil within plant groups in the conditioning phase, using the independent t-test analysis in SPSS version 23.

Three-Way (Three Factor) Analysis of Variance to test the Main Effects on Biomass and Feedback Values

A general linear model with the main effects phytometer species, conditioning species and soil treatment was analyzed to determine the feedback of growing phytometers in natural field soil, sterilized soil and inoculum added soil, using a three-way analysis of variance (ANOVA) in SPSS version 23. Three-way ANOVA comparisons were computed across phytometers to compare biomass per plant across phytometers after the normality and homogeneity of variance assumptions were met for the original biomass data sub-set by phytometer plant species.

The feedback calculations were based on biomass variable and feedback variables to i^{th} individual observations. The biomass variable was calculated as follows: $(O_i - F_i)$ pairwise as described by Brinkman et al. (2010). The feedback variables were calculated as follows; (a) impact of soil sterilization = $[(A_i - B_i)/\text{maximum biomass value of either } (A_i \text{ or } B_i)]$, where A_i is biomass of the phytometer species grown in non-sterilized soil and B_i is the biomass of the phytometer species grown in sterilized soil and (b) impact of soil inoculum amendment = $[(A_i - C_i)/\text{maximum biomass value of either } (A_i \text{ or } C_i)]$, where A_i is biomass of the phytometer species grown in soil biota without inoculum and C_i is the biomass of the phytometer species grown in soil biota with inoculum as described by Brinkman et al. (2010).

The strength of PSFs were categorized into non-significant, significant, highly significant and very highly significant, where $p > 0.05$, $p \leq 0.05$, $p < 0.01$ and $p < 0.001$ (IBM Corp. Released, 2015; R Core Team, 2018), respectively.

Paired t-test for differences between 'Own' and 'Foreign' Biomass Data within Plant Species

Paired differences in biomass per phytometer species group were tested for normality and equal variance assumptions after ignoring significant outliers in the dataset. The biomass data did not violate these assumptions in the paired t-test calculations to determine the strength of plant-soil

feedback responses in; (a) 'own' relative to 'foreign' soil biota and nutrients, (b) 'own' relative to 'foreign' soil nutrients, and (c) 'own' relative to 'foreign' soil biota.

Kruskal-Wallis test for differences in Soil Nitrogen between Nine Soil Origins

Box plots were used to visualize the descriptive statistics of soil nitrogen, FDA activity and active carbon legacies data using the 'Car' package in R 3.5.2 software. Where soil nitrogen data violated the normality assumption, the Kruskal-Wallis test was performed to test for differences in soil nitrogen legacies among nine categories of conspecific/heterospecific soil origin. Where significant differences were detected, pairwise contrasts were calculated to reveal the treatments responsible for the differences.

One-Way ANOVA test for differences in Soil Active Carbon and FDA Hydrolyzed between Nine Soil Origins

The ANOVA test for normality was met for both soil active carbon (mg/kg) and FDA hydrolyzed ($\mu\text{g}/\text{mg}$ soil) using the Shapiro-Wilk test. The significant ANOVA test results were analyzed using multiple comparisons based on the Tukey's honestly significant difference (Tukey HSD) test across soil conditioning and feedback phase's legacies in SPSS version 23.

Ordination Technique to rank PLFA Data to reveal relationships among selected Soil Legacies

Multivariate analysis of conspecific/heterospecific soil legacies data for PLFA biomass was visualized using non-metric multidimensional scaling (NMDS) ordination diagram of the Bray-Curtis dissimilarities, with two computed axes of soil fungal and bacterial biomass across treatments (Canoco 5 version 5.11; ter Braak & Šmilauer, 2018).

5.4 Results

5.4.1 Climate Variables between March and September 2018

The mean daily temperature ranges per month for phase-1 was three times that of phase 2 (Figure S5.1a). However, the daily mean temperatures between seeding and harvesting

decreased steadily in phase-1, but increased relatively from the seeding until harvest in phase-2. In Figure S5.1b, the calculated daily temperature ranges (10.5 to 14.0 °C) were strongly influenced by the minima values. In phase-2 (Figure S5.2a-d), relative humidity ranged from 20 to 70 % with a mean of 44 %, while the average solar intensity at 10:00 was 7900 Lux and the dew point mean was 14 °C.

5.4.2 Plant Performance

The Spearman non-parametric correlation results indicate that biomass of the first phase and the second phase were not correlated in either sterilized soils ($r_s = 0.095$, $n = 128$, $p > 0.05$) or natural field soils ($r_s = 0.094$, $n = 128$, $p > 0.05$), implying that it is not necessary to include the impact of the first phase nutrient depletion on plant performance in the second (test) phase. In the conditioning phase, *G. max* total mass was superior to all three other plant species in both field and sterilized soil (Figure S5.3a-c). Differences in root to shoot ratios within groups of plant species were not significant, except for *Z. mays* (Table S5.1, Figure S5.3d).

5.4.3 Plant Performance in response to Soil Inoculum Addition and Sterilization

The biomass differences among the individual plants of *Z. mays* growing in field soil were found to be significant, while *P. vulgaris*, *H. annuus* and *G. max* were not. The biomass of *Z. mays*, *H. annuus*, *P. vulgaris*, and *G. max*, growing in inoculated soil (Figure 5.2d) did not differ among each phytometer species. In terms of plant production in all soil treatments, *G. max* produced the highest total dry matter in g per plant as shown in Figure 5.2d. *Zea mays* and *P. vulgaris* grew more root than shoot biomass, whereas *H. annuus* and *G. max* produced more aboveground than belowground dry matter at the end of the feedback phase.

5.4.4 Comparisons of Plant Performance across Phytometers

A three-way ANOVA was used to compare the performance of phytometers in the three soil types; natural field soil, sterile soil and inoculated soil (Table S5.2). The biomass differed between the feedbacks in one or more experimental phytometer groups except *P. vulgaris*

(ANOVA test Table S5.2). Using a three-way ANOVA and post-hoc pairwise tests, the significant differences in *Z. mays* feedbacks were found to emanate from mean differences in monoculture and *H. annuus* conditioned soils in phase-1. Significant differences in *H. annuus* feedback came from mean differences in monoculture. In *G. max* performance, significant differences in feedback can be explained by mean differences of *Z. mays* conditioned soil and monoculture soil.

5.4.5 Analysis of Feedback Experiments in Monoculture (Own) versus Crop Rotation (Foreign) Soil

The same plant species were cultivated under similar conditions using different feedback approaches, that is, in sterilized field soil, non-sterilized soil and soil with inoculum. *Phaseolus vulgaris* consistently showed positive feedbacks in response to both sterilization treatment (Figure 5.2b and c) and inoculum addition treatment (Figure 5.2c). The *paired t-test* analysis (deviation from 0) of the findings revealed that plant-soil feedbacks varied in direction and strength (Figure 5.2). *Glycine max* showed a weak negative PSF ($p > 0.05$) in total biomass (g per plant) (Figure 5.2a) while *Z. mays* ($p < 0.001$) and *H. annuus* ($p < 0.01$) displayed a strong negative PSF. A strong positive PSF was only observed in *P. vulgaris* ($p < 0.001$) (Figure 5.2a). The positive PSF performance of *P. vulgaris* in own relative to foreign soil nutrients was very weak, while strong and negative for both *Z. mays* and *H. annuus*. The PSF magnitude of *G. max* was neutral (Figure 5.2b).

All plant species grew better in their own relative to foreign soil biota (Figure 5.2c), although with weak positive PSF values for *Z. mays*, *G. max* and *H. annuus*. On the other hand, a strong positive PSF value was observed for *P. vulgaris* (Figure 5.2c).

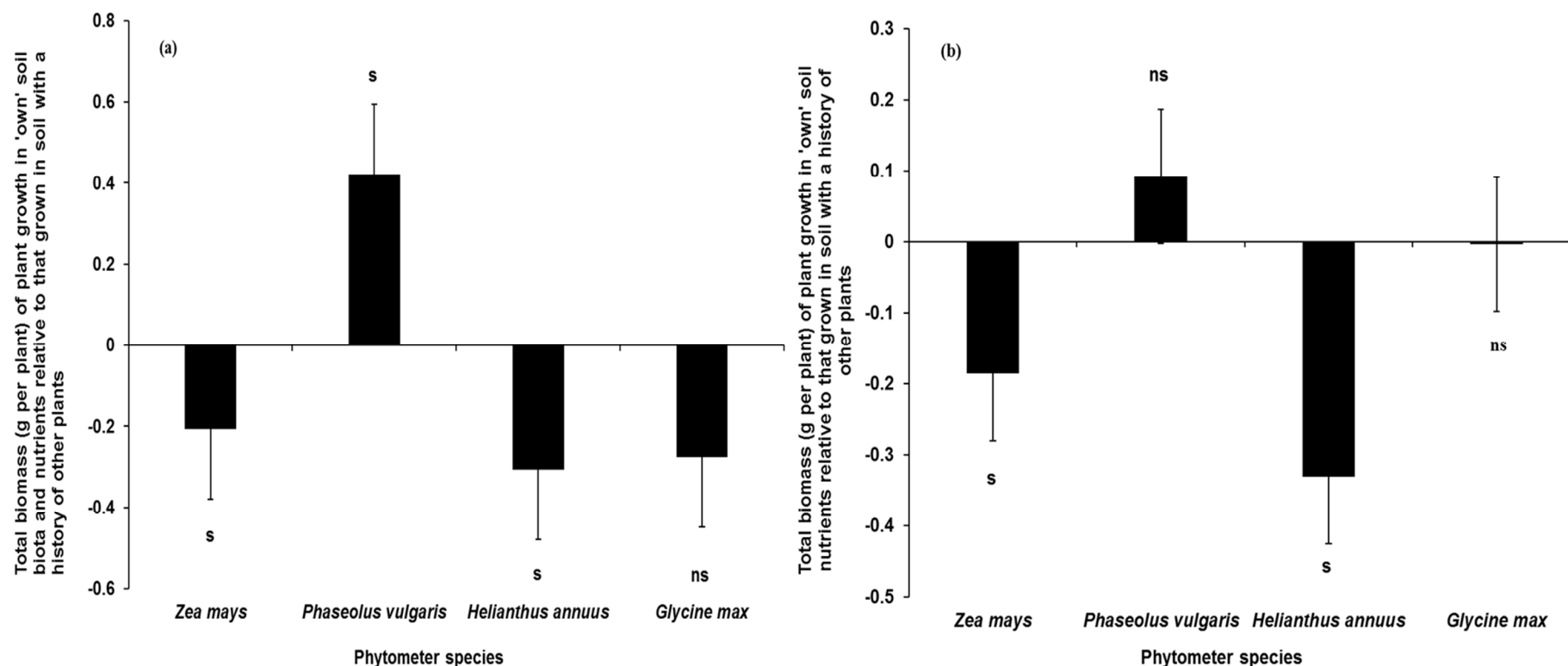


Figure 5.2 The strength of plant-soil feedback (total biomass) responses of four crop species grown in: (a) 'own' relative to 'foreign' soil biota and nutrients using natural field soil and (b) 'own' relative to 'foreign' soil nutrients using sterilized field soil. All bars represent the standard error of the mean (n = 24 for (a) to (b)).

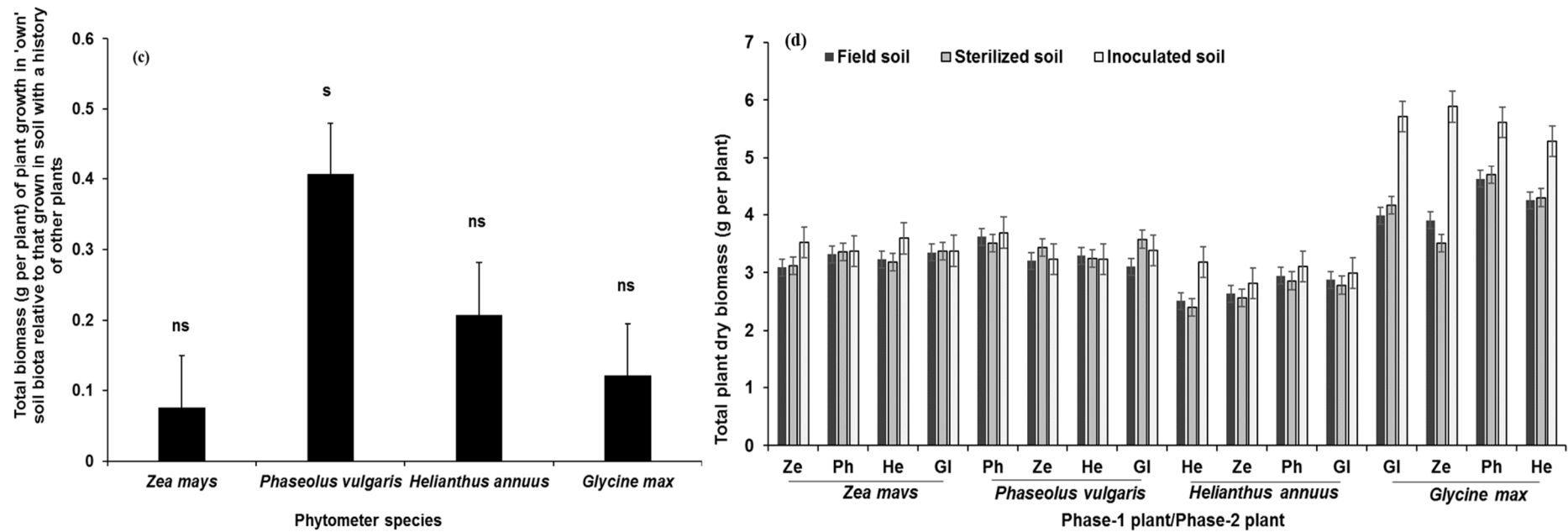


Figure 5.2 The strength of plant-soil feedback (total biomass) responses of four crop species grown in: (c) ‘own’ relative to ‘foreign’ soil biota using sterilized soil with inoculum added. ‘s’ represents significant differences ($p \leq 0.05$) in biomass or a strong PSF value while ‘ns’ represents non-significant differences ($p > 0.05$) in biomass or a weak PSF value based on the *paired t-test* analysis (deviation from zero). (d) The effect of soil treatments on total mass for four phytometer species grown in soils cultivated by their ‘own’ or ‘foreign’ species. *Glycine max* had the highest biomass at the end of the feedback phase. All bars represent the standard error of the mean ($n = 24$ for (c), $n = 8$ for (d)).

Soil treatment (sterilization and inoculum amendment) effects on biomass were significantly different according to three-way analysis of variance (ANOVA) (Table 5.2).

Table 5.2 Three-way ANOVA table of general linear models for effects on total dry biomass and feedback values associated with soil sterilization and adding soil inoculum. Statistics parameters represented degrees of freedom (d.f), F-statistic and p-values (p). Significant p values are < 0.05.

Effect	d.f.	Soil sterilization application				Soil inoculum amendment			
		In-transformed biomass		Feedback values ^a		In-transformed biomass		Feedback values ^b	
		F	P	F	p	F	P	F	p
Phytometer species (P)	3	97.4	<0.001	33.4	<0.001	186	<0.001	0.834	0.478
Conditioning species (C)	3	8.20	<0.001	2.07	0.108	4.35	0.005	0.800	0.496
P × C	9	1.77	0.076	3.17	0.002	0.515	0.863	0.815	0.604
Soil treatment (S)	1	0.021	0.884			55.4	<0.001		
S × P	3	0.725	0.538			18.8	<0.001		
S × C	3	0.538	0.657			1.70	0.169		
S × P × C	9	0.479	0.888			2.47	0.011		

^aFeedback values represent $(A_i - B_i)/\max(A_i, B_i)$ where A_i is biomass of the phytometer species grown in non-sterilized soil and B_i is the biomass of the phytometer species grown in sterilized soil. ^bFeedback values represent $(A_i - C_i)/\max(A_i, C_i)$ where A_i is biomass of the phytometer species grown in soil biota without inoculum and C_i is the biomass of the phytometer species grown in soil biota with inoculum.

The Table 5.2 summerizes the effect of phytometer species (P; phase-2 *Zea mays* L., *Helianthus annuus* L., *Phaseolus vulgaris* L., *Glycine max* L.), conditioning species (C; phase-1 *Zea mays* L., *Helianthus annuus* L., *Phaseolus vulgaris* L., *Glycine max* L.), soil treatment (S; non-sterilized soil/soil biota without inoculum/sterilized soil/ soil biota with inoculum), interactions

(between; $P \times C$, $S \times P$, $S \times C$, $S \times P \times C$). For phytometer species, the results of soil sterilization and soil inoculum experimental approaches using log-transformed total biomass data gave similar results, but very different results in feedback values (Table S5.2 and Table 5.2).

5.4.6 Characterization of Soil Legacies in Active Carbon and Enzyme Activity following the Conditioning and Feedback Phase

The average soil legacies in active carbon and enzyme activity following cultivation in phase 1 with all four plant species were 1026.08 mg/kg and 2.32 FDA hydrolyzed $\mu\text{g}/\text{mg}$ soil (Figure 5.3a and 5.3b), respectively. Using the ANOVA test across soil conditioning legacies, the differences in soil active carbon (mg/kg) were not significant ($F_{4,10} = 0.550$; $p > 0.05$) while the enzyme activity in FDA hydrolyzed ($\mu\text{g}/\text{mg}$ soil) was found to be significantly different ($F_{4,10} = 5.10$, $p \leq 0.05$). The *post hoc* multiple comparisons (Tukey's HSD at $p = 0.05$) for the significant differences in soil FDA hydrolyzed are summarized in Table S3. Feedback soils previously conditioned by *H. annuus* (Table S5.4) were not significantly different in soil active carbon level but differed significantly in FDA hydrolyzed enzyme activity. The patterns of these significant differences are shown in Figure 5.3a and 5.3b boxplots.

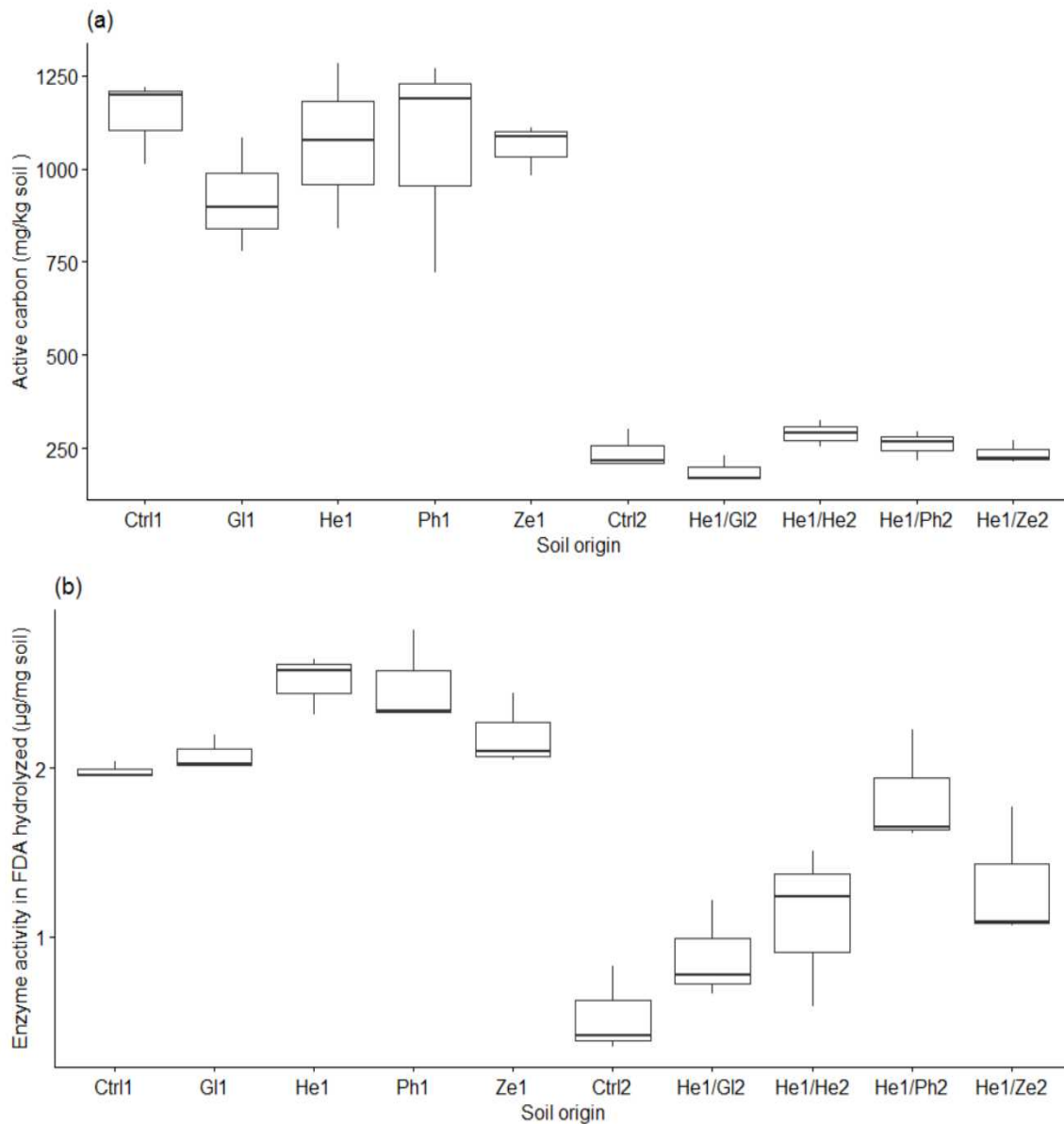


Figure 5.3 Legacies in soil (a) active carbon (mg/kg) and (b) FDA hydrolyzed ($\mu\text{g}/\text{mg}$ soil) at the end of the conditioning (phase 1=1) and feedback (phase 2=2) phases. After harvest, the active carbon stocks in conditioned soils are lower than the fallow soil (control). The soil enzyme activity (FDA hydrolyzed) legacy is high in all conditioned soils and diminished in the fallow bare soil. Feedback phase legacies were from soils conditioned by *H. annuus* in phase 1. Ctrl – procedural bare control soil, Gl – *G. max*, He – *H. annuus*, Ph – *P. vulgaris*, and Ze – *Z. mays*, He/Ze heterospecific *Z. mays* feedback, He/He conspecific *H. annuus* feedback, He/Ph heterospecific *P. vulgaris* feedback and He/Gl heterospecific *G. max* feedback, horizontal heavy bar = median, box=lower and upper quartiles, whiskers=minimum and maximum values, filled dot=outliers.

5.4.7 Characterization of Soil Legacy in Nitrogen following the Feedback Phase

Having calculated the averages in soil nitrogen legacies after the second phase, total soil N in control soil increased to 4.62 mg/l (Figure 5.4d) from the initial 1.95 mg/l total soil N in field-sampled soil (Table 5.1). Soil total nitrogen legacies were on average highest in control natural field soil (4.62 mg/l), followed by *Z. mays* conspecific soil (1.06 mg/l), *P. vulgaris* conspecific (0.81 mg/l) and heterospecific soils (0.78 mg/l), as shown by boxplots in Figure 5.4d. The Linnaeus grass (*Z. mays*; Ze) and legumes (*P. vulgaris*; Ph and *G. max*; Gl) had a relatively high total nitrogen legacy compared to the forb (*H. annuus*; He). This suggests that plant growth in this experiment was not limited by soil nitrogen availability during the feedback phase experiment.

In the Kruskal-Wallis test results, the level of soil nitrogen was not the same ($p \leq 0.05$, Table S5.5) among the nine categories of soil origin. The pairwise contrasts (Table S5.6) show that these significant results in soil nitrate nitrogen feedbacks were between the *H. annuus* heterospecific soil and conspecific *Z. mays* soil pair, *H. annuus* and control natural field soil pair, including the pair of heterospecific *G. max* soil and control natural field soil. There was a significant difference in soil ammonium nitrogen legacy (Figure 5.4c, Table S5.6) between *H. annuus* conspecific soil and natural field soil.

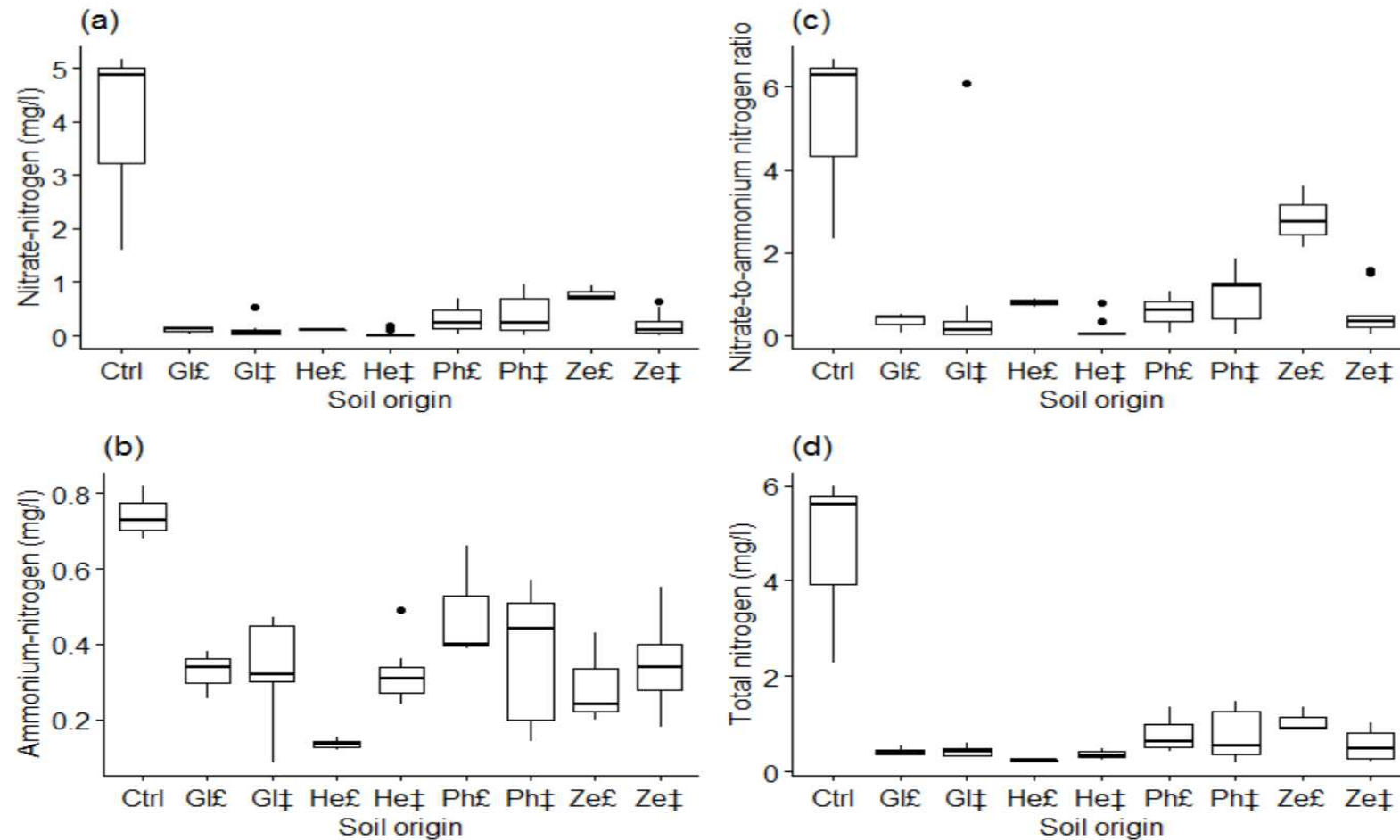


Figure 5.4 Feedback phase legacy of soil nitrogen in conspecific (£, $n = 3$) and heterospecific (‡, $n = 9$) plant-soil feedbacks. The soil origin legacies are significantly different in (a) nitrate-nitrogen in mg/l ($H = 25.4$; d.f = 8; $p < 0.01$), (b) ammonium-nitrogen in mg/l ($H = 18.9$; d.f = 8; $p < 0.01$), (c) nitrate-to-ammonium nitrogen ratio ($H = 25.4$; d.f = 8; $p < 0.01$), (d) total nitrogen in mg/l ($H = 24.0$; d.f = 8; $p < 0.01$). Horizontal heavy bar = median, box=lower and upper quartiles, whiskers=minimum and maximum values, filled dot=outliers.

5.4.8 Soil Microbial-Biomass Legacy following the Conditioning and Feedback Phase

The total PLFA biomass patterns and very low ratios for bacteria indicate that all soils were dominated by Gram-negative (GN) bacteria biomass compared to Gram-positive (GP) bacteria (Table S5.7). As expected, in the first phase, fungal biomass dominated over bacteria in all the soil histories except control soil (Table S5.7). However, there was a shift towards bacterial dominance at the end of phase 2 for all heterospecific soils conditioned by *H. annuus* in phase 1 (Table S5.7). *Zea mays* supported more biomass of viable bacteria and fungi than the three other plant species.

Surprisingly, some fungal PLFA biomarker legacies for C18:3 ω 3c were reduced in all treatments, except conspecific *H. annuus* (He/He), while others like C18:1 ω 9t disappeared completely in all treatments of phase- 2 soils previously conditioned by *H. annuus* (Table S5.8). In bacterial PLFA biomarker legacies, a-C15:0 and i-C15:0 disappeared in all except heterospecific *Z. mays* (He/Ze) soil; markers C15:0, i-C16:0 and C17:0 disappeared in all the conspecific and heterospecific treatment soils conditioned by *H. annuus*; biomarker i-C17:0 was absent in both phases and biomarker C18:17 appeared in the second phase although it was absent in phase- 1 in all soil treatments.

Fungal and bacterial PLFA biomarkers from soils conditioned in phase- 1, and conspecific and heterospecific feedback soils conditioned by *H. annuus* in phase- 1 revealed that C16:19 biomarker was two-fold higher in bare control soil, while C17:0 was absent at the end of the conditioning phase (Table S5.8). Fungal biomarker C18:3 ω 3c was unique to *H. annuus* conspecific soils while the marker C18:2 ω 6c was 10-times higher in conspecific *H. annuus* soil than the remaining heterospecific soils. Bacterial markers i-C15:0 and a-C15:0 were unique to heterospecific *Z. mays* soils. The bacterial C14:0 biomarker was absent in both conspecific *H. annuus* soil and heterospecific *P. vulgaris* soils (Table S5.8).

Similarities among phase 1 conditioned soils (Figure 5.5a) and among phase 2 soils conditioned by *H. annuus* (Figure 5.5b) were based on soil microbial biomass legacies. The unlabeled first NMDS axes are strongly correlated with fungal and bacterial biomass legacies in soil (Figure 5.5), with highest dissimilarities between the bare control soil and soils conditioned by the four plant species (Figure 5.5a; stress: 0.0001, $k = 2$).

However, microbial biomass legacies in soils conditioned by the plant species were ordinated closer to each other, suggesting that they were less dissimilar. After the feedback phase, the microbial biomass legacies in conspecific *H. annuus* soil were dissimilar from other heterospecific feedbacks. Moreover, the heterospecific *Z. mays* soil was dissimilar from the cluster of heterospecific *G. max*, *P. vulgaris* and bare control soil (Figure 5.5b; stress: 0.0000, $k = 2$).

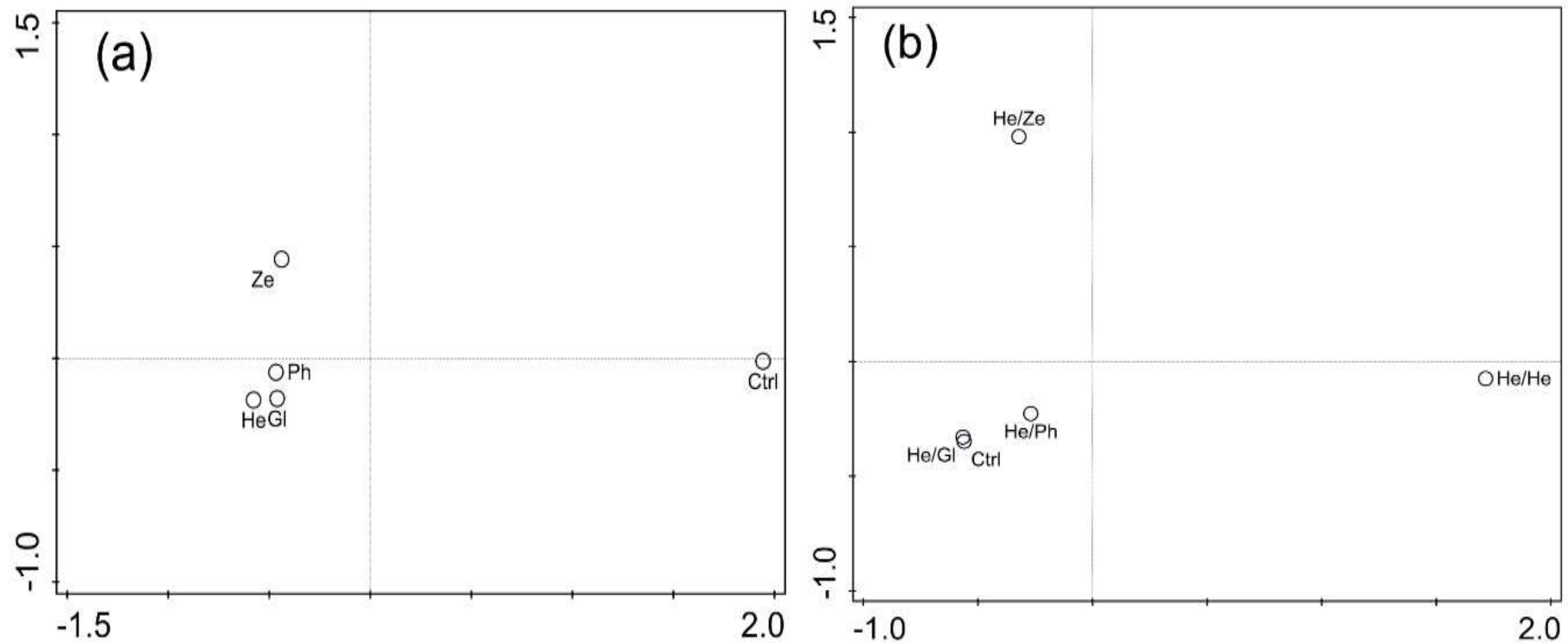


Figure 5.5 Non-metric multidimensional scaling (NMDS) ordination diagram of the Bray-Curtis dissimilarities with two computed axes of microbial biomass of fungi and bacteria PLFAs in soils after the conditioning phase (a) and the conspecific *H. annuus* and heterospecific soils after the feedback phase (b). Ctrl – procedural bare control soil, Gl – *G. max*, He – *H. annuus*, Ph – *P. vulgaris*, and Ze – *Z. mays*, He/Ze heterospecific *Z. mays* feedback, He/He conspecific *H. annuus* feedback, He/Ph heterospecific *P. vulgaris* feedback and He/Gl heterospecific *G. max* feedback

5.5 Discussion

5.5.1 Plant Performance

In this greenhouse study, live and sterilized field soil were conditioned under mean daily temperature of 28 °C and soil moisture regime which were optimum for plant growth, preexisting soil pathogens and mutualists (in live soils), including the symbiont *B. japonicum* (for *G. max*). However, these favorable glasshouse conditions are known to trigger transition of arbuscular mycorrhizal associations from mutualistic to neutral and eventually to parasitic trophic relationships with plant partners (Klironomos, 2003). The superior total dry biomass of *G. max* is worth considering for soils requiring increased litter inputs for organic matter improvements.

5.5.2 Feedback Experiments

In the present study, the high values in biomass feedbacks in soil with added inoculum compared to those in sterilized soils indicate that microbiota support all crop species at larger magnitudes in live soils, confirming that microbes play a role in PSFs (Figure 5.2). Furthermore, these observed positive effects in own soil microbiota suggest that mutualists had a greater impact on plant performance than pathogens. The biological activities of the soil organisms in the rhizosphere correlate to a decrease or increase in plant performance (De Long et al., 2019). However, the overall soil biotic effects in plant-soil feedback depend on the net-activity of pathogens, antagonists, mutualists and decomposers (van der Putten et al., 2013). Destroying these groups of soil biota, for instance through soil sterilization, minimizes the impact of negative plant-soil feedback on plant performance (Cesarano et al., 2017).

Glycine max unexpectedly showed negative, neutral and positive feedbacks despite artificial inoculation with symbiotic *B. japonicum* in field, sterilized and inoculum added soils, respectively (Figure 5.2). However, other studies have shown that leguminous nitrogen-fixing species also develop negative plant-soil feedbacks under monoculture through self-inhibitory effects (Cesarano et al., 2017).

For *P. vulgaris*, it is clear from parallel experimental approaches, soil sterilization, addition of soil inoculum and soil conditioning by ‘own’ vs. ‘foreign’ plant species, that the consistent positive soil-feedback results were strongly associated with its own beneficial soil microbiota which in turn promoted plant growth (Baxendale et al., 2014). In other words, the conditioning phase legacy of mutualists and decomposers were more significant than pathogens (Brinkman et al., 2010). On the other hand, the rest of the phytometer species were negatively affected by the combination of conspecific soil microbiota and nutrients (Figure 5.2a). This seems to indicate that *Z. mays*, *H. annuus* and *G. max* perform best in soils cultivated in plant rotations by avoiding injuries due to species-specific pathogens or allelopathy common in monoculture (Bardgett & Wardle, 2012). We propose that this negative PSF was possibly due to host-specific pathogens and host-specific shifts in microbial biomass and composition (Bardgett, 2009).

In this study, the inoculum-addition approach produced feedback results that were more positive in all plant species than in the sterilization approach (Figure 5.2b and c), suggesting that the effects of mutualists/beneficials are less density-dependent than are soil pathogen effects (Brinkman et al., 2010). The inoculum-addition approach can isolate microbial effects from physicochemical effects, although the resulting soil conditions will favor fast-growing microbes and plant species (Forero et al. 2019).

Zea mays and *P. vulgaris* had a consistently higher root-mass than *H. annuus* and *G. max* in all soil treatments at the end of the feedback phase. This high root-trait response is associated with adaptation to low nutrient supply and soil carbon budgets (Lambers et al., 2008). Other studies show that the high specific root length (SRL) and finer roots of grasses increase their susceptibility to pathogens and the ability to acquire nutrients from soil (Bergmann et al., 2016) in conspecific soils, while promoting positive plant-soil feedbacks in heterospecific soils (Kos et al., 2015). Monocot grasses have a more fibrous root system than most eudicots, which have

a less dense but deeper root system (Fageria, 2013). Deep roots of forb species in conspecific soils reduce nutrient availability and increase species-specific pathogenic fungi responsible for negative plant-soil feedbacks (Kos et al., 2015). Results showed that there was no correlation between phase-1 and phase-2 biomass responses, indicating that soil nutrient depletion in the conditioning phase did not go on to affect the phytometer species in the feedback phase (Kardol et al., 2006). The overall feedback effects suggest mutualistic interactions between crops and soil organisms in combination with the abundance of these specific soil biota (Brinkman et al., 2010).

5.5.3 Soil Legacies

Zea mays cultivated soils had similar high soil active carbon stocks to *H. annuus* and *P. vulgaris*. C4 plants such as *Z. mays* have a high turnover of carbon and their root exudates are rich in amino acids, sugars and organic acids (Baudoin et al., 2003). These simple soluble substrates provide nourishment for pioneer saprophytic fungi (Deacon, 2006).

From our observations, the low active carbon stocks in soils cultivated with *G. max* at the end of the conditioning phase (Figure 5.3a) may predispose microbes to mineralization of the dissolved organic N and excretion of ammonium ions. In this way, microbes meet their energy needs for growth and increase nitrogen legacies in the soil (Bardgett, 2009).

Soil legacies of microbial communities supported by plant roots include the dominating and fast-growing Gram-negative bacteria (de Boer et al., 2005) which possibly survive on relatively labile active carbon (Fanin et al., 2019). The relative fungal to bacterial biomass ratio in conspecific *H. annuus* soils compared to heterospecific soils of *Z. mays*, *G. max* and *P. vulgaris* showed that this forb species had either unique or abundant soil fungal and bacterial composition. In addition, conspecific *H. annuus* soils had the highest legacy of soil microbial enzyme activity in the first phase, which in turn possibly created high legacy of active carbon stocks at the end of phase-2. Increasing soil fungal to bacteria ratios are linked to litter

decomposition and increasing carbon storage (Malik et al., 2016). The decomposition process is also favored over plant biomass growth by abiotic factors such as high moisture and mean daily temperatures (FERTASA, 2016).

Disappearance of some fungal and bacterial PLFA biomarkers in soils at the end of the second phase indicate that they were sensitive to a factor(s) not identified in this study. Moreover, we did not record changes in soil pH or the accumulation of allelochemicals. However, bacterivorous nematodes of the genera *Acrobeloides*, *Cephalobus* and *Acrobeles* were the most prevalent in these soils (Marais et al. data not published) and probably contributed in-part to the disappearance and reduction of bacterial biomarkers. Zhang et al. (2015) also reported that *Z. mays*-*G. max* rotations supported dominance of *Acrobeloides* in the soil. Generally, all treatments had a higher soil fungal and bacterial biomass legacy than bare control soil, indicating that the plant-derived substrates from rhizodeposition supported an increase in soil microbial composition and biomass (Baudoin et al., 2001).

Our results also detected a 10-fold increase in the fungal lipid biomarker linoleic acid (C18:2 ω 6c, methyl cis-9-12-octadecadienoate) in soil for *H. annuus* monoculture, compared to the remaining heterospecific soils. This is a biomarker of saprophytic fungi (Frostegard & Bååth, 1996; Tavi et al., 2013; Willers et al., 2015; Schwab et al., 2017) whose activities are to mineralize nutrients necessary for plant growth, which in turn support soil biological communities (Bardgett & Wardle, 2012). *Helianthus annuus* monoculture also established a unique linolenic acid (C18:3 ω 3c, methyl cis-9-12-15-octadecatrienoate) soil fungal biomarker (Schwab et al., 2017). The relative increase in common and reliable fungal markers in *H. annuus* conspecific soils compared with heterospecific soils may explain the observed high feedbacks in soil enzyme activity and active carbon stocks. These soil legacies are essential ecosystem services in building organic matter for plant nutrition, especially in dryland sandy soils of South Africa, where farmers are faced with the problem of naturally low soil organic

matter varying between 0.5 and 3% (FERTASA, 2016). The PLFA data from this greenhouse study were unreplicated, as such, extra caution must be taken to avoid misinterpreting results. However, these PLFA findings confirmed patterns which were observed in the field during the growing season in February 2018 from *Z. mays* monoculture and crop rotation systems replicated 8 times (Steyn et al., 2019).

It is possible that the root traits of *H. annuus* enabled the crop to efficiently extract mineral nutrients, leading to the observed poor soil nitrogen legacies and an increase in fungal to bacterial biomass ratio. Soil carbon legacies of a grass monoculture favor bacteria, actinomycetes and fungal biomass growth more than a legume and a forb (Ladygina & Hedlund, 2010). This is in agreement with our results whereby *Z. mays* grass had the highest total PLFAs relative to the other three species. The decline in soil active carbon and PLFA legacies after the second phase suggests that remaining microbial groups overcame possible selection pressure due to competition for diminishing labile carbon stocks in the decomposition sequence during microbial succession (Deacon, 2006).

In the conditioning phase, the higher FDA hydrolysis in soil legacies for *P. vulgaris* and *H. annuus* than the other two species indicate that they are capable of cultivating suppressiveness to pathogens in soil associated with relatively high enzyme activity (Janvier et al., 2007). The soil legacies in nitrate nitrogen and ratio of nitrate nitrogen-to-ammonium nitrogen were significantly higher in conspecific *Z. mays* soil than *H. annuus* heterospecific soil (Figure 4), suggesting that monoculture of *Z. mays* is better at increasing nitrate stocks, which in turn can alkalize the rhizosphere better than *H. annuus* (Ryan et al., 2009). This indicates that *Z. mays* monoculture removes less nitrogen from the soil than other plants, hence, rotating *Z. mays* with nitrogen-fixing legume species, specifically *G. max*, will be a good option for growers as a way of reducing costs on nitrogen spending in fertilizer programmes (FERTASA, 2016).

The microbial biomass legacies in *H. annuus* soil under monoculture were different from other heterospecific soil feedbacks. The heterospecific *Z. mays* soil was also dissimilar from heterospecific *G. max*, *P. vulgaris* and bare control soils (Figure 5.5). It is still difficult to generalize that soil fungal to bacteria biomass ratios can act as indicators of soil health in PSFs since thresholds are not yet clear for their transitions in soils (Bardgett & McAlister, 1999). Additional information is required on the taxonomy and microbial activities of these true markers for fungi in *H. annuus* monoculture using culture-dependent and –independent techniques in soil microbial ecology. In summary, *H. annuus* seems to cultivate a significantly high biomass of viable saprophytic fungal markers in addition to an enzymatically active soil legacy that may be crucial in building soil organic matter under monoculture. However, there is evidence that positive PSFs are associated with the influence of soil mutualists (Brinkman et al., 2010).

5.6 Conclusion

These greenhouse-measured PSFs demonstrate that plant species induce changes in legacies of soil fungi, bacteria, active carbon and enzyme activity that may in-turn influence overall soil health and plant health in soil–plant systems. Following the sterilization and inoculum addition approaches, the direction and strength of PSF results were similar for *P. vulgaris*, but different for *Z. mays*, *H. annuus* and *G. max*. However, the consistent positive PSF results in ‘own’ compared to ‘other’ soils under the inoculum addition approach suggest that soil microbial effects were largely due to mutualist effects, rather than species-specific pathogens. In sterilized soils, only *P. vulgaris* grows well in monoculture through positive PSFs, while the other remaining three species grow best in crop rotations through neutral to negative PSFs. Our results show that *H. annuus* was superior to other crop species in cultivating relatively high active carbon stocks and an enzymatically active soil for the next crop. In this context, increasing the frequency of *H. annuus* in rotation sequences can raise fungal relative to

bacterial biomass in soils, while *Z. mays* can increase total microbial biomass. However, more studies using Koch's postulates and plant tissue analysis are needed to evaluate whether the effects of elevated fungal to bacterial biomass ratios in soils promote or depress plant performance and nutrition under different environments. In addition, *in vitro* plate assays and soil bioassays are necessary to test soil legacies for suppressing soil-borne pathogens. In spite of the need to confirm these greenhouse findings under field conditions, we make three suggestions to growers, especially in dryland cropping systems. Firstly, *H. annuus* can recruit and feed decomposer fungi, raise soil enzyme activity and build active carbon stocks. Secondly, *Z. mays* will likely enrich the soil with a balanced microbial community and diversity (Baudoin et al., 2001) and minimize removal of soil nitrogen from sandy soils with low organic matter. Thirdly, *G. max* may increase the quantity of litter inputs at the end of the harvest period.

5.7 Acknowledgements

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Conflict of interest

Authors have no conflict of interest to declare.

CHAPTER 6

Soil Resilience in Greenhouse Rotations of Four Dryland Crop Species via Plant-Soil Feedbacks

6.1 Abstract

Background: A mechanistic understanding of crop rotation system resilience and resistance to disturbance is crucial to successional planting in dryland systems. However, plant-soil health studies of dryland crop rotations in response to cropping sequences are scarce, despite the vulnerability of crops to the environmental stresses experienced in rain-fed farming systems.

Aims: The purpose of this study was to determine soil resilience using plant-soil feedback responses. The plant biomass response to a crop rotation treatment can be different on soils which had different pre-treatments prior to the time of imposition of the crop. We hypothesized that plant-soil feedbacks (PSFs) can estimate the ability of crop rotation sequences to recover (resilience) from and resist (resistance) monoculture disturbance under greenhouse conditions.

Methods: We estimated the resistance to phytopathogenic soil microbiota of dryland crops such as *Zea mays* L., *Helianthus annuus* L., *Phaseolus vulgaris* L., and *Glycine max* L. (Merr.) using repeated monocultures. Resilience or recovery after monoculture was estimated using biomass of the test crop at the final phase of a three-phase PSF study (training phase 1, training phase 2 and feedback phase).

Results: Baseline PSF studies on biomass indicate that *P. vulgaris* cultivates soil legacies that improve plant performance in subsequent cultivation of other dryland crops under greenhouse conditions. The PSF results suggest that legume rotations are better than monoculture for dryland production. In particular, *P. vulgaris* is ideal for *H. annuus* rotations, while *G. max* is

an ideal rotation crop for *Z. mays*. The PSF results suggest that *P. vulgaris* in *H. annuus* crop rotations offers soil resilience to pathogens associated with *H. annuus* monoculture. *Helianthus annuus* relative to the other selected dryland crop species seems to be highly efficient in the uptake of trace and macronutrient elements from the same soil environment. The *in vitro* assays show that *H. annuus* and *G. max* crop rotations seem to yield both soil microbiota and nutrient legacies that relatively inhibit the mycelial growth of *R. solani* AG 2-2 IIIB. The findings indicate that growers can include *H. annuus* and *G. max* in dryland crop rotations to create disease suppressiveness to *R. solani* AG 2-2IIIB.

Conclusion: A crop rotation system consisting of *H. annuus* and *G. max* will improve soil resistance and resilience to *R. solani* infestation. *Glycine max* is ideal for *Z. mays* plant production systems while *P. vulgaris* will be ideal for improving plant production in *H. annuus*. More so, growers can use these legume options to design the sequential planting and soil fertility programmes compatible with their strategic goals.

Key words

Zea mays L. (maize); *Helianthus annuus* (sunflower); *Glycine max* (soybean); *Phaseolus vulgaris* (sugar bean); soil resilience; plant-soil feedbacks.

6.2 Introduction

Plant-soil feedbacks (PSFs) are crucial for understanding the mechanism by which dryland crop-soil legacies alter soil functions in crop rotation systems under greenhouse conditions (Benitez et al., 2017; Seipel et al., 2019; Mushonga et al., 2020; Zhao et al., 2021b). Soil microbiota are key in maintaining soil functions after natural or man-made disturbance or environmental change (Nannipieri et al., 2003; Griffiths & Philippot, 2013). In this study, a three-phase PSF experiment was used for estimating the resilience of soils by comparing plant growth in sterilized soils (a disturbance or soil treatment which destroyed soil microbiota) versus field-collected living soils (unsterilized soil treatment).

Resilience of soil is quantified using the logarithmic ratio of the plant biomass harvested in live soils to plant biomass in sterilized soils as an indicator, or quantitative estimate, of recovery mechanisms after disturbance under greenhouse conditions. The PSF estimate value for resilience represents the capacity of soil to recover after sterilization (Seybold et al., 1999). Soil sterilization is a disturbance that disrupts soil ecosystem services by destroying soil microbiota (Griffiths & Philippot, 2013). The residual legacies in soils after PSFs were tested for the suppression of *R. solani* AG 2-2 IIIB using fungistasis assays. The *in vitro* suppression of *R. solani* was hypothetically taken as an estimate of soil health legacies dependent on diversity and successional plantings in crop rotations. The change in the soil's capacity to suppress mycelial growth relative to a reference or baseline condition (control sterilized sand soils) is a measure of the dynamic quality of soil to function due to management activities (Seybold et al., 1999).

We hypothesized that under a controlled environment, soil resilience is caused by biological and physicochemical properties of soil as a result of plant effects on soil followed by soil effects on subsequent plant biomass of the phytometer or test plants (Griffiths & Philippot, 2013; Polain et al., 2020). In other words, soil resistance is the capacity of a soil to continue to function without change throughout a disturbance (Seybold et al., 1999). Soil microbial composition is as important as crop species for mechanisms or processes predicting soil resilience (Allison & Martiny, 2008). Soil resistance was estimated by comparing phytometer biomass yield in monoculture versus crop rotations. Monoculture i.e. cultivating the same crop species sequentially on the same soil removes competing plant species but builds up a soil legacy of plant-specific pathogens (Seybold et al., 1999).

The positive response for phytometer species grown in soils with a history of crop rotation compared to monoculture represented soil resistance. Soil quality or health is associated with soil resilience on the basis of the recovery of soil functions while soil resistance relates to soil

quality based on the degree of change in soil functions as a consequence of a disturbance (Seybold et al., 1999). Soil quality parameters of soil resilience and resistance are influenced by soil type and vegetation, disturbance patterns, land use and scale (Seybold et al., 1999). At field scale, disturbance is associated with heavy farm machinery, soil tillage, monoculture and the application of chemical fertilizers and pesticides (Seybold et al., 1999; Liu et al., 2020).

During growth, plants release rhizodeposits (i.e. root exudates) into the rhizosphere where soil-microbe nutrient feedbacks mediate plant-soil interactions that drive the plant nutrient acquisition strategy (Geisseler & Scow, 2014; Zhao et al., 2021b). The objective was to assess soil resilience to the destruction of soil microbiota using steam sterilization. After the destruction of soil microbiota, soil health is influenced largely by soil resilience (Seybold et al., 1999). Plant uptake of soil minerals into tissue biomass is a means of soil degradation which reduces the capacity of soil function to supply nutrients to subsequent plants (Seybold et al., 1999). However, plant nutrient acquisition has trade-offs, like the phytoremediation of nutrient pollution in soil and water environments, which assists soil or water resources to recover their functions following degradation (Lambers et al., 2008). The impact of individual crops in crop rotation sequences on the crop yields has been attributed to both crop induced soil biota and soil nutrient status (Dias et al., 2015). The crop rotation effects induce changes in the structure and composition of rhizospheric biota which in turn condition the soil such that it becomes more conducive to crop productivity and soil health.

Plant-soil feedback experiments have the potential to reveal whether crop-specific soil biota changes or soil nutrients influence crop productivity in crop rotations. To investigate plant-soil interactions in rotation feedbacks, we performed greenhouse feedback or soil sickness experiments to recognize and reveal complex above and belowground interactions, associated with microbial communities. We sought to understand the feedbacks of beneficial or harmful soil microbiota to plant biomass and tissue concentration of mineral nutrients.

The decomposition of organic matter and nutrient cycling in soil are associated with soil recovery processes or mechanisms driven by biological activity, biological diversity, and plant growth (Seybold et al., 1999). *Rhizoctonia solani* AG groups in dryland systems of South Africa reduce potato production which leads to yield losses (Van der Waals et al., 2016). Although the selected dryland crops are not produced under controlled environments, there is need for a mechanistic understanding of plant-soil interactions. Disease-suppressive soils have biotic and abiotic properties that restrict the establishment of a plant pathogen or reduce disease incidence caused by the pathogen (Vega et al., 2019). The cultivation of different plant species in rotations is the most popular practice in reducing soil borne pathogen inoculum. Following interesting two-phase PSFs results (Mushonga et al., 2020), we extended the study of the PSFs with a third phase of phytometer/test plants namely; maize (*Zea mays* L.), sunflower (*Helianthus annuus* L.), sugar bean (*Phaseolus vulgaris* L.), and soybean (*Glycine max* L. (Merr.)) to determine the impact of soil legacies from PSFs on plant biomass.

6.3 Materials and Methods

6.3.1 Soil Sampling

Soil collection was done as described in Mushonga et al. (2020) from a *Zea mays* uniformity trial situated at Petrus Steyn in the eastern Free State (EFS) province of South Africa (S27° 53' 45.5" E28° 12' 59.8", Elevation 1705 m). After the training phase 2 in plant–soil feedback experiments, soil analysis showed that N forms were significantly different according to soil origin (Table S6.1, Mushonga et al., 2020).

6.3.2 Plant-Soil Feedback Experimental Design

Greenhouse experiments were conducted at the University of Pretoria (Wheatboard Greenhouse and Labs, S 25°45'21" E 28°13'51", South Africa) from 7 March 2018 to 10 December 2018. A three-phase experiment was performed; training phase 1, training phase 2 and feedback phase (Figure 1) (Mushonga et al., 2020). The PSFs for all cropping sequences

were closed as three-course rotations using the same crop as in phase 1. The direction and strength of plant–soil feedbacks were investigated (Figure 6.1).

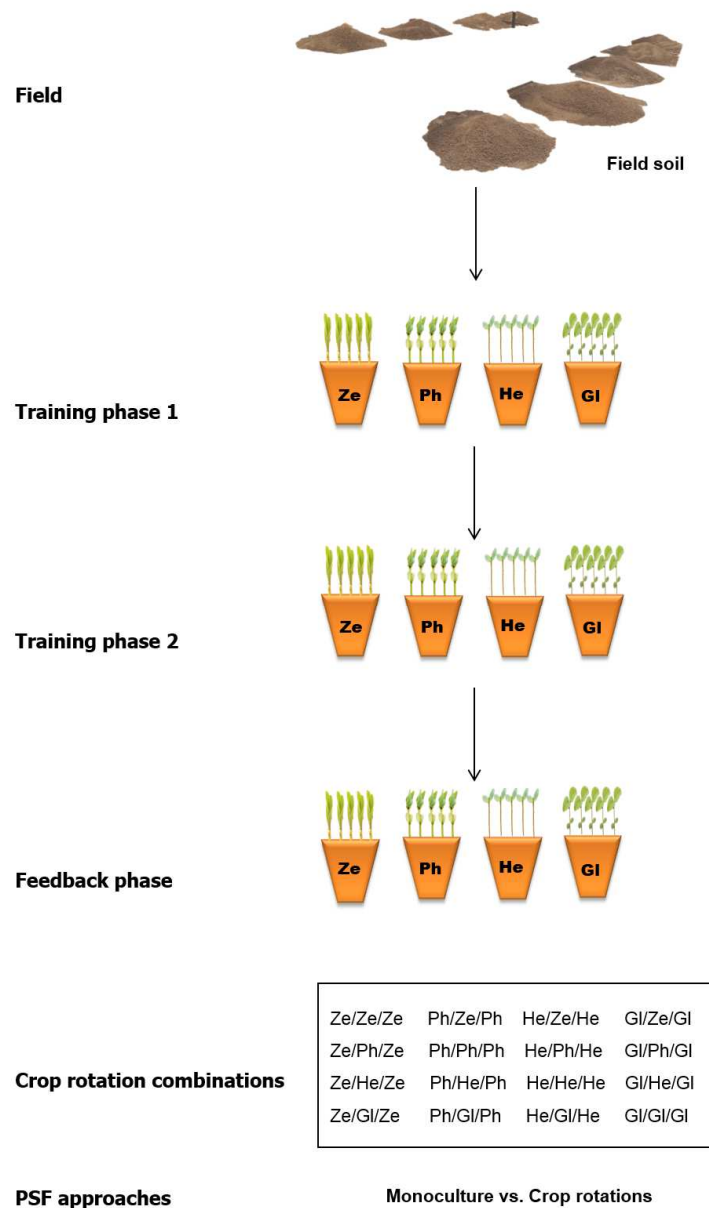


Figure 6.1 Conceptual diagram of the PSF experimental design made up of the training phase 1, training phase 2 and feedback phase. In the greenhouse, training phase 1 involved cultivating *Z. mays* (Ze), *P. vulgaris* (Ph), *H. annuus* (He), and *G. max* (Gl) in live field soil and sterilized soil simultaneously. In training phase 2, the training phase 1-conditioned soils were planted with the same four plant species to give all possible soil permutations and combinations. In the feedback phase, the training phase 2-conditioned soils were planted with the same four plant species considered here as phytometers to give all possible soil combinations of crop rotations. Drawing courtesy of Mr. Fungai Mushonga.

Table 6.1 lists all the different experiments and parameters related to plant performance that were tested in each experiment. For example in Experiment B, disease-suppressive soils were converted to disease-conducive soils by pasteurization (treatment at about 100 °C, 60 kg per h). In Experiment C, the inoculum was found to have high levels of *Proteobacteria* associated with plant fitness and plant growth promotion including species from the genera, *Bacilli*, *Burkholderia*, *Pseudomonas*, *Serratia*, *Streptomyces*, and *Stenotrophomonas* (Dube et al., 2019). Previous studies have shown that when this inoculum was re-introduced into sterilized field soil it rendered the soils suppressive again (Deacon, 2006).

Table 6.1 The different experiments and parameters tested in the three-phase PSF experiments made up of the training phase 1, training phase 2 and feedback phase (Figure 6.1).

Experiments	Plant-soil feedbacks	Parameters tested
Experiment A , natural live field soil	Monoculture relative to crop rotation soil biota and nutrients	• Plant performance • PSF ratios of resilience to soil sterilization • Crop rotation resistance to monoculture disturbance
Experiment B , sterilized field soil	Monoculture relative to crop rotation soil nutrients	• Plant performance • Crop rotation resistance to monoculture disturbance
Experiment C , inoculated sterilized field soil	Monoculture relative to crop rotation soil biota	• Plant performance • PSF ratios of resilience to soil sterilization • Crop rotation resistance to monoculture disturbance

6.3.3 Plant Nutrient Analysis

Macro- and micro-nutrient analysis of plant material were measured using standard procedures at Nvirotek Laboratories (Pty) Ltd (ISO/IEC 17025 international standard for Testing laboratories Accredited Testing Laboratory, certified SANAS and SADCAS assessors,

certified AgriLASA Fertilizer Laboratory, Unit 6, Nviro Business Hub, Ifafi, 0260, South Africa). Plant leaf analysis was done using Total Nitrogen – DUMAS, Calcium by ICP-OES, Magnesium by ICP-OES, Potassium by ICP-OES, Sodium by ICP-OES, Sulphur by ICP-OES, Phosphorus by ICP-OES, Iron by ICP-OES, Manganese by ICP-OES.

6.3.4 Physiological Measurements

Harvesting was done at physiological maturity after flowering of all crops after 10 weeks of growth. Fresh weight determinations were done using a weighing scale while dry weight determination was estimated by drying crop biomass samples at 62 °C in a forced-air oven for 48 hours till constant weight is achieved. The total crop biomass, aboveground and belowground biomass in fresh and dry weight (g) were measured using a laboratory scale.

6.3.5 Pathogen Suppression using *in vitro* Growth Assays

The aim of the *in vitro* growth assay was to determine the effect of the crop rotation soil legacies on mycelial growth measured mycelial extension of reference *R. solani* AG 2-2 IIIB J.G. Kühn culture strain CBS 101765 on the surface of soil samples in Petri dishes. In the present study, *R. solani* AG 2-2 IIIB was chosen because it is one of the aggressive root-rot pathogens and leading cause of damping-off problems for several dryland crops in the Free State province (Muzhinji et al., 2015; Dube, 2020). Elsewhere, *R. solani* AG 2-2 IIIB is an important soil borne pathogen that causes root rot disease in vegetables, cereal, sugar bean, soybean and sugar beet production systems (Muyolo et al., 1993; Brown et al., 2021). The field soil samples used in this assay were sieved using an SABS-certified 2 mm stainless steel wire cloth sieve (Labotec Test Sieve Toetssif, Industria, South Africa). The assay for soil suppression of *R. solani* AG 2-2 IIIB mycelial growth was performed as described by Ko & Ho (1983) and De Boer et al. (1998). Approximately 50 g composite soil samples were added to 9 cm diameter-Petri dishes and evenly spread in an 8 mm smoothened layer. The soils were moistened by adding 10 ml sterile distilled water and covered with a thin layer of water agar. A 7-mm-

diameter potato dextrose agar (PDA) disc from the margin of actively growing fungus colony was inverted and placed at the centre on top of the soil-water agar surface. Control soil samples were prepared similarly by using sterilized sand soil (autoclaved twice at 121 °C for 15 minutes) instead of rotation soils. The bulk soil samples from undisturbed field soil were also prepared in the same way as composite rotation soils. The Petri dishes were sealed with Parafilm M® and incubated at 24 °C in darkness. The growth of *R. solani* was located and measured on soil-water agar surfaces on days 2, 3 and 4 using a Zeiss stereomicroscope (WILD M7A model, Leitz, Heerbrugg, Switzerland) at the magnification of 20X powered by a model MTR 28 transformer (0 - 7V total max 50 VA, WILD, Heerbrugg, Switzerland). The surface growth of the reference fungus on crop rotation legacy soil samples was compared with that on sterile sand control treatment. The percentage of reference *R. solani* growth suppression was calculated as:

$$\% \text{ Suppression} = 100 \times \left(1 - \frac{\text{Mycelial extension on crop rotation soil legacies}}{\text{Mycelial extension on conducive sterile sand soil}}\right).$$

6.3.6 Data Processing and Statistical Analysis

The percentage increase in phytometer biomass for crop rotation against monoculture at the end of the feedback phase were computed and analysed using the *post hoc* Tukey's honestly significant difference (Tukey HSD) test in SPSS version 23 (IBM Corp. Released, 2015). In this *post-hoc* test, an ANOVA F test was done to determine the significance of crop rotation treatment effects while the Tukey HSD *post-hoc* test was done to group the treatment means (Steel & Torrie, 1960) strictly for soil and plant health data (or transformed data) of equal sample sizes that meet the normality and homogeneity of variance assumptions. Overall, this *post-hoc* test provides answers to specific meaningful questions, which were after all the objectives of the study in comparing all pairs of crop rotation treatments (Ruxton and Beauchamp, 2008; Kozak & Powers, 2017). The ratios represent PSF which are independent of plant size allowing comparisons between crop rotation combinations (Brinkman et al., 2010;

Seipel et al., 2019) observing that the ratios and log ratios of normally distributed random variables oftentimes violate the normality assumption (Rinella and Reinhart, 2018). Resilience or recovery was estimated using natural log-ratio between phytometer biomass harvested in active soil (*i*) and sterilized soil (*j*) within the same crop rotation as represented in the following equation:

$$PSF_{ij} = \ln\left(\frac{\text{biomass}_i (\text{active soil})}{\text{biomass}_j (\text{sterilized soil})}\right).$$

Resistance to the effect of harmful soil microbiota on plant growth was estimated using repeated monocultures. Crop rotation resistance to monoculture disturbance was hypothetically formulated according to Nannipieri et al. (2003) definition for resistance in the following equation:

$$\% \text{ Increase in Rotation biomass} = \frac{100 \times (\text{Rotation biomass} - \text{Monoculture biomass})}{\text{Rotation biomass}}.$$

Significant paired differences in phytometer growth using feedback ratios were based on the *post hoc* Tukey's honestly significant difference (Tukey HSD) test while the differences in rotation resistance data were also analysed using One-way ANOVA based on *post hoc* Tukey HSD test conducted using SPSS version 23 (IBM Corp. Released, 2015).

The surface growth of the reference fungus on crop rotation legacy soil samples were compared with that on conducive sand control treatments to obtain an estimate of percent suppression values. Significant differences in *Rhizoctonia solani* AG 2-2 IIIB *in vitro* (a) colony diameter and (b) % suppression of mycelial growth were based on ANOVA with Tukey HSD *post hoc* multiple comparisons, conducted using SPSS version 23 (IBM Corp. Released, 2015).

Data analyses were carried out using R 3.5.2 (R Core Team, 2018), SPSS version 23 (IBM Corp. Released, 2015) and Canoco 5 version 5.11 (ter Braak & Šmilauer, 2018). Time series of climate characteristics were plotted using the “ggplot2” and “tseries” packages in R. Boxplots were drawn in SPSS version 23 for all recorded data to detect outliers in the dataset.

Multivariate analysis of plant mineral nutrient legacies data were visualized using principal component analysis (PCA) and non-metric multidimensional scaling (NMDS) ordination diagram of the Bray–Curtis dissimilarities, with two computed axes of plant macro- and micro-nutrient biomass across soil treatments (Canoco 5 version 5.11; ter Braak & Šmilauer, 2018).

6.4 Results

6.4.1 Climate Characteristics

In Figure S6.1a, the calculated daily temperature ranges (19.4 to 37.3°C) were higher than for training phases 1 and 2. In the feedback phase (Figure S6.1b), relative humidity ranged from 30.6% to 82.6% with an average of 54.9%, and the maximum solar intensity was 32,280 Lux (Figure S6.1c).

6.4.2 Effects of Plant-Soil Feedback Legacies on Plant Performance

Crop rotations did differ significantly in plant biomass of *P. vulgaris* and there was an increase from monoculture of between 26 – 90% ($p < .05$, Table 6.2). *Post hoc* analysis showed that the PhHePh rotation significantly increased yield compared to PhPhPh monoculture ($p < .05$). The ZePhZe rotation significantly increased biomass by 45% while ZeGlZe significantly decreased biomass by 9% ($p < .05$, Table 6.2). For the ZeHeZe rotation, the biomass feedback was similar to the ZeZeZe monoculture and ZeGlZe ($p > .05$, Table 6.2).

There was no significant increase in the phytometer *G. max* biomass between crop rotations ($p > .05$, Table 6.2). Compared to the other two *H. annuus* rotations, there were increases of 6% and 13% in biomass for the HeZeHe and HeGlHe rotations, respectively ($p > .05$, Table 6.2). The results in Table 6.2 suggest that legume rotations are better than monoculture for dryland production, in particular *G. max* is good for *H. annuus* rotations while *P. vulgaris* is an ideal option for *Z. mays* rotations for growers.

Table 6.2 Total biomass (g per plant) of *Zea mays* (Ze), *Phaseolus vulgaris* (Ph), *Glycine max* (Gl) and *Helianthus annuus* (He) in monoculture and rotation systems in three-phases from 7 March 2018 to 10 December 2018. Mean $\pm$ SEM. % **Increase** = $[(r - m)/m] * 100$, where biomass **r** is for cropping system and **m** is for monoculture. Different letters in log-transformed biomass indicate significant differences within crop rotations based on *post hoc* Tukey's HSD test after one-way ANOVA for plant biomass with crop rotation as a fixed factor.

Crop rotation	Feedback phase	
	Biomass	% Increase
Maize ($p < .05$)		
ZeZeZe	$0.82 \pm 0.050a$	0
ZePhZe	$1.19 \pm 0.099b$	45
ZeGlZe	$0.75 \pm 0.040a$	-9
ZeHeZe	$0.82 \pm 0.036a$	0
Sugar bean ($p < .05$)		
PhPhPh	$0.68 \pm 0.015a$	0
PhZePh	$1.01 \pm 0.093bc$	50
PhGlPh	$0.85 \pm 0.044ab$	26
PhHePh	$1.29 \pm 0.128c$	90
Soybean ($p > .05$)		
GlGlGl	$1.48 \pm 0.096a$	0
GlZeGl	$1.74 \pm 0.195a$	18
GlPhGl	$1.43 \pm 0.146a$	-3
GlHeGl	$1.83 \pm 0.190a$	23
Sunflower ($p > .05$)		
HeHeHe	$0.43 \pm 0.018a$	0
HeZeHe	$0.46 \pm 0.033a$	6
HePhHe	$0.38 \pm 0.020a$	-14
HeGlHe	$0.49 \pm 0.064a$	13

All crops showed significant differences in total biomass across PSF phase (P) and cropping sequence (C) ($p < .001$, Table 6.3) while the cropping sequences within legumes were not significantly different ($p > .05$, Table 6.3). Interactions between phase and cropping sequence were significant for all crops ($p < .05$, Table 6.3) except for *G. max*.

Table 6.3 Two-way ANOVA for log-transformed total biomass (g per plant) of the four crop species with PSF phase and cropping sequence as fixed factors.

Crop	ANOVA, Variable	d.f.	<i>F</i>	<i>P</i>
<i>Zea mays</i>	Phase (P)	2	448.289	< .001
	Cropping sequence (C)	3	7.346	< .001
	P * C	6	5.232	< .001
<i>Phaseolus vulgaris</i>	Phase (P)	2	261.262	< .001
	Cropping sequence (C)	3	2.555	.061
	P * C	6	6.650	< .001
<i>Glycine max</i>	Phase (P)	2	88.860	< .001
	Cropping sequence (C)	3	0.749	.526
	P * C	6	2.135	.058
<i>Helianthus annuus</i>	Phase (P)	2	1044.261	< .001
	Cropping sequence (C)	3	6.968	< .001
	P * C	6	3.958	.002

6.4.3 PSF Ratios, Resilience and Resistance

6.4.3.1 PSF Ratios and Resilience

The natural log-transformed ratios are centred on zero when plant biomass of phytometers grown in (a) field soil (soil microbiota and nutrients) and (b) inoculated soil (soil microbiota only) are equal to plant biomass in phytometers grown in sterilized soil controls (Figures 6.2 to 6.6). A positive ratio suggests the presence of beneficial soil microbiota associated with positive plant growth while a negative ratio suggests the presence of harmful soil microbiota associated with negative plant growth. Baseline studies on biomass indicate that *P. vulgaris* cultivates soil legacies that improve plant performance in subsequent cultivation of other dryland crops under greenhouse conditions (Mushonga et al., 2020). Tukey's HSD test was performed in all PSFs within each phytometer after one-way ANOVA for plant biomass with crop rotation combinations as a fixed factor ($p < .05$, $p < .001$).

In field soils, the ZeHeZe and ZePhZe crop rotations displayed negative plant-soil feedbacks which did not differ significantly from positive feedback in the ZePhZe rotation ($p > .05$, Figure 6.2a). On the other hand, in inoculated soils, ZeGlZe rotation had very strong positive plant-soil feedback compared to ZeHeZe and ZePhZe rotations ($p < .05$, Figure 6.2b). The *post hoc*

Tukey's HSD test of the *Z. mays* phytometer findings revealed that plant–soil feedbacks varied in direction and strength as shown in Figure 6.2. In field soils, the PhHePh crop rotation showed a very strong positive plant-soil feedback that was significantly different from the negative feedback for PhPhPh monoculture ($p < .05$, Figure 6.3a). On the other hand, in inoculated soils, all *Z. mays* rotations had similar weak positive plant-soil feedback ($p > .05$, Figure 6.3b). The post hoc Tukey's HSD test of the *P. vulgaris* phytometer findings revealed that plant–soil feedbacks varied in direction and strength as shown in Figure 6.3.

In field soils, the GlZeGl and GlHeGl crop rotations showed very strong positive plant-soil feedbacks while the GlGlGl & GlPhGl crop rotations had very strong negative plant-soil feedbacks ($p < .05$, Figure 6.4a). On the other hand, all *G. max* rotations had similar weak negative plant-soil feedback in inoculated soils ($p > .05$, Figure 6.4b). These results suggest *Z. mays* creates soil legacies that are conducive to pathogens common in repeated *G. max* monocropping. The post hoc Tukey's HSD test of the *G. max* phytometer findings revealed that plant–soil feedbacks varied in direction and strength (Figure 6.4). In field soils, the HeZeHe crop rotations showed a negative plant-soil feedback compared to the positive plant-soil feedback in HeHeHe monoculture ($p > .05$, Figure 6.5a). On the other hand in inoculated soils, the greatest magnitude of positive plant–soil feedbacks were observed in HePhHe crop rotation ($p < .05$, Figure 6.5b). These results suggest that *P. vulgaris* in *H. annuus* crop rotations offers resilience to pathogens associated with *H. annuus* monoculture. The post hoc Tukey's HSD test of the *H. annuus* phytometer findings revealed that plant–soil feedbacks varied in direction and strength (Figure 6.5).

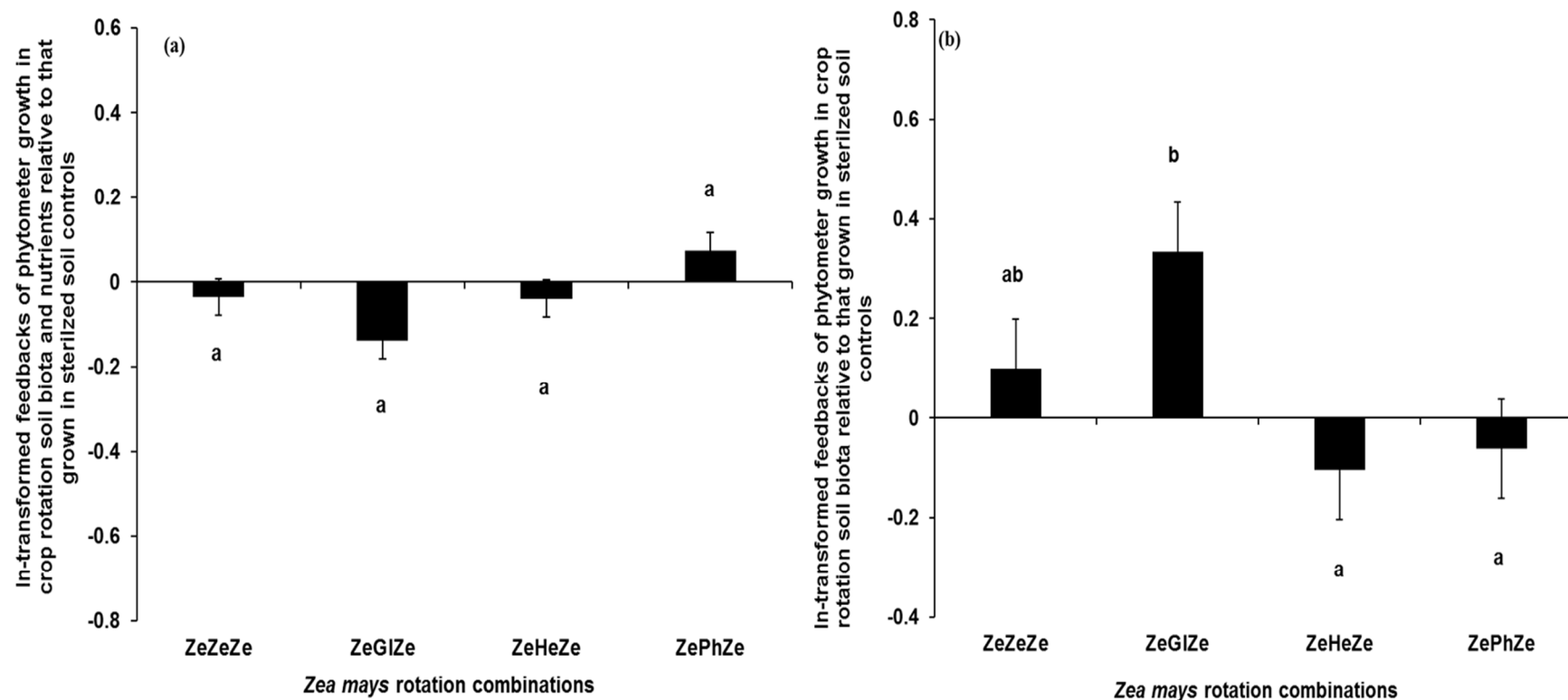


Figure 6.2 The strength of plant–soil feedback responses of *Zea mays* by crop rotation in (a) soil biota and nutrients and (b) soil biota only relative to that grown in sterilized soil controls. Different letters represent significant differences ($p < .05$) among crop rotation combinations or a strong PSF value, while shared letters represent non-significant differences ($p > .05$) among crop rotation combinations or a weak PSF value based on the Tukey's HSD test. All bars represent the standard error of the mean ($n = 8$). *Zea mays* (Ze), *Phaseolus vulgaris* (Ph), *Glycine max* (Gl) and *Helianthus annuus* (He).

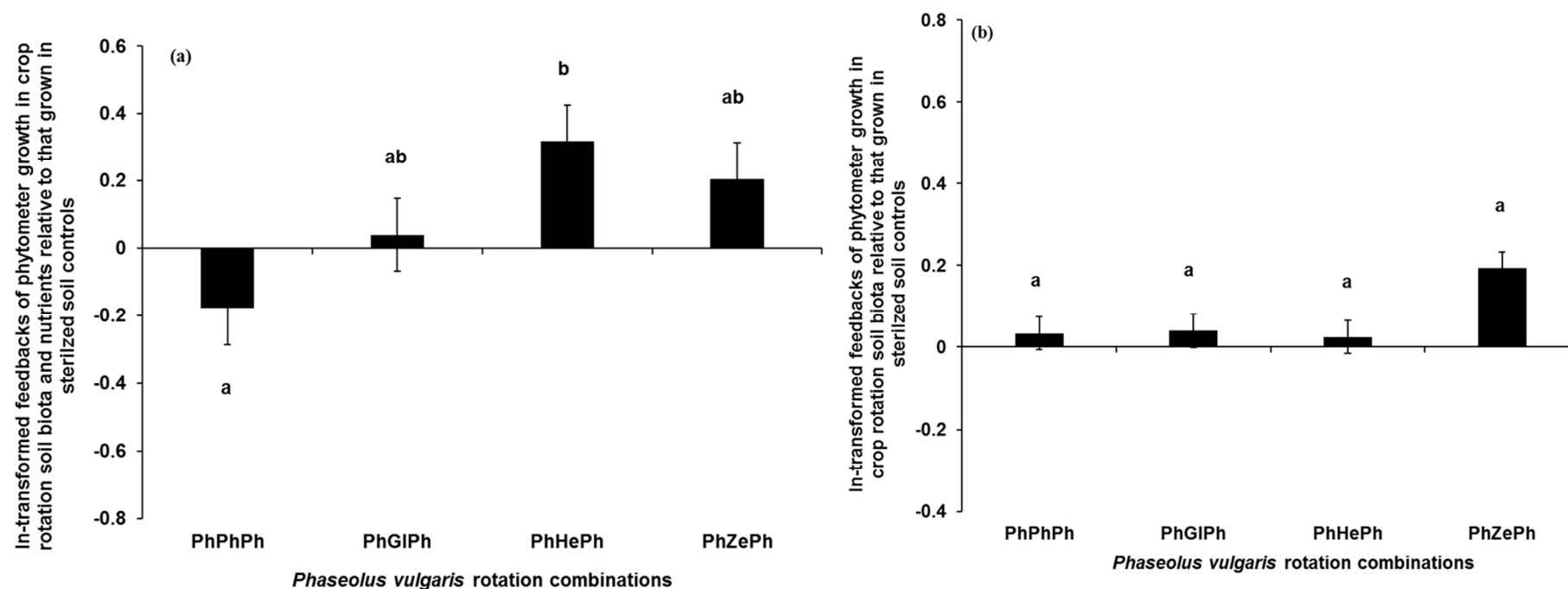


Figure 6.3 The strength of plant–soil feedback responses of *Phaseolus vulgaris* by crop rotation in (a) soil biota and nutrients and (b) soil biota only relative to that grown in sterilized soil controls. Different letters represent significant differences ($p < .05$) among crop rotation combinations or a strong PSF value, while shared letters represent non-significant differences ($p > .05$) among crop rotation combinations or a weak PSF value based on the Tukey’s HSD test. All bars represent the standard error of the mean ($n = 8$). *Zea mays* (Ze), *Phaseolus vulgaris* (Ph), *Glycine max* (Gl) and *Helianthus annuus* (He).

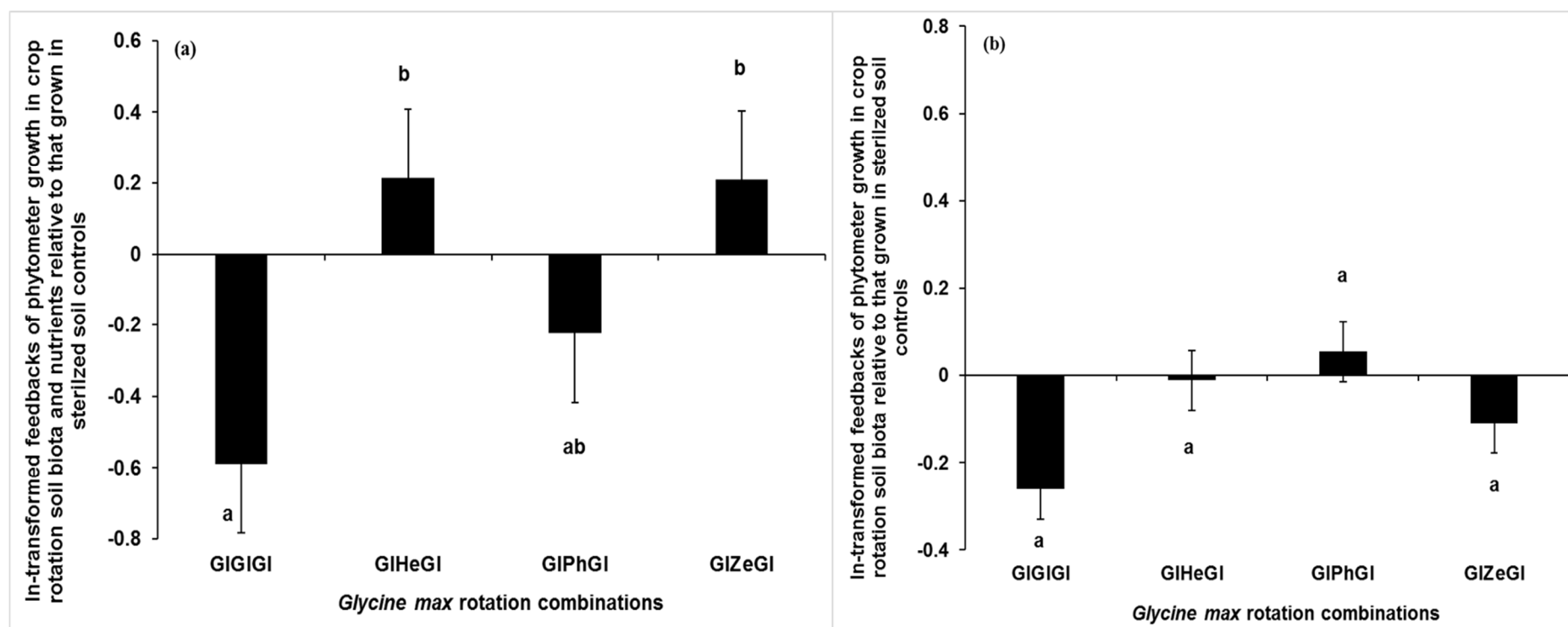


Figure 6.4 The strength of plant–soil feedback responses of *Glycine max* by crop rotation in (a) soil biota and nutrients and (b) soil biota only relative to that grown in sterilized soil controls. Different letters represent significant differences ($p < .05$) among crop rotation combinations or a strong PSF value while shared letters represent non-significant differences ($p > .05$) among crop rotation combinations or a weak PSF value based on the Tukey's HSD test. All bars represent the standard error of the mean ($n = 8$). *Zea mays* (Ze), *Phaseolus vulgaris* (Ph), *Glycine max* (Gl) and *Helianthus annuus* (He).

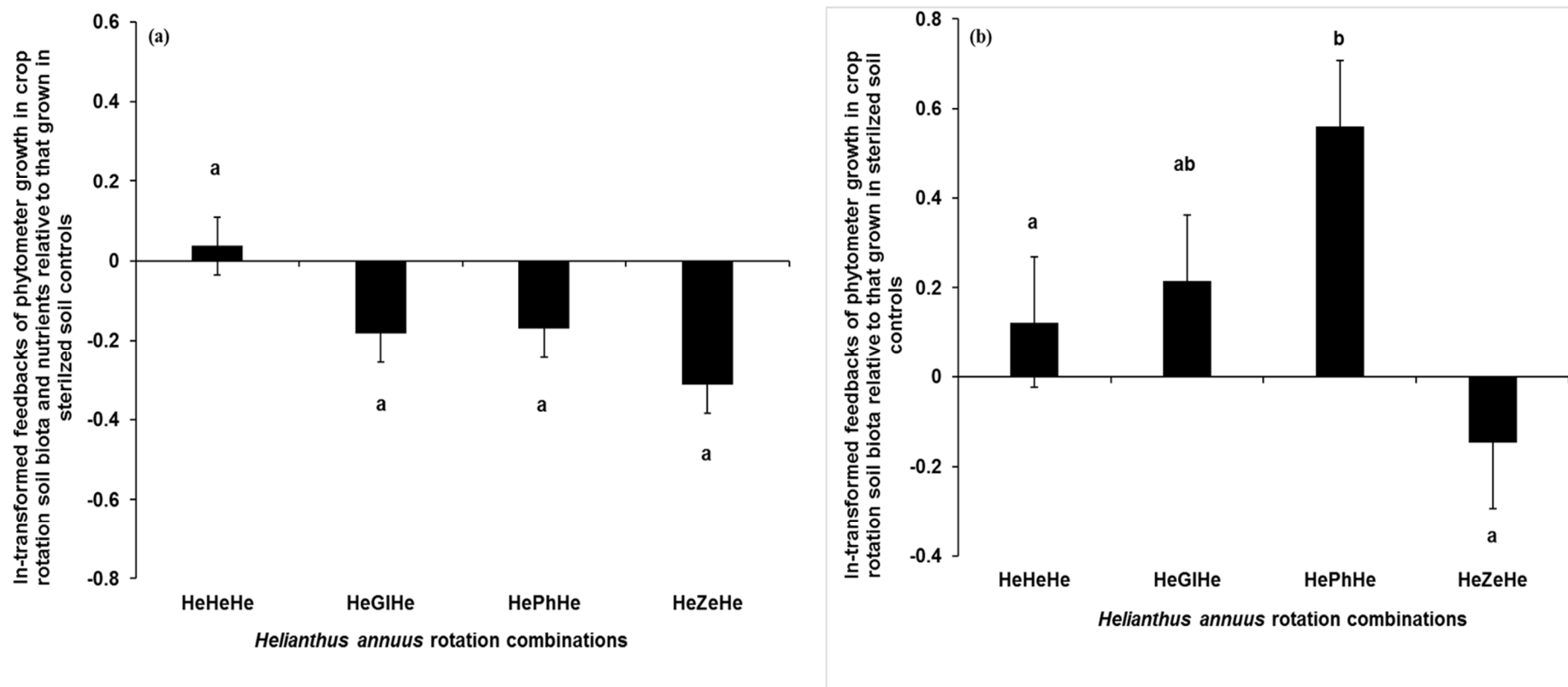


Figure 6.5 The strength of plant–soil feedback responses of *Helianthus annuus* by crop rotation in (a) soil biota and nutrients and (b) soil biota only relative to that grown in sterilized soil controls. Different letters represent significant differences ($p < .05$) among crop rotation combinations or a strong PSF value while shared letters represent non-significant differences ($p > .05$) among crop rotation combinations or a weak PSF value based on the Tukey’s HSD test. All bars represent the standard error of the mean ($n = 8$). *Zea mays* (Ze), *Phaseolus vulgaris* (Ph), *Glycine max* (Gl) and *Helianthus annuus* (He).

6.4.3.2 Resistance

Multiple comparisons indicate the ZePhZe rotation has the highest resistance value under soil biota and nutrients legacies ($F_{2, 21} = 7.576$, $P = .003$, Figure S6.2a) and soil nutrients legacies ($F_{2, 21} = 5.452$, $P = .012$, Figure S6.2b) while rotations ZeGlZe and ZeHeZe had the lowest mean resistance. In soil biota legacies, the ZeGlZe rotation resistance to monoculture was significantly different from the other two rotations ($F_{2, 21} = 4.422$, $P = .025$, Figure S6.2c). In *P. vulgaris* rotations, the PhHePh rotation has the highest resistance value under soil biota and nutrients legacies ($F_{2, 21} = 3.601$, $P = .045$, Figure S6.3a) while in soil nutrients legacies ($F_{2, 21} = 0.318$, $P = .731$, Figure S6.3b) and soil biota legacies the rotations showed similar resistance values ($F_{2, 21} = 0.369$, $P = .696$, Figure S6.3c). All *G. max* rotations did not significantly promote resistance to monoculture under legacies of soil biota and nutrients ($F_{2, 21} = 0.927$, $P = .412$, Figure S6.4a), soil nutrients ($F_{2, 21} = 1.465$, $P = .254$, Figure S6.4b) and soil biota ($F_{2, 21} = 1.289$, $P = .296$, Figure S6.4c). *Helianthus annuus* crop rotations did not significantly promote resistance to monoculture under soil biota and nutrients legacies ($F_{2, 21} = 1.336$, $P = .284$, Figure S6.5a) and soil nutrients legacies ($F_{2, 21} = 3.300$, $P = .057$, Figure S6.5b). In soil biota legacies, the HeZeHe rotation resistance to monoculture was significantly different from the other two rotations ($F_{2, 21} = 3.614$, $P = .045$, Figure S6.5c).

6.4.4 Plant Tissue Nutrient Concentration Analysis

Plant nutrient analysis of the last crop in the rotation sequence that is the phytometer indicate that relative to other crop species, *H. annuus* has root traits that enable it to be the best “miner” of most mineral elements (macro-and micronutrients). While *Z. mays* accumulates mostly Fe, *G. max* is a superior miner of N and *P. vulgaris* of P obtained from soil with a history of crop rotation (Table 6.4 and Table 6.5). The concentration of mineral macronutrients in plant material after growing test crops in three soils with a legacy of crop rotation are illustrated in Table 6.4.

Table 6.4 Plant tissue concentrations (in the last crop of the rotation sequence) of macronutrients (Mean $\pm$ SE) obtained from the soil with a history of crop rotation. Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S). N = 3 composite samples. SE = standard error. *Zea mays* (Ze), *Phaseolus vulgaris* (Ph), *Glycine max* (Gl) and *Helianthus annuus* (He). Field soil (§), sterilized soil (£) and inoculated soil (§).

Plant origin	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
GlGlGl§	2.67 ± 0.06	0.163 ± 0.00	0.803 ± 0.03	1.77 ± 0.03	0.27 ± 0.03	0.063 ± 0.00
GlHeGl£	3.43 ± 0.11	0.18 ± 0.01	0.503 ± 0.02	2.16 ± 0.06	0.373 ± 0.02	0.077 ± 0.01
GlHeGl§	3.11 ± 0.17	0.183 ± 0.01	0.62 ± 0.05	1.93 ± 0.08	0.427 ± 0.00	0.07 ± 0.01
GlHeGl§	2.6 ± 0.23	0.143 ± 0.01	0.463 ± 0.02	1.95 ± 0.05	0.297 ± 0.00	0.063 ± 0.00
HeHeHe£	1.48 ± 0.06	0.26 ± 0.01	1.9 ± 0.06	4.61 ± 0.10	0.707 ± 0.02	0.16 ± 0.01
HeHeHe§	1.18 ± 0.04	0.293 ± 0.01	1.86 ± 0.13	4.57 ± 0.03	0.657 ± 0.04	0.14 ± 0.01
HeHeHe§	1.4 ± 0.00	0.263 ± 0.01	1.7 ± 0.02	4.68 ± 0.14	0.757 ± 0.01	0.127 ± 0.01
PhHePh£	1.53 ± 0.09	0.47 ± 0.05	0.943 ± 0.21	3.34 ± 0.24	0.31 ± 0.01	0.077 ± 0.00
PhHePh§	1.12 ± 0.05	0.37 ± 0.01	0.77 ± 0.08	3.1 ± 0.30	0.31 ± 0.01	0.063 ± 0.00
PhHePh§	1.59 ± 0.18	0.437 ± 0.01	1.19 ± 0.12	2.91 ± 0.24	0.33 ± 0.02	0.073 ± 0.01
ZeHeZe£	1.99 ± 0.65	0.237 ± 0.00	0.803 ± 0.05	0.657 ± 0.06	0.303 ± 0.01	0.057 ± 0.01
ZeHeZe§	0.647 ± 0.04	0.22 ± 0.01	1 ± 0.04	0.5 ± 0.02	0.253 ± 0.00	0.05 ± 0
ZeHeZe§	0.813 ± 0.04	0.247 ± 0.01	0.75 ± 0.04	0.62 ± 0.03	0.323 ± 0.02	0.053 ± 0.00
ZeZeZe£	0.703 ± 0.04	0.237 ± 0.00	0.733 ± 0.03	0.603 ± 0.03	0.347 ± 0.02	0.053 ± 0.00
ZeZeZe§	0.683 ± 0.05	0.21 ± 0.01	0.913 ± 0.11	0.477 ± 0.03	0.263 ± 0.01	0.057 ± 0.01
ZeZeZe§	0.8 ± 0.03	0.233 ± 0.01	0.823 ± 0.08	0.683 ± 0.10	0.35 ± 0.02	0.06 ± 0.01

Table 6.5 Plant tissue concentrations (in last crop in the rotation sequence) of micronutrients (Mean $\pm$ SE) obtained from the soil with a history of crop rotation. Sodium (Na), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), molybdenum (Mo) and boron (B). N = 3 composite samples. SE = standard error. *Zea mays* (Ze), *Phaseolus vulgaris* (Ph), *Glycine max* (Gl) and *Helianthus annuus* (He). Field soil ($\ddagger$), sterilized soil (£) and inoculated soil (§).

Plant origin	Na mg/kg	Fe mg/kg	Mn mg/kg	Cu mg/kg	Zn mg/kg	Mo mg/kg	B mg/kg
GlGlGl $\ddagger$	94.3 ± 2.33	72 ± 8.74	426 ± 37.1	3 ± 0	50.3 ± 4.26	0.553 ± 0.04	50.3 ± 3.18
GlHeGl£	92.3 ± 1.45	51 ± 4.58	584 ± 33.6	3.33 ± 0.33	56.7 ± 5.21	0.567 ± 0.16	62.7 ± 1.2
GlHeGl§	109 ± 15.8	70 ± 5.51	471 ± 27.4	3 ± 0	49 ± 1	0.387 ± 0.03	56.7 ± 1.33
GlHeGl $\ddagger$	91 ± 1.53	53 ± 1.15	510 ± 15.3	3 ± 0	51.7 ± 0.88	0.323 ± 0.04	55.7 ± 4.33
HeHeHe£	125 ± 6.64	228 ± 38.4	1379 ± 44.2	12.7 ± 0.88	64 ± 2.31	10.3 ± 0.27	155 ± 0.58
HeHeHe§	98.3 ± 0.33	115 ± 13.4	1394 ± 270	12.7 ± 0.33	42 ± 5.13	8.96 ± 0.94	143 ± 4.63
HeHeHe $\ddagger$	113 ± 11.6	133 ± 10.7	1095 ± 95.2	14 ± 0.58	61 ± 7.51	10.8 ± 0.82	170 ± 9.07
PhHePh£	105 ± 2.08	148 ± 13	1333 ± 234	6.33 ± 0.67	44.7 ± 7.51	1.22 ± 0.30	62.7 ± 6.57
PhHePh§	106 ± 2.31	112 ± 8.45	1330 ± 93.7	6.33 ± 0.88	34.7 ± 4.98	0.803 ± 0.28	55.7 ± 5.17
PhHePh $\ddagger$	110 ± 6.36	126 ± 5.17	921 ± 136	6.33 ± 0.88	37.3 ± 6.89	1.27 ± 0.25	55.7 ± 5.24
ZeHeZe£	94.7 ± 1.45	167 ± 20.5	223 ± 28	3.33 ± 0.33	15.3 ± 1.2	2.34 ± 0.25	23.7 ± 1.86
ZeHeZe§	100 ± 6.84	169 ± 31.9	240 ± 18.6	3 ± 0	13.3 ± 0.33	2.25 ± 0.11	21.7 ± 0.33
ZeHeZe $\ddagger$	94.3 ± 4.84	189 ± 16.7	205 ± 11.9	3 ± 0	14 ± 0.58	2.49 ± 0.21	24.3 ± 0.33
ZeZeZe£	90.3 ± 2.19	131 ± 10.3	174 ± 14.4	3 ± 0	16 ± 0	2.53 ± 0.22	21.7 ± 1.76
ZeZeZe§	90.7 ± 0.67	116 ± 18.5	190 ± 25.4	3.33 ± 0.33	15.7 ± 0.67	1.58 ± 0.07	21.7 ± 1.76
ZeZeZe $\ddagger$	90 ± 2.31	141 ± 14.6	190 ± 11.5	3 ± 0	16.7 ± 0.67	2.29 ± 0.12	27.3 ± 3.33

6.4.5 Comparison of Plant Tissue Nutrient composition across and within Phytometers in response to Crop Rotation in Field Soil, Sterilized and Inoculated Soil

Phytometer data differed significantly in tissue concentration of most macronutrients following cultivation in field soil, sterilized soil and inoculated soil ($p < .05$, Table S6.2) except S in field soil ($p > .05$, Table S6.2). Likewise, phytometers differed significantly for most trace elements following cultivation in field soil, sterilized soil and inoculated soil ($p < .05$, Table S6.3) except Na and B for phytometers cultivated in field soil ($p > .05$, Table S6.3). Comparison of plant tissue nutrient composition within *Z. mays* rotations in response to crop rotation in field soil, sterilized and inoculated soil indicate that only Mg concentration differed significantly ($p < .05$, Table S6.4) while no significant differences were detected for micronutrient compositions ($p > .05$, Table S6.5).

Plant tissue nutrient composition within *P. vulgaris* rotations in response to crop rotation in field soil, sterilized and inoculated soil did not differ significantly in macro- or micro-nutrient concentrations ($p > .05$, Table S6.6 and Table S6.7). Comparison of plant tissue macronutrient composition within *G. max* rotations showed significant differences in response to crop rotation in field soil, sterilized soil and inoculated soil ($p < .05$, Table S6.8) except in N and S concentrations. There were no significant differences in plant micronutrient concentrations among soils ($p > .05$, Table S6.9). Comparison of plant tissue nutrient composition within *H. annuus* rotations in response to crop rotation in field soil, sterilized and inoculated soil showed no significant differences ($p > .05$, Table S6.10 and Table S6.11).

Non-metric multidimensional scaling (NMDS) displayed that the plant nutrient concentration was impacted by phytometers cultivated in three different soil treatments with a history of crop rotations performed under greenhouse conditions. There were clear statistically significant differences between phytometers separated in Figure 6.6 by macronutrients and in Figure 6.7 by micronutrients ($p < .05$, Table S6.2). The unlabelled first NMDS axes are strongly correlated

with macronutrient legacies in harvested plant material (Figure 6.6; stress: 0.0576, k = 2).

Figure 6 shows the NMDS of the macronutrient concentrations in plant tissue after growing test crops in three soils with a legacy of crop rotation.

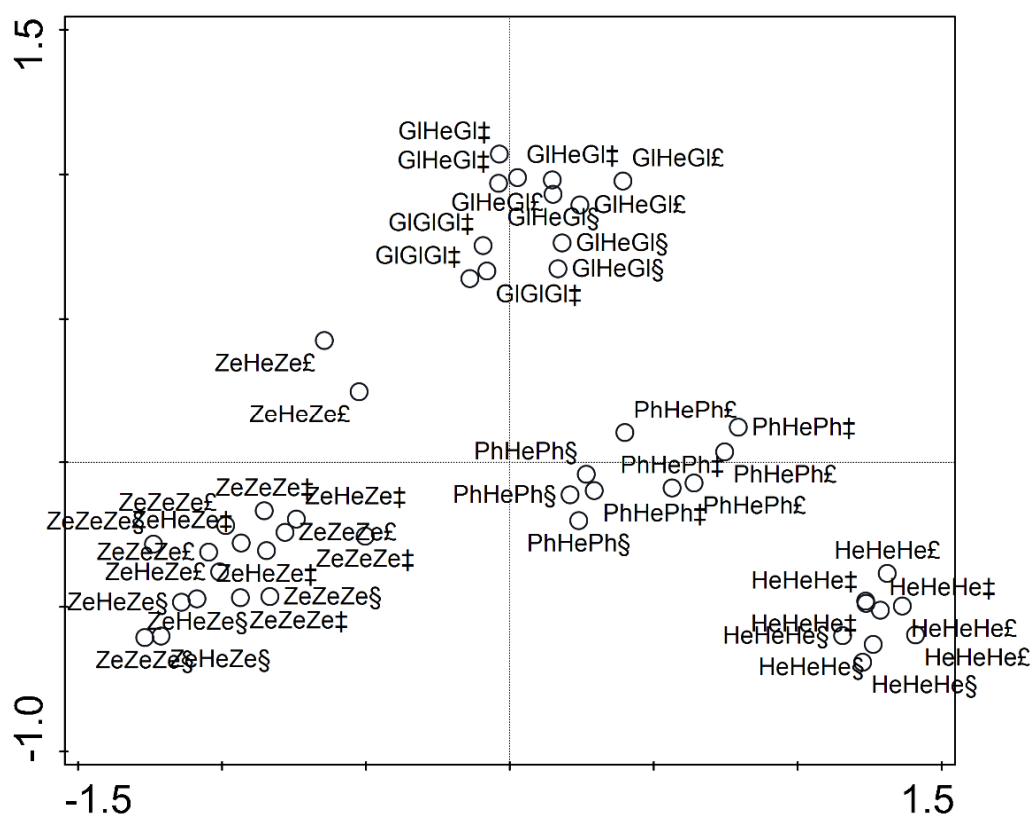


Figure 6.6 Non-metric multidimensional scaling (NMDS) ordination diagram of the Bray–Curtis dissimilarities with two computed axes of macronutrient (N, P, K, Ca, Mg and S) concentrations in plant tissue after cultivation of phytometers (last crop in the rotation sequence) in; field soil (‡), sterilized soil (£) and inoculated soil (§) cultivated in various crop rotations (O) under greenhouse conditions. *Zea mays* (Ze), *Phaseolus vulgaris* (Ph), *Glycine max* (Gl) and *Helianthus annuus* (He).

The unlabelled first NMDS axes are strongly correlated with micronutrient legacies in harvested plant material (Figure 6.7; stress: 0.0429, k = 2). The NMDS of the trace elements or micronutrient concentrations in plant tissue clustered by test crops in three soils with a legacy of crop rotation (Figure 6.7). The major observation of the nutrient concentrations in plant tissue show that some dryland crop species have a higher efficiency of uptake of mineral elements from the same soil than others (Fageria et al., 2010; Fageria, 2013; Fageria, 2016).

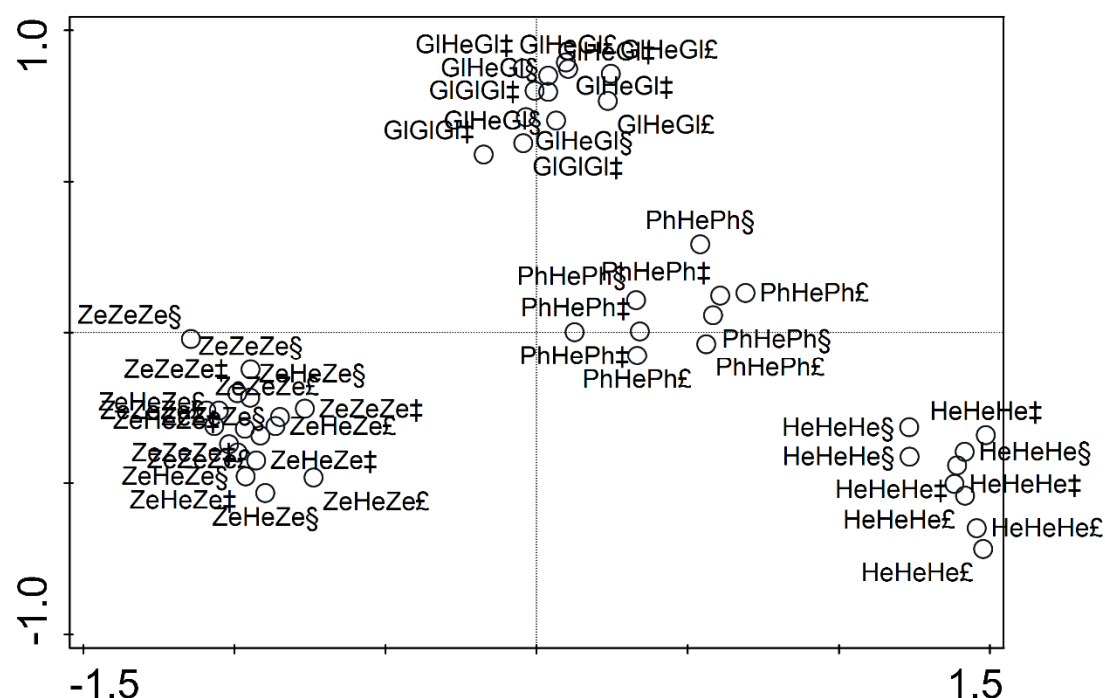


Figure 6.7 Non-metric multidimensional scaling (NMDS) ordination diagram of the Bray–Curtis dissimilarities with two computed axes of micronutrient (Na, Fe, Mn, Cu, Zn, Mo and B) concentrations in plant tissue after cultivation of phytometers (last crop in the rotation sequence) in; field soil (‡), sterilized soil (£) and inoculated soil (§) cultivated in various crop rotations (O) under greenhouse conditions. *Zea mays* (Ze), *Phaseolus vulgaris* (Ph), *Glycine max* (Gl) and *Helianthus annuus* (He).

The high efficiency of uptake of N by *G. max* was observed in sterilized soil and inoculated soil cultivated with historical legacies of GlHeGl crop rotation (Figure 6.8). On the other hand, all *Z. mays* crop sequences are ordinated and located opposite to the N property suggesting relatively low uptake of N by *Z. mays* in the same soils (Figure 6.8).

Figure 6.8 shows the Principal Component Analysis (PCA) of the macronutrient (N, P, K, Ca, Mg and S) concentrations in plant tissue after growing test crops (last crop in the sequence) in three soils with a legacy of crop rotation. There was high efficiency of uptake of P and K by *P. vulgaris* observed in field soil and sterilized soil cultivated with historical legacies of PhHePh rotation (Figure 6.8).

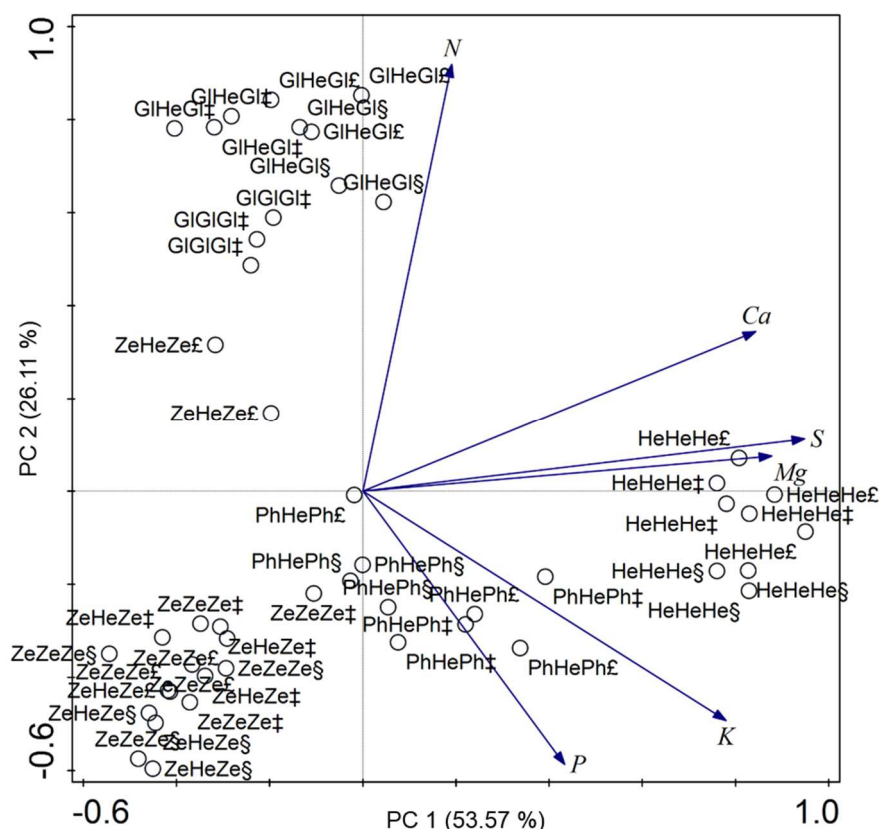


Figure 6.8 Principal component analysis biplot diagram separating various crop rotations (O) based on data scores from macronutrient concentrations in plant tissue after cultivation of phytometers (last crop in the rotation sequence) in; field soil (†), sterilized soil (£) and inoculated soil (§) under greenhouse conditions. *Zea mays* (Ze), *Phaseolus vulgaris* (Ph), *Glycine max* (Gl) and *Helianthus annuus* (He). The blue arrows represent the plant element content legacies where:

N – Nitrogen (%)	P – Phosphorus (%)	K – Potassium (%)
Ca – Calcium (%)	Mg – Magnesium (%)	S – Sulphur (%)

In the same rotation, a high efficiency of uptake by *P. vulgaris* was also observed for Zn in the sterilized and inoculated soil (Figure 6.9). The *H. annuus* crop displayed the most popular and highest efficiency of uptake of macronutrients such as Ca, Mg, S in the sterilized and inoculated soil (Figure 6.8) and high micronutrient uptake for Fe, Cu, Na, Mo, Mn and B in all the soils cultivated in historical legacies of the HeHeHe crop rotation (Figure 6.9).

Figure 6.9 shows the PCA of the trace elements or micronutrients (Na, Fe, Mn, Cu, Zn, Mo and B) concentrations in plant tissue after growing test crops (last crop in the sequence) in three soils with a legacy of crop rotation.

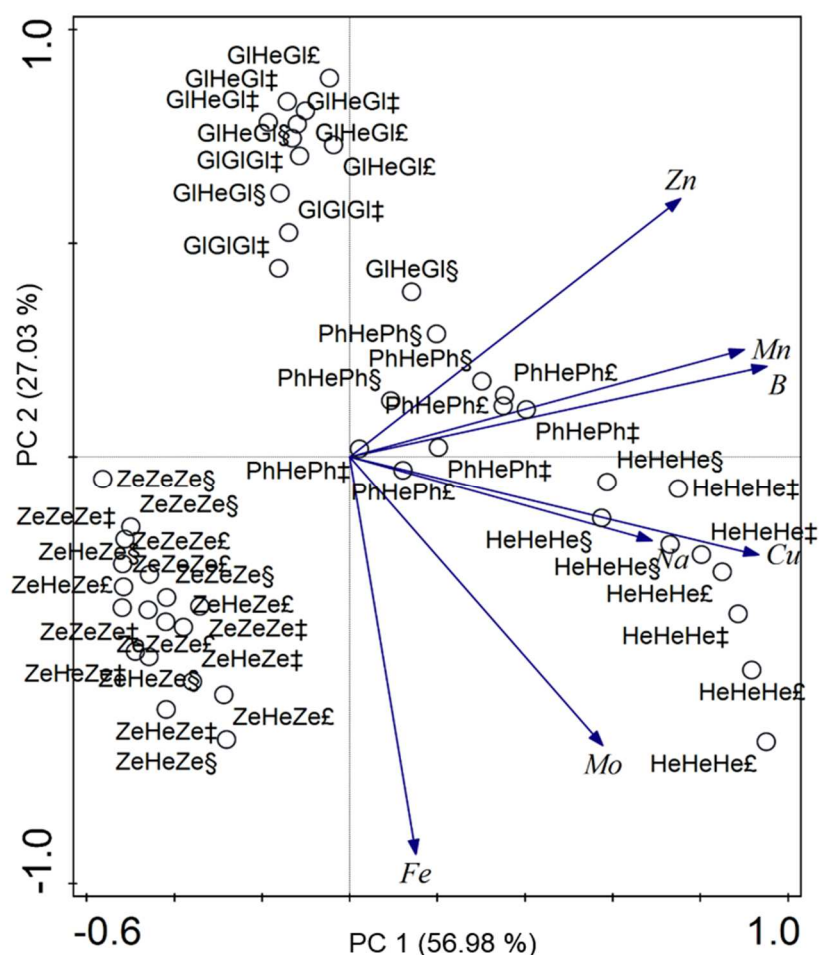


Figure 6.9 Principal component analysis biplot diagram separating various crop rotations (O) based on data scores from micronutrient concentrations in plant tissue after cultivation of phytometers (last crop in the rotation sequence) in; field soil (§), sterilized soil (£) and inoculated soil (§) under greenhouse conditions. *Zea mays* (Ze), *Phaseolus vulgaris* (Ph), *Glycine max* (Gl) and *Helianthus annuus* (He). The blue arrows represent the plant element content legacies where:

Na – Sodium (mg/kg)	Fe – Iron (mg/kg)	Mn – Manganese (mg/kg)
Cu – Copper (mg/kg)	Zn – Zinc (mg/kg)	Mo – Molybdenum (mg/kg)
B – Boron (mg/kg)		

The high efficiency of uptake of Fe by *Z. mays* was observed in field soil, sterilized soil and inoculated soil cultivated with historical legacies of ZeHeZe crop rotation (Figure 6.9).

Overall plant nutrient analysis results suggest that *H. annuus* seems to be a highly efficient dryland crop species in the uptake of trace and macronutrient elements in the same soil.

6.4.6 Suppression of *R. solani* AG 2-2 IIIB Mycelial Growth in Three Soils with a Legacy of Greenhouse Crop Rotations using the *in vitro* Growth Assay

There was higher inhibition of mycelial growth of *R. solani* AG 2-2 IIIB within soils originating from *G. max* and *H. annuus* crop rotations than in soils from *P. vulgaris* and *Z. mays* rotations. All soils were significantly different in *R. solani* AG 2-2 IIIB colony diameter and % suppression from the control ($p < .05$) according to Tukey's HSDtest (Figure 6.10 to Figure 6.13). However, comparisons of differences in % suppression of mycelial growth were done according to crop type across three soils; field soil (§) contained microbiota and nutrients, sterilized soil (£) contained nutrients only and inoculated soil (§) contained microbiota only. Figure 6.10 shows boxplots of colony diameter (mm) and percent suppression of mycelial growth of *R. solani* AG 2-2 IIIB in three soils with a legacy of *Z. mays* crop rotation using the *in vitro* growth assay.

Biotic soil content legacies (the inoculated soil treatment) of *Z. mays* monoculture were most conducive to mycelial growth of *R. solani* AG2-2IIIB (Figure 6.10) whereas the microbiota cultivated in *H. annuus* and *G. max* monocultures were the most suppressive (Figure 6.12 and Figure 6.13). Soil nutrient content legacies (in sterilized soil treatment) of *P. vulgaris* crop rotations were most conducive to mycelial growth of *R. solani* AG2-2IIIB (Figure 6.11) while the most suppressive of monocultures in the same soil treatment were *G. max* and *H. annuus* (Figure 6.12 and Figure 6.13). Figure 6.11 shows boxplots of colony diameter (mm) and percent suppression of mycelial growth of *R. solani* AG 2-2 IIIB in three soils with a legacy of *P. vulgaris* crop rotation using the *in vitro* growth assay.

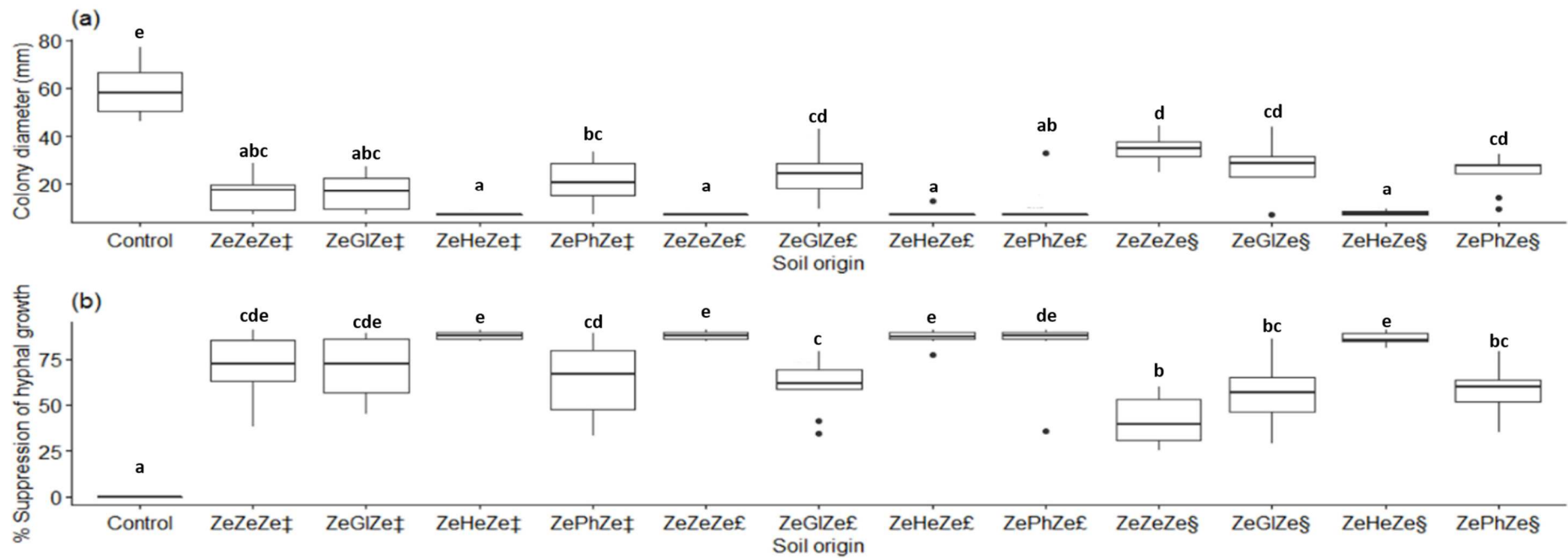


Figure 6.10 Boxplots of *Rhizoctonia solani* AG 2-2 IIIB *in vitro* (a) colony diameter and (b) % suppression of mycelial growth in; field soil (‡), sterilized soil (£) and inoculated soil (\$) cultivated in *Zea mays* crop rotations under greenhouse conditions. Control (sterile sand), *Zea mays* (Ze), *Phaseolus vulgaris* (Ph), *Glycine max* (Gl) and *Helianthus annuus* (He). Different letters represent significant differences ($p < .05$) among crop rotation combinations or a strong PSF value while shared letters represent non-significant differences ($p > .05$) among crop rotation combinations or a weak PSF value based on Tukey's HSD test ($p < .05$, $p < .001$). Each rotation sequence treatment had 10 replications. Horizontal heavy bar = median, box=lower and upper quartiles, whiskers=minimum and maximum values, filled dot=outliers.

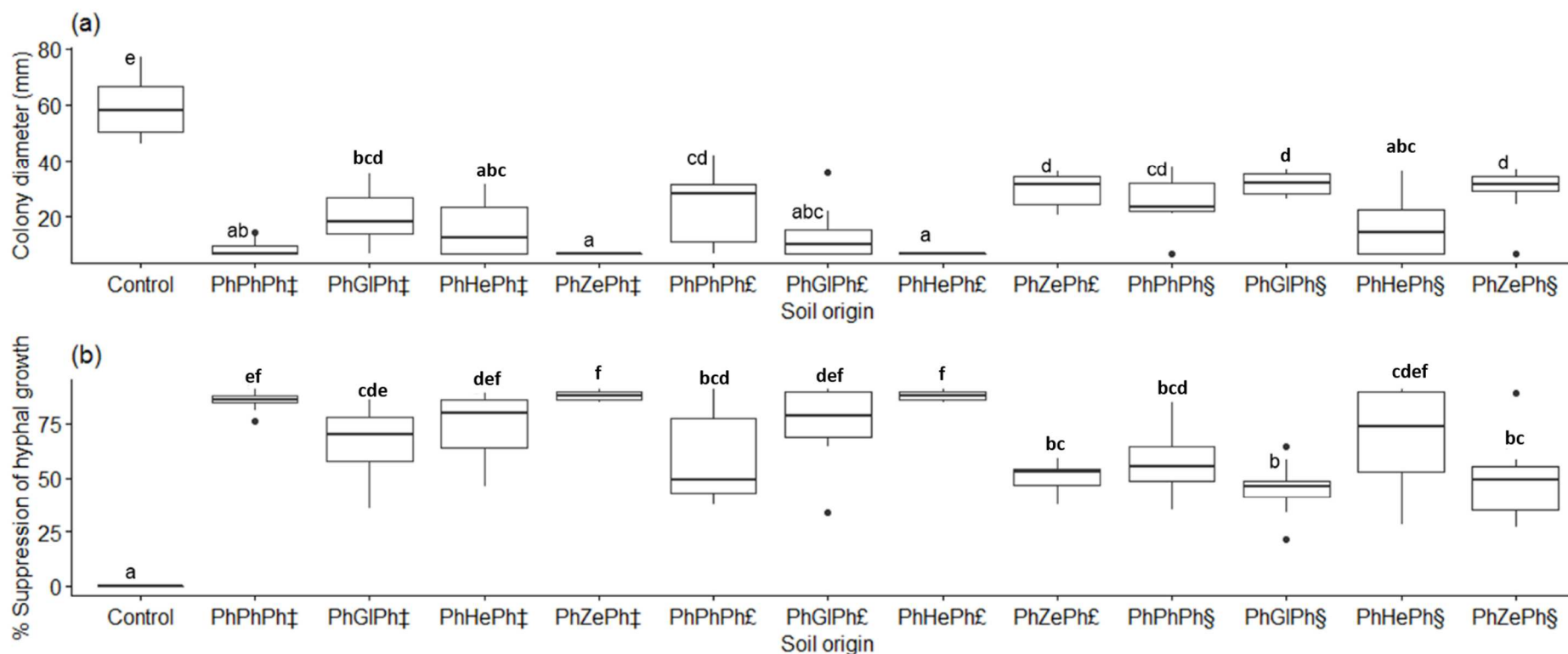


Figure 6.11 Boxplots of *Rhizoctonia solani* AG 2-2 IIIB *in vitro* (a) mycelial diameter and (b) % suppression of mycelial growth in; field soil (‡), sterilized soil (£) and inoculated soil (§) cultivated in *Phaseolus vulgaris* crop rotations under greenhouse conditions. Control (sterile sand), *Zea mays* (Ze), *Phaseolus vulgaris* (Ph), *Glycine max* (Gl) and *Helianthus annuus* (He). Different letters represent significant differences ($p < .05$) among crop rotation combinations or a strong PSF value while shared letters represent non-significant differences ($p > .05$) among crop rotation combinations or a weak PSF value based on Tukey's HSD test ($p < .05$, $p < .001$). Each rotation sequence treatment had 10 replications. Horizontal heavy bar = median, box=lower and upper quartiles, whiskers=minimum and maximum values, filled dot=outliers.

Combined soil nutrients and biota content legacies (in field soil treatment) of *G. max* crop rotations were most conducive to mycelial growth of *R. solani* AG 2-2IIIB (Figure 6.12) whereas the most suppressive were *Z. mays* and *H. annuus* rotations (Figure 6.10 and Figure 6.13). Figure 6.12 shows boxplots of mycelial growth (mm) and percent suppression of mycelial growth of *R. solani* AG 2-2 IIIB in three soils with a legacy of *G. max* crop rotation using the *in vitro* growth assay.

The boxplots display the differences in mycelial growth (mm) and percent suppression of mycelial growth of *R. solani* AG 2-2 IIIB in three soils with a legacy of *H. annuus* crop rotation using the surface petri-plate method (Figure 6.13).

From the *in vitro* suppression assay results (Figure 6.10 to Figure 6.13), *H. annuus* and *G. max* crop rotations seem to cultivate soil microbiota legacies that relatively inhibit the mycelial growth of *R. solani* AG 2-2 IIIB. In addition, *H. annuus* and *G. max* crop rotations seem to cultivate soil nutrient legacies that relatively inhibit the mycelial growth of *R. solani* AG 2-2 IIIB. *Zea mays* and *H. annuus* crop rotations seem to cultivate both soil nutrient and microbiota legacies that relatively inhibit the mycelial growth of *R. solani* AG 2-2 IIIB. The result indicates that commercial growers can include *H. annuus* and *G. max* in dryland crop rotations to cultivate a disease suppressive soil against *R. solani* AG 2-2IIIB.

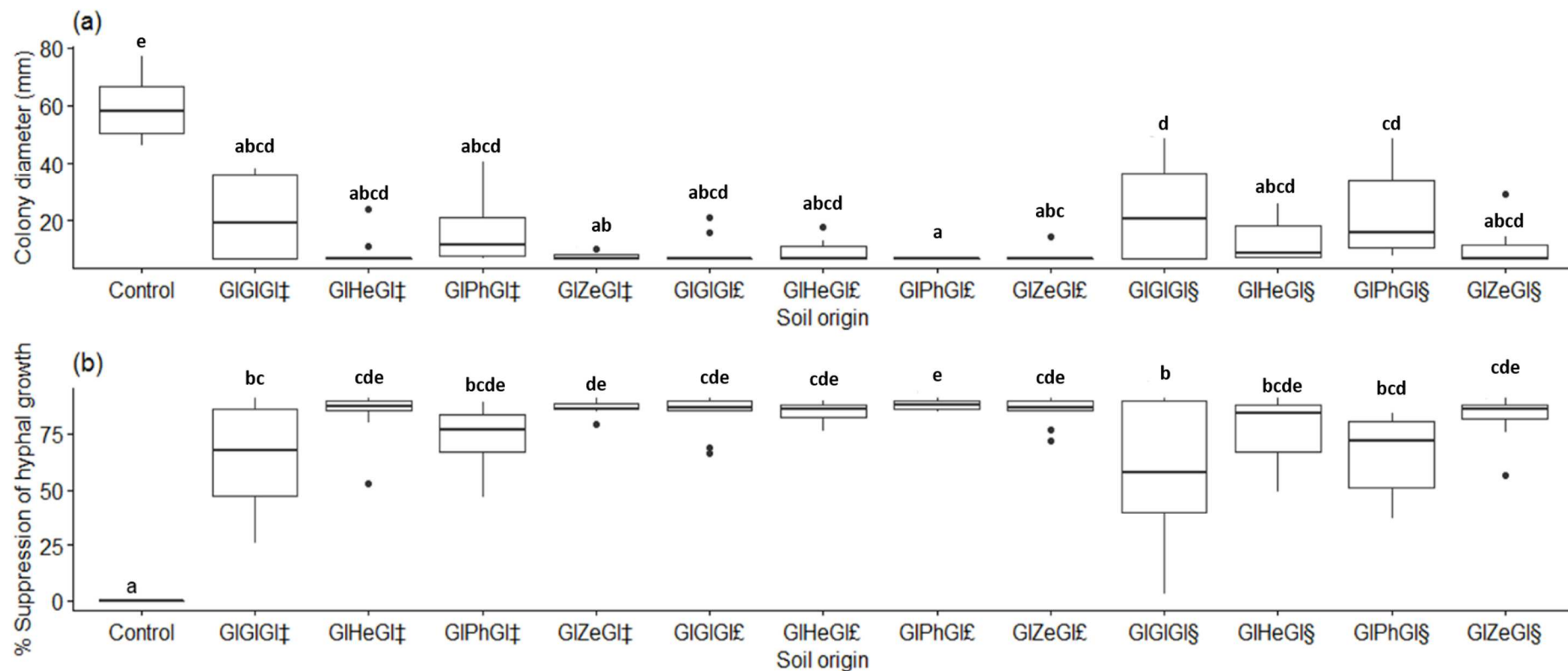


Figure 6.12 Boxplots of *Rhizoctonia solani* AG 2-2 IIIB *in vitro* (a) mycelial diameter and (b) % suppression of mycelial growth in; field soil (‡), sterilized soil (£) and inoculated soil (§) cultivated in *Glycine max* crop rotations under greenhouse conditions. Control (sterile sand), *Zea mays* (Ze), *Phaseolus vulgaris* (Ph), *Glycine max* (Gl) and *Helianthus annuus* (He). Different letters represent significant differences ($p < .05$) among crop rotation combinations or a strong PSF value while shared letters represent non-significant differences ($p > .05$) among crop rotation combinations or a weak PSF value based on the Tukey's HSD test ($p < .05$, $p < .001$). Each rotation sequence treatment had 10 replications. Horizontal heavy bar = median, box=lower and upper quartiles, whiskers=minimum and maximum values, filled dot=outliers.

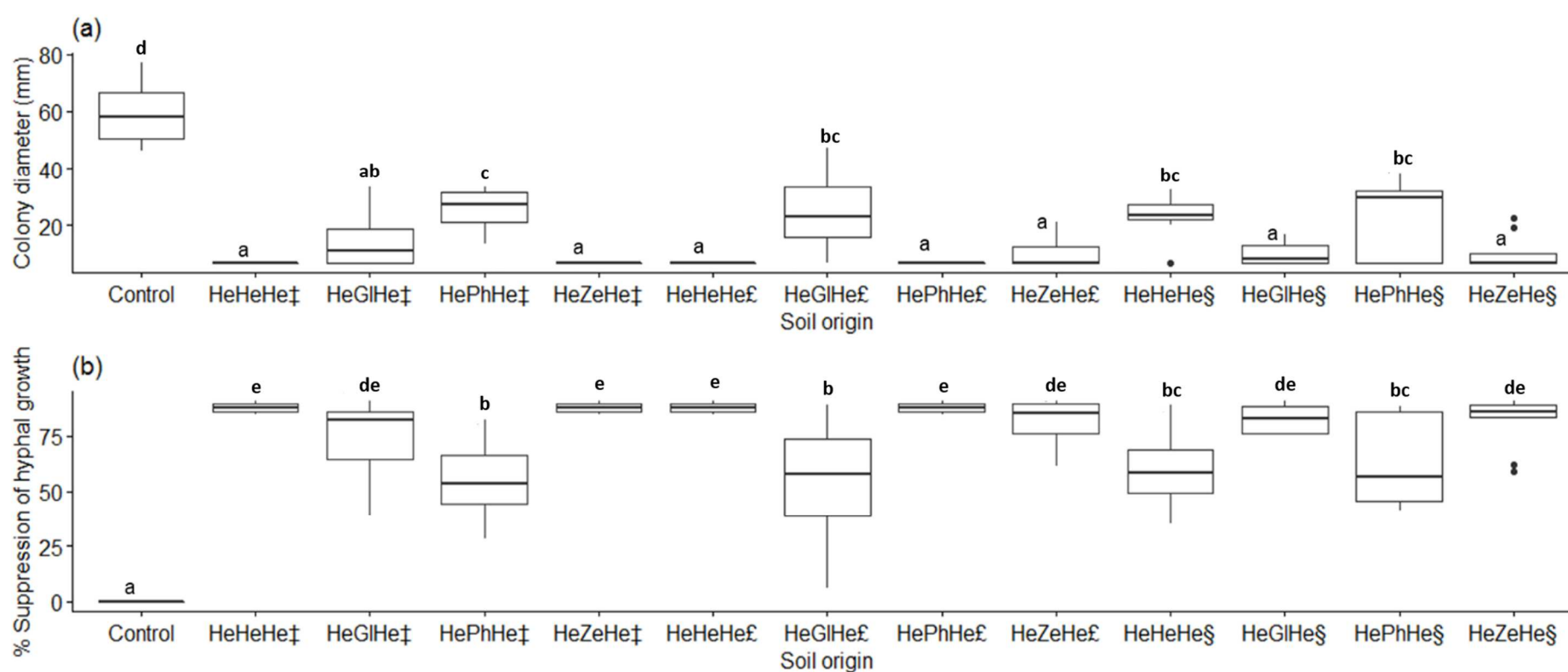


Figure 6.13 Boxplots of *Rhizoctonia solani* AG 2-2 IIIB *in vitro* (a) mycelial diameter and (b) % suppression of mycelial growth in; field soil (‡), sterilized soil (£) and inoculated soil (\$) cultivated in *Helianthus annuus* crop rotations under greenhouse conditions. Control (sterile sand), *Zea mays* (Ze), *Phaseolus vulgaris* (Ph), *Glycine max* (Gl) and *Helianthus annuus* (He). Each rotation sequence treatment had 10 replications. Different letters represents significant differences ($p < .05$) among crop rotation combinations or a strong PSF value while shared letters represents non-significant differences ($p > .05$) among crop rotation combinations or a weak PSF value based on the Tukey's HSD test ($p < .05$, $p < .001$). Horizontal heavy bar = median, box=lower and upper quartiles, whiskers=minimum and maximum values, filled dot=outliers.

6.5 Discussion

6.5.1 Effects of Plant-Soil Feedback Legacies on Plant Performance

Crop rotations were significantly dissimilar in phytometer biomass of *P. vulgaris* and there was a 26–90% increase from monoculture. Compared to monoculture biomass, the ZePhZe rotation significantly increased biomass by 45% while ZeGlZe significantly decreased biomass by 9%. There were increases in biomass for the HeZeHe and the HeGlHe rotations of 6% and 13%, respectively while biomass was reduced in HePhHe rotation relative to monoculture biomass. Similar research has indicated that more diverse rotations increase *Z. mays* and *G. max* yields by 15-25% compared to less diverse rotations (Benitez et al., 2021). The phytometer results suggest that legume rotations are better than monoculture for dryland production, in particular *G. max* is good for *H. annuus* rotations while *P. vulgaris* is an ideal rotation crop for *Z. mays* growers. In other studies, *G. max* yields increased by 16% in crop rotations compared to monoculture (Kelley et al., 2003). In other studies, *G. max* yields increased by 16% in crop rotations with grasses (wheat and sorghum) compared to monoculture (Kelley et al., 2003). In summer rainfall area of SA, the fallow, wheat, sunflower and soybean rotation effects include changes in water availability and nitrogen, soil microbial composition, weeds pressure and diseases that increase maize yields more than in maize monoculture cropping by maintaining a healthy root system (Nel & Loubser, 2004; Nel, 2005).

6.5.2 Crop Rotation Resilience and Resistance to Monoculture under different Soil Health Legacies

Plant-soil interactions at the rhizosphere interface are crucial for plant growth and performance. Sugar beans cultivate soil legacies that improve plant performance in subsequent cultivation of other dryland crops under greenhouse conditions (Mushonga et al., 2020). These results suggest that *P. vulgaris* in *H. annuus* crop rotations offers resilience to pathogens associated with *H. annuus* monoculture. Contrary, the soil legacies from *G. max* and *H. annuus* crop rotations

were slightly affected after continuous cultivation and were thus highly resilient compared to soil originating from *P. vulgaris* and *Z. mays* rotations, an example of general soil resilience (Seybold et al., 1999). Different plant types cultivate specific soil microbial diversity legacies of mutualists, pathogens and decomposers, which affect subsequent plant performance in the short- or long term (Veen et al., 2019). Sunflower is resilient in a warm dry climate and in drought years (Mathews, 2018), suggesting that plant-soil feedback effects of sunflower would more likely be a result of root physiology than drought effects (Fry et al., 2018; Bardgett & Caruso, 2020).

6.5.2.1 Inoculated Soils (Soil Microbiota Effects)

In inoculated soils, the ZeGlZe rotation had very strong positive plant-soil feedback and high resistance to monoculture while the ZeHeZe and ZePhZe rotations had weak positive plant-soil feedback and low resistance. The greatest magnitude of positive plant–soil feedbacks was observed in the HePhHe crop rotation, possibly due to beneficial soil microbiota or biocontrol agents from the *Sphingomonas* and *Rhizobium* genera (Oberholster et al., 2018). In a related field study in the Eastern Free State, agricultural soils had high levels of *Proteobacteria* associated with plant fitness and plant growth promotion including *Bacilli*, *Burkholderia*, *Pseudomonas*, *Serratia*, *Streptomyces*, and *Stenotrophomonas* species (Dube et al., 2019).

According to Stirling et al. (2016), soils with higher populations of antifungal- or antibiotic-producers support more plant growth than soils with low population levels of the Pseudomonads. These bacteria outcompete fungal pathogens in capturing mineral Fe (via siderophores) and they also attack fungal pathogens via antibiotics phenazine-1-carboxylic acid (PCA) and 2,4-diacetylphloroglucinol (DAPG) at the root interface (Deacon, 2006). Soil biodiversity enhances soil health by suppressing soil borne pathogens through competition, predation, parasitism, antibiosis, toxin production and lysis (Stirling et al., 2016). Soil biota enhance plant biomass, nutrient contents, and crop yields (Bender et al., 2015).

6.5.2.2 Field Soils (Soil Nutrients and Microbiota Effects)

Soil sterilization showed that under monoculture *G. max* phytometer biomass increased significantly compared to unsterilized soil of the same field soil origin, suggesting that harmful soil microbiota were responsible for soil sickness and subsequent negative plant growth in *G. max* monoculture (Dias et al., 2015; Liu et al., 2020). Plant–soil feedbacks in field soils were negative for PhPhPh monoculture and positive for the PhHePh and PhZePh crop rotations.

The magnitude of PSF results in field soils were low for *Z. mays* and *H. annuus* monoculture suggesting resistance to pathogens following repeated cultivation of *Z. mays* and *H. annuus*, respectively. According to Stirling et al. (2016), soil borne pathogens cause a 15-30% reduction in yields and sometimes lead to total crop loss. Resistance to and recovery are improved in *Z. mays* and *H. annuus* when rotated with legumes under controlled conditions. Increasing diversity of crops in rotation improves resilience in agroecosystems (Seipel et al., 2019).

6.5.3 Comparison of Plant Tissue Nutrient Composition across and within Phytometers in Response to Crop Rotation in Field Soil, Sterilized and Inoculated Soil

Tissue nutrient analysis indicated that relative to other crop species, *H. annuus* has root traits that enabled it to be the best “miner” of most mineral elements (macro-and micronutrients) while *Z. mays* accumulates mostly Fe, *G. max* is a superior miner of N and *P. vulgaris* of P obtained from soil with a history of crop rotation. The quantity and the uptake properties of root surface area determines nutrient acquisition from the substrate (Lambers et al., 2008).

There was evidence of high efficiency in the uptake of N by *G. max* under the GIHeGI crop rotation system possibly due to biological N from the *B. japonicum* WB74 strain which is the preferred soil inoculant for commercial *G. max* production (van der Hoven, 2018). On the other hand, *P. vulgaris* is generally inoculated by the N-fixing *Rhizobium phaseoli* species (Fageria et al., 2010) but has poor and variable N fixation compared to *G. max* (Slatni et al., 2008). On

the other hand, all *Z. mays* crop sequences showed relatively low uptake of N by *Z. mays* in the same soil treatments.

There was a high efficiency of uptake of P and K by *P. vulgaris* phytometer observed in field soil and sterilized soil cultivated with historical legacies of the PhHePh rotation. Nutrient acquisition for example of immobile nutrients like P in beans is associated with soil colonization by plant roots (Fageria et al., 2010). In the same rotation, a high efficiency of uptake by *P. vulgaris* phytometer was also observed for Zn in the sterilized and inoculated soil. The high concentration of K and P in plant material of *P. vulgaris* from the PhHePh rotation may be ideal for reducing the negative impact of *Rhizoctonia*. Studies on sheath blight suggest that high K tissue concentrations in rice increase resistance to *Rhizoctonia* damage (Schurt et al., 2015; Gupta et al., 2017). In addition, root rot disease by *Rhizoctonia* was reduced in wheat with high tissue concentrations of P (Kirkegaard et al., 1999; Gupta et al., 2017).

The *H. annuus* phytometer crop displayed high efficiency in the uptake of macronutrients such as Ca, Mg, S in the sterilized and inoculated soil and high micronutrient uptake for Fe, Cu, Na, Mo, Mn and B in all the soils cultivated in historical legacies of HeHeHe monoculture. In other studies, N composition in *H. annuus* straw averaged 0.69-1.26% (Keipp et al., 2019), which is comparable to 1.18-1.48% in plant tissue concentrations for *H. annuus* in the present study. Although N uptake is dynamic in space and time, our findings suggest that sunflower in crop rotation maintains its inherent physiological root activity traits of resource uptake. Plant root activity is dynamic; differing among soil horizons and varying spatially and temporally among species and functional types (Freschet et al., 2021). There was a high efficiency of uptake of Fe by *Z. mays* phytometer in the ZeHeZe crop rotation. Overall plant nutrient analysis results suggest that *H. annuus* is a highly efficient dryland crop species in the uptake of trace and macronutrient elements from the same soil.

6.5.4 Suppression of *R. solani* AG 2-2 IIIB Mycelial Growth in Three Soils with a Legacy of Greenhouse Crop Rotations *in vitro* Growth Assay

A typical suppressive soil consistently lowers the incidence of disease in certain patches of the field (Ko & Ho, 1983; De Boer et al., 1998). In the present study, field soils were cultivated with different crop treatment legacies, we thus hypothesized that suppressiveness to *R. solani* will vary from one treatment to another. There was higher inhibition of mycelial growth of *R. solani* AG 2-2 IIIB within soils originating from *G. max* and *H. annuus* crop rotations than in soils from *P. vulgaris* and *Z. mays* rotations. All soils were significantly different when compared to the control in suppressing *R. solani* AG 2-2 IIIB colony diameter and % suppression. Soil-borne pathogen suppressive soils have microbial composition, diversity and activities which can delay disease manifestation despite the presence of the pathogen and a susceptible host (Larkin, 2017).

Biotic soil legacies (the inoculated soil treatment) of *Z. mays* monoculture were most conducive to mycelial growth of *R. solani* AG2-2IIIB whereas the microbiota cultivated in *H. annuus* and *G. max* monocultures were the most suppressive. Suppression of *R. solani* has been reported with various fungi and bacteria viz: *Bacillus* spp. (Sneh et al., 1996), the genus *Lysobacter* species (Ciancio et al., 2016), *Pseudomonas fluorescence* (Sneh et al., 1996) and fungi of *Gliocladium* spp. (Lewis and Lumsden, 2001), *Trichoderma* spp. (Lamichhane et al., 2017) and *Verticillium biguttatum* (Sneh et al., 1996); mycophagous nematodes (Sneh et al., 1996) and binucleate *Rhizoctonia* (Sneh et al., 1996). Antibiotic compounds, removal of pathogen stimulating compounds, nutrient competition and extracellular lytic enzymes by ‘rhizotypes’ such as *Proteobacteria*, *Firmicutes* and *Ascomycota* impart soil suppressiveness to soil-borne pathogens (Raaijmakers et al., 2009; Berendsen et al., 2012; Dube et al., 2019).

In our previous two-phase PSF study, *H. annuus* was superior to other dryland crops in creating an enzymatically active soil, active carbon stocks and elevated fungal to bacterial ratios while *Z. mays* supported the highest total fungal and bacterial biomass in soil (Mushonga et al., 2020). *Helianthus annuus* and *G. max* crop rotations seem to cultivate soil microbiota legacies that relatively inhibit the mycelial growth of *R. solani* AG 2-2 IIIB. In addition, *H. annuus* and *G. max* crop rotations seem to cultivate soil nutrient legacies that inhibit the mycelial growth of *R. solani* AG 2-2 IIIB. *Zea mays* and *H. annuus* crop rotations seem to cultivate both soil nutrient and microbiota legacies that relatively inhibit the mycelial growth of *R. solani* AG 2-2 IIIB. These results indicate that by including *H. annuus* and *G. max* in dryland crop rotations to promote disease suppressiveness of the soil environment against *R. solani* AG2-2IIIB. *Pseudomonas fluorescens* and *P. aureofaciens* suppress rhizosphere-linked root diseases caused by plant pathogenic fungi (Deacon, 2006). Beneficial pseudomonas usually survive in cultivated dry soil environments and produce phenazines which are effective against *Rhizoctonia* damping-off diseases (Berendsen et al., 2012). One of the main challenges with biological control agents is that establishment in soil is delayed and thus lags behind necrotrophs that grow fast and attack young plants (Lamichhane et al., 2017).

The *in vitro* pathogen suppression assay findings indicate that soil legacies from *P. vulgaris* cultivation in *H. annuus* rotations and the cultivation of *G. max* in *Z. mays* rotations improves soil disease suppressiveness against *R. solani* AG2-2IIIB. Contrary, field studies suggested that potatoes-maize-sugar beans-maize rotations seem to cultivate soil legacies that relatively inhibit the growth of *R. solani* AG 2-2 IIIB (Mushonga et al., unpublished). The differences between these two studies could have been spatial and temporal in terms of for example, litter inputs, human management, climate and plant development (Kardol et al., 2006; Kulmatiski & Kardol, 2008; Veen et al., 2021).

6.6 Conclusion

The PSFs soil microbiota legacies of legumes in crop rotations are better than in monoculture, in particular, *P. vulgaris* cultivation is ideal in *H. annuus* rotation sequences while *G. max* cultivation is ideal in *Z. mays* rotation sequences. The PSF results also suggest that *P. vulgaris* in *H. annuus* crop rotations offers soil resilience to pathogens associated with *H. annuus* monoculture. Our results show that *Z. mays* creates soil legacies that are conducive to pathogens common in repeated *G. max* monoculture. Plant nutrient analysis results suggest that *H. annuus* seems to be a highly efficient dryland crop species in the uptake of trace and macronutrient elements from the same soil. Pending the confirmation of these greenhouse findings under field conditions, we suggest that growers, especially in dryland cropping systems can improve *Z. mays* rotations by including *G. max* crop species, while for *H. annuus* rotations including *P. vulgaris* will be an ideal crop option. The *in vitro* pathogen suppression assay results indicate that soil suppressiveness was associated with *H. annuus* and *G. max* soil legacies. In dryland crop rotations, such suppressive activity can provide a potential control of *R. solani* AG2-2IIIB soil infestation.

6.7 Acknowledgements

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Conflict of interest

Authors have no conflict of interest to declare.

CHAPTER 7

Region-Specific Crop Rotation Effects on Soil Health and Crop Yield in a Dryland System

7.1 Abstract

Background: Sustainability of annual crop rotations under rain-fed or dryland field conditions is crucial for improving soil and plant health.

Aim: The goal of the study was to test the impact of short rotations on soil physical, chemical and biological properties under field conditions. Soil biota exert direct effects on plants by feeding upon roots and forming antagonistic or mutualistic relationships with their host plants while indirect effects are due to living roots, rhizodeposits and dead plant matter (litter). Weed biomass and counts at harvest in the final season, were investigated to determine the impact of crop rotations on weed suppression. A long-term trial consisting of four crop rotation systems (Potatoes-Maize-Maize-Teff, Potatoes-Maize-Sugar beans-Maize, Potatoes-Maize-Soybeans-Maize, and Potatoes-Maize-Sunflower-Maize) was conducted to determine their short-term effects on selected biotic and abiotic soil conditions over three consecutive growing seasons.

Methods: The trial layout was divided equally into four crop rotation treatments across eight blocks and planted with teff and maize cultivars in the final season in a randomized complete block design (RCBD). At the end of the 2019 growing season, soil samples were collected from the root-zone and analysed using standard soil chemical, physical and biological tests. Using a Poisson sampling strategy, three weed species (*Amaranthus hybridus*, *Senecio consanguineus*, and *Taraxacum officinale*) were hand-collected, counted and weighed at the end of the four-year rotation cycle to estimate the residual effects of the different rotation cycles on weed

occurrence and density. Dry yield and yield corrected for 12.5% moisture and its components were determined using a combine harvester and the crop cut method, respectively.

Results: Soil organic matter content was generally low (0.3 ± 0.1 %) across all crop rotations. Most soil legacies in micronutrient concentrations of concern to the growers such as B, Zn and Cu in crop rotations (rhizosphere disturbed soils) were on average lower than in non-rhizosphere bulk/loose field soils located within the field. This suggests that growers must apply soil micronutrient amendments annually to manage farm soil deficiencies. Compared to other dryland crops, our findings demonstrate that the inclusion of teff could significantly reduce the populations and density of *S. consanguineus* and *T. officinale* weed species. The dry grain yield of the follow-up maize crop was strongly associated with a linear decline in soil concentration legacies of Zn, P (Mehlich 3) and phosphorus (Bray 2). Soil pH had a very high positive and significant association with soil legacies in % Ca and % Mg, while a highly significant negative association was observed with soil nitrate-N legacies. The Solvita soil microbial respiration had a significant and positive association with soil density.

Conclusion: The inclusion of sunflower in rotations will possibly create a legacy of a loose soil type which supports shallow rooting dryland crops in subsequent plantings. Teff will on the other hand minimize soil erosion by promoting a consolidated soil. Teff and sunflower are thus ideal break crops for the studied weed species. Overall findings on soil health and weed suppression suggests that there is an urgent need to raise soil pH and increase soil organic matter and micronutrients in the sandy soils of the Eastern Free State province of South Africa under rain-fed conditions. This will assist growers to achieve the goal of soil health for plant and human health.

Key words

Crop rotation; dryland crops; soil health; rain-fed systems; soil legacies; weeds.

7.2 Introduction

Soil health represents a balanced state of soil properties that nourishes soil biodiversity and protects the soil community from harmful factors and mismanagement (Vieweger & Döring, 2015). Unlike soil quality, which focuses on yield traits, soil health links multivariate soil factors to multiple ecosystem services for plant, animal and human health (Friedrichsen et al., 2021; Pawlett et al., 2021). The profitability of crop production in general, but especially that of potato production, is consistently on the decline due to the continuous rise in input costs which have increased out of proportion compared to product price inflation. Another contributing factor is lower productivity due to increased disease pressure, particularly in the case of potatoes (Steyn et al., 2020).

Several papers have hinted that the response of crops to different rotation and tillage systems may vary, depending on the specific crops used, as well as soil and climatic conditions. The development of region-specific rotation programmes is therefore needed to ensure the successful implementation of this practice. This warrants urgent attention and requires long-term research, observation and adaptations to ensure more sustainable cropping systems in future.

Sunflower (*Helianthus annuus* L.) is cultivated as a dryland crop in South Africa. It is grown under temperate, subtropical and tropical climates and, ranked third relative to oilseed crops in the world (Pilorgé, 2020). It is the third most cultivated grain crop after maize and wheat in South Africa (Ma'ali, 2017). The 'crop rotation effect' is defined as "a yield benefit that results from crop rotation even in the apparent absence of diseases or any other detectable production limitations" (Whiting & Crookston, 1993; Bennett et al., 2012). Farming practices such as ploughing, planting, fertilization and cropping generally follow conventional tillage in the Eastern Free State, however, for this field trial we did not apply fungicides, herbicides or pesticides. We hypothesized that conventional tillage and crop rotations would cultivate

legacies or residual effects, which will manifest and persist to reduce dicot weeds and disease under monocots *Z. mays* and *Eragrostis tef* mono-cultivation thereby improving soil health. Weed residue biomass contributes to total carbon in tropical soils under maize cultivation (Barthes et al., 2004) but some weeds hosts phytopathogens which reduce crop yields (FERTASA, 2016). Weeds suppression is an indicator of soil health and sustainability in crop production systems (Lehmann et al., 2020; Karlen et al., 2021). In particular, weed suppression provides the farmer with the option of minimizing soil disturbance and wheel traffic compaction during secondary tillage practices in weed-problematic fields. Also, weed suppression minimizes weeds competition with crops for soil nutrient and water resources. However, to achieve this positive goal, there is need for the systematic sampling of weed suppression and necessary data to provide important information on the association between weeds and soil health. *Zea mays* was planted in 24 plots as described in the maize uniformity trial in Chapter 3 while the remaining eight plots were planted with *Eragrostis tef*. In SA, the teff grass is a good nurse crop against soil erosion in well-drained soils and cultivated pastures (Smith, 2006). The Potatoes SA stakeholders decided to investigate the role of teff as a nurse crop in providing; ground cover, organic plant-litter inputs and the protection of the soil surface against high dryland soil temperatures and erosion (Gert Bester-Research committee & EFS Potatoes SA Workgroup, personal communication).

Sustainable weed control using crop rotation requires the active participation of growers in determining cropping sequences, crop allocation across the field plots and tillage systems ideal in both an environmental and economic context (González-Díaz et al., 2012). The greatest weed suppression was observed in long rotations with many crop species (González-Díaz et al., 2012), compared to monocultures and narrow rotations (Bowles et al., 2020; Van der Putten, 2021). It is known from literature that dryland fields in the Eastern Free State have the potential to support dense weed populations of *Senecio consanguineus*, *Amaranthus hybridus*

and *Taraxacum officinale* (Raimondo et al., 2009; Hugo, 2013; Hugo & Van der Walt, 2016).

Using weeds suppression is an indicator of soil health, requires weed sampling to be done more systematically every season to generate enough time series data instead on the weed sampling that was done at one sampling date.

The field experiment experienced hailstorm in late December 2017 which had a negative impact on crop yield (kg/ha). Hail damage, in terms of financial income terms, in the season 2018 was an estimated; 90% for soybeans and sugar beans, 70% for sunflower and 60% for maize (Steyn, personal communication). Several papers have suggested that the response of crops to different rotation and tillage systems may vary, depending on the specific crops used, as well as the soil and climatic conditions. Therefore, it is necessary to develop region-specific rotation programmes to ensure successful implementation of climate-smart dryland production systems. This warrants urgent attention and requires long-term research to ensure more sustainable cropping systems.

The impact of edaphic factors and agricultural practices on soil microbial diversity and function, including the mechanisms of promoting plant development and defence against abiotic stressors are reviewed in Abdul Rahman et al. (2021). The aim of this study was therefore to assess how different cropping sequences will affect chemical, physical and microbial properties of soil over time and weed suppression at the end of rotations in order to find the most optimal crop combinations that will ensure long-term sustainability of crop production in the Eastern Free State province of South Africa. In this study, the goal of investigating the impact of crop rotation on weed management in a conventional tillage system was achievable considering that the confounding effects of herbicide-use were removed from the field trial (Légère & Stevenson, 2002).

7.3 Materials and Methods

7.3.1 Field Site Description and Sample Collection

A long-term trial consisting of four five-year crop rotation systems was initiated in the Petrus Steyn district of the Eastern Free State during the 2014/15 summer cropping season. The trial unfortunately had to be relocated to a new site due a change in land ownership, and a nearby field that was already planted to potatoes was selected for the new trial (2015/16 season - Year 1). The trial consists of four four-year crop rotation systems replicated eight times, giving a total of 32 plots (35 m x 21.91 m in size each) as detailed in Table 1.1 (refer to Chapter 1). Potatoes in year 1 were followed by maize on all plots in 2016/17 (Year 2/ season 2017), when the physical, chemical and microbial observations of the relevant soils commenced. Third year rotation crops were planted in 2017/18 (season 2018) and the 2018/19 summer growing season was designated year 4 (season 2019) of the four-year rotation. In this chapter, the season 2018/19 maize and teff data will be used for analysis of the field trial (refer to Table 1.1, Chapter 1)

The trial was conducted as a randomized complete block design (RCBD) comprising 32 plots of similar rectangular shape (35 m x 21.91 m) and size 766.85 m² across a total area of 2.98 ha situated near Petrus Steyn in South Africa (27° 53' 45.5" E 28° 12' 59.8", Elevation 1705 m). The soil was an arenosol greyish sandy soil (81.3±1.47% sand, 9.8±1.34% silt, 8.9±1.01% clay). In the first two seasons i.e. seasons 2016 and 2017, the fields were homogenized by planting potatoes and maize uniformity trials, respectively. In the 2018 growing season, the field was planted with eight replications of each crop/treatment (maize-*Zea mays*, sugar bean-*Phaseolus vulgaris*, soybean-*Glycine max* and sunflower-*Helianthus annuus*) following dryland conventional tillage. In the 2019 growing season, 24 plots were managed uniformly and planted with the same maize cultivar was planted cross the maize plots following the standard practice of conventional tillage (Table S7.1). The remaining eight plots were managed

uniformly and planted with the same teff variety which was broadcast at 7 kg/ha on 17 January 2019 (Table S7.1). Table S7.1 shows the crop and financial management of the four crop rotations and their respective seasons.

All soil sampling activities were georeferenced with the aid of a handheld global positioning system device (GPS) (eTrex Legend® HCx, Garmin International Inc., Olathe, Kansas, USA). Field soil sampling was done in a spatial W-shaped pattern covering 2 m² per plot area at physiological maturity at the R growth stage (178 days after planting). Rhizosphere soil samples were recovered by brushing off soil attached to grub-up roots and pooled to make one sample per plot. Bulk non-rhizosphere soils were collected as control treatments. All rhizosphere soil samples were collected in sterile zip-lock plastic bags, stored at 4 – 5 °C and used to determine contamination by soil borne pathogens. On 29 July 2019, soil from the root zone was collected as composite samples from the topsoil 0 cm to 20 cm depth in each plot using a 5 cm diameter by 8 cm height soil core and analysed for macro-and micronutrients. Pseudoreplication in sampling for rhizosphere health analysis was avoided by sampling only three plants per replicate/block, followed by pooling these samples into one composite replicate/block sample sent for downstream DNA extraction. Composite soil core samples and rhizosphere soil samples were placed in separate individual sterile zip-lock plastic bags, stored at 4 – 5 °C for soil analysis and at – 80 °C for DNA analysis of soilborne pathogens, respectively.

The maize planted was an early medium-maturity cultivar grown under dryland climate conditions. After 7 months and 17 days (29 July 2019), maize was harvested from all blocks using the same combine and driver, recording on average 12 % grain moisture content at full physiological maturity. The teff crop was harvested after 27 weeks 4 days (29 July 2019) at full physiological maturity. Crop rotation effects on yield and soil properties for the follow-up crops (maize and teff) grown in season 2018/19 were evaluated by monitoring physical,

chemical and biological properties of the topsoil (20 cm depth) using standard procedures. This included soil penetrometer resistance, bulk density, pH and nutrient status. Chemical soil analyses were performed by Nvirotek Laboratories (Pty) Ltd. Changes in physical and chemical properties, as well as soil nutrient depletion or increase over time were then assessed for each rotation system.

Yield data was collected at the end of the growing season and analysed using the Sampling for Harvest Unit technique described by Sapkota et al. (2016). Eight of the 24 plant rows of each crop per plot were harvested using a combine harvester, where after the grains were weighed and moisture content (%) per plot was determined on-site. Yields were then adjusted to 12.5% moisture content for comparison.

Weeds were collected in all plots on 29 July 2019, using the Poisson sampling strategy. Each field plot (766.92 m²) was assigned with three randomly placed quadrats per plot on the assumption that a single 1 m² quadrat fulfilled the homogeneity of variances and normally distributed errors criteria of a Poisson process. A weed specialist (Wilbri Vorster) identified and sorted the weed species into annual weeds (*Senecio consanguineus* and *Amaranthus hybridus*) and one perennial weed (*Taraxacum officinale*).

7.3.2 Whole Field Yield Data collection using a Combine Harvester

Using the sampling technique described by Sapkota et al. (2016), eight out of 24 rows (only four plots had 16 rows) of maize plants per plot were harvested with a combine harvester and the grains were measured for weight and % moisture content per plot on-site. The total yield or productivity per plot was computed by multiplying the yield of eight rows by the total area of the plot divided by the sampled harvest plot area of the eight rows, as recommended by Castellanos-Navarrete et al. (2013).

7.3.3 Soil Physicochemical and Biological Analysis

Soil physical, chemical and biological properties were measured using standard procedures at Nvirotek Laboratories (Pty) Ltd (ISO/IEC 17025 international standard for Testing laboratories Accredited Testing Laboratory, certified SANAS and SADCAS assessors, certified AgriLASA Fertilizer Laboratory, Unit 6, Nviro Business Hub, Ifafi, 0260, South Africa).

The soil samples were dried, sieved and analysed for density prior to preparation of the 1:2 water extract slurry used to measure macro- and micronutrients using the Mehlich III extraction method (FERTASA, 2016). Total P was determined using the P Bray 2 method using hydrochloric acid and ammonium fluoride in a 1:7.5 extraction ratio (mass) for all soils with pH (KCl) < 7 (FERTASA, 2016).

Active C was measured using the permanganate-oxidizable active C method (Weil et al., 2003). Soil respiration was estimated using the standard protocol of the Solvita CO₂-burst test method based on that described by Haney et al. (2015), following manufacturer's instructions. Solvita CO₂ test estimates the microbial activity in the soil at field capacity and is positively related to soil fertility (Haney et al., 2015; Stott, 2019). The soil organic matter content was estimated using the Walkley-Black method based on the oxidation with potassium dichromate (FERTASA, 2016).

Soil penetration resistance was measured (*in situ*) in-rows, between rows and on compressed tractor lanes during dry soil conditions. Readings were taken at 1 cm increments to a soil depth of 50 cm in every plot on 30 July 2019 using a Geotron Hand Penetrometer Model P5 (Geotron Systems, Potchefstroom, South Africa) up to 5000 kPa. The soil bulk density was obtained from undisturbed soil samples collected at 20 cm and 40 cm depths following the procedures described earlier in section 3.3.4.

7.3.4 Spatial Data Collection

Logging of spatial sampling of soil properties and maize yield components was done using a handheld global positioning system (GPS) device (eTrex Legend® HCx, Garmin International Inc., Olathe, Kansas, USA). The distribution of soil biological and physicochemical factors, climate, fungal and bacterial diversity, soil-borne pathogen DNA and yield components data was subjected to spatial analysis to determine the localization, geographic pattern of soil health. Spatial relationships using principal coordinates of neighbour matrices were visualized using symbol and contour plots based on the loess model as described in Chapter 3 subsection 3.3.8.3 (Lepš & Šmilauer, 2014; ter Braak & Šmilauer, 2018).

7.3.5 Data Analysis

Data analysis was conducted to compare the temporal and spatial impact of treatment legacies on soil health and plant health in a dryland field. The test statistics included; one-way analysis of variance (ANOVA), multivariate and spatial analysis, log linear models, repeated measures ANOVA, and linear mixed effects models. A RCBD layout was selected to compare the rotation sequence factor as different treatments. Data analyses were carried out using R 3.5.2 (R Core Team, 2018), SPSS version 23 (IBM Corp. Released, 2015) and Canoco 5 version 5.11 (ter Braak & Šmilauer, 2018). Graphics, linear mixed models and log-linear models were done using the “ggplot2”, “lme4” packages in R 3.5.2 and SPSS version 23, respectively. Boxplots were drawn in SPSS version 23 for all recorded data to detect outliers in the dataset. Canoco 5 version 5.11 was useful for analysis of multivariate and spatial field data.

7.3.5.1 One-Way Analysis of Variance (ANOVA) test for differences between Crop Rotations in terms of Crop Yield and Weed Responses

All one-way ANOVA and *post hoc* statistical analyses were done in R version 3.5.2 (R.Core.Team, 2018), assuming that: treatment effects were constant over the different blocks, block effects were also constant for all plots within a replication/block, and that the errors

followed a normal distribution with mean of zero and variance of σ^2 . Soil biology data were analysed by season and across seasons with the one-way ANOVA procedure.

7.3.5.2 Multivariate and Spatial Analysis for ordering and classifying Soil Properties, Crop Yield and Weed responses

Principal component analyses (PCA), symbol/contour plots and canonical correspondence analysis (CCA) were done using a multivariate analysis approach from Canoco 5 version 5.11 (ter Braak & Šmilauer, 2018) to display responses of crop yield and weeds to soil factors. The Spearman's correlation (ρ , r_s) coefficients were used to rank the relationships among soil physicochemical and biological compositions in SPSS version 23.

7.3.5.2 Log-Linear Model for Weed responses to Crop Rotation Treatments

Poisson sampling at a field scale involved randomly sampling weed species for a quadrat fixed area (1 m²) followed by cross-classifying them according to the levels of four crop rotation treatment legacies (X) and three weed species (Y) where total number of observations n was a random variable. This way, the odds ratio became a valid measure of the magnitude of association among X and Y categorical variables for the Poisson sampling design and the validity of using the log-linear model (LLM) for the analysis of categorical data X and Y. The structures of associations among two independent categorical variables i.e. crop rotations and weed count were analysed using the Pearson's chi-square test while the magnitude of the associations was estimated using the Odds-ratio in a log-linear model (LLM) in SPSS version 23. The Poisson regression model (Equation 7.1) and the Negative Binomial model (Equation 7.2) were represented by the log-linear predictors:

$$\log \mu_{ij} = \mu + \lambda_i^X + \lambda_j^Y, \text{ where } X \text{ and } Y \text{ are independent variables} \quad \text{(Equation 7.1)}$$

$$\log \eta_{ij} = \mu + \tau_i + \rho_j, \text{ where } \lambda \text{ is distributed as } \text{Gamma}(\mu, \alpha) \quad \text{(Equation 7.2)}$$

Due to the presence of overdispersion in weed data (variance greater than the mean) the Poisson regression (Equation 7.1) was switched to a Poisson/Gamma mixing model for the same

clumped weed counts data (Equation 7.2) in our final regression model (Schabenberger & Pierce, 2002).

The sample size n was a random variable, which was not pre-determined prior to sampling. Since the sample size was very large ($N = 869$), a dissimilarity index was used to avoid rejecting the goodness of fit test even though the deviations between the LLM and the data were insignificant practically or agronomically.

The dissimilarity index (Equation 7.3) indicates a good fit even if the goodness of fit test rejects the null hypothesis.

$$\text{Dissimilarity Index } (\hat{\Delta}) = \frac{1}{2n} \sum_{\text{all cells}} |O - E|, \quad (\text{Equation 7.3})$$

There was no record of any sampling or structural zeros in our sampling design. The data collected was cross-classified into a complete contingency table made up of four rows (Crop rotations: **R1** Potatoes-Maize-Maize-Teff, **R2** Potatoes-Maize-Sugar beans-Maize, **R3** Potatoes-Maize-Soybeans-Maize and **R4** Potatoes-Maize-Sunflower-Maize) and three columns (Weeds species: **W1** *Amaranthus hybridus* L. subsp. *hybridus* var. *hybridus*, **W2** *Senecio consanguineus* DC and **W3** *Taraxacum officinale*) for log-linear modelling. Essentially, the null hypothesis was that crop rotation effects are statistically independent of weed populations while the alternative hypothesis was that crop rotation effects and weed populations are dependent/related. The two-way chi-square test was used to determine whether the model predicts both presence and absence occurrences better than random predictions across the same field plots since the sample size was very large ($N = 869$).

7.3.5.3 Repeated measures analysis of variance (ANOVA) using univariate and multivariate approaches in search for potential soil health indicators

Repeated measures ANOVA was performed on the soil health data measurements done successively in three seasons (2016/17, 2017/18 and 2018/19) ordered along a temporal scale from the same observational unit (field plots within blocks). The 18 soil properties commonly

reported in agronomy trials by Potatoes South Africa were selected in the search for potential soil health indicators. Time variable was included as a treatment in the preliminary search for potential soil health indicators. The first aim sought to determine the impact of crop rotation sequence on the composition legacies of soil health factor(s) at the end of season 2018/19. After data were log-transformed, univariate analysis of variance was performed using the formula:

$$LOG(\text{soil factor values in season '2019'}) = CROP ROTATION \quad \textbf{Equation for Aim 1}$$

The second aim sought to determine the fastest rate of compositional changes in soil health factor(s) over the first two seasons (difference between seasons 2016/17 and 2017/18). After data were log-transformed, univariate analysis of variance was performed using the formula:

$$LOG(\text{soil factor season \{'2018' - '2017'\}}) = CROP ROTATION \quad \textbf{Equation for Aim 2}$$

The third aim sought to determine the impact of grasses on the rate of compositional change in soil health factor(s) over the last two seasons (difference between seasons 2017/18 and 2018/19). After data were log-transformed, univariate analysis of variance was performed using the formula:

$$LOG(\text{soil factor season \{'2019' - '2018'\}}) = CROP ROTATION \quad \textbf{Equation for Aim 3}$$

The fourth aim sought to test whether there were differences in soil health factor(s) composition across the three seasons among the four crop rotations. After data were log-transformed, multivariate analysis of variance was performed using the formula:

$$LOG(\text{soil factor season '2017','2018','2019'}) = CROP ROTATION \quad \textbf{Equation for Aim 4}$$

The assumption was that if a plot was relatively high or low in a specific soil factor at the end of season 2016/17, then it was likely to remain relatively high or low throughout the experiment until the end of season 2018/19. We also treated the three season data points per plot as independent observations by adjusting the analysis for the 'plot' effect whereby one plot corresponded to one data point to avoid inflating the sample size of the field experiment.

Shapiro-Wilk test and histogram of residuals were used to test for the normality assumption. The homogeneity of variances were tested using residual plots by the “lsmeans” package in R 3.5.2 software (R Core Team, 2018).

Aims 1 to 3 were analysed using one-way ANOVA while for Aim 4, the Pillai’s criterion was used in estimating the analysis of variance with multiple response variables (called ‘MANOVA’). We drew conclusions from jointly analysed dependent variables, to possibly capture the effect of an explanatory variable on other variables and to minimize bias of taking advantage of chance inherent in single test for each variable (Snijders & Bosker, 2011).

7.3.5.4 Linear Mixed Models (LMMs) for selected Soil Health Legacies

The analyses of crop rotation effects on soil health composition data were modelled as a clustered longitudinal experiment using linear mixed models (or hierarchical linear models) in R version 3.5.2 using the “lme4” package (R Core Team, 2018). Unlike repeated measures models where cluster effects do not change with time, the longitudinal models use time as a pivotal explanatory variable of within cluster variation related to changes in soil health composition legacies (Schabenberger & Pierce, 2002). The primary aim was to study the trends in selected soil health factors to estimate conclusions about soil biology and soil acidity over three years/seasons in a region-specific crop rotation under dryland production.

In simple terms, we were interested more in the fixed-effect predictors (soil health property and year/season) and less on the random-effect predictors (plot and block) over time ($t = 1, 2$ corresponding to years 1 and 2) in an observational study. Table 7.1 illustrates the clustered longitudinal data for the crop rotations across three seasons measured annually at harvest.

Table 7.1 Parameters for fitting LLMs to clustered longitudinal data in which the unit of analysis (field plots as a random-effect predictor) are nested within clusters (field block as random-effect predictor), and repeated measures are done on the field plots over seasons (years as fixed-effect predictor).

Level of data		Crop rotation data
Cluster	Cluster ID variable (random effect)	Block
Unit of analysis	Unit of analysis ID variable (random effect)	Plot
Time	Response variable (fixed effect)	Soil health property
Time	Time variable (fixed effect)	year = annual season

The observations and measurements were done over three annual growing seasons in the years 2016/17 (as baseline), 2017/18 and 2018/19 across a randomized complete block design made up of 32 field plot units clustered across eight blocks. In our hypothesis we test whether the fixed-effect predictors associated with the two-way interactions between ‘year’ and the other fixed covariate (baseline value for soil health property) are all equal to zero, using the maximum likelihood (ML) estimation. The distribution of the test statistic under the null hypothesis is a χ^2 (Chi-squared test) with 1 degree of freedom, corresponding to a single fixed effect set to zero under the null hypothesis.

Null hypothesis (H_0) : There are no changes/differences in soil health legacies after keeping fixed effects associated with all two-way interactions (Level-three variance: $\beta_1 = \beta_2 = \beta_3 = 0$).

Alternative hypothesis (H_a): There are changes/differences in soil health legacies after keeping fixed effects associated with all two-way interactions (Level-three variance: $\beta_1 \neq 0$, $\beta_2 \neq 0$, $\beta_3 \neq 0$).

The random effects, u_{0j} , u_{1j} , $u_{(0i|j)}$ and ε_{ij} are assumed to be independent, normally distributed with means 0 and variances , $\sigma^2_{(intercept:block)}$, $\sigma^2_{(year:block)}$, $\sigma^2_{(intercept:plot(block))}$ and σ^2 , respectively. The interaction effects β_1 , β_2 , β_3 are assumed to be random effects, independent and normally distributed. The proposed models for Solvita soil respiration, soil active C and soil pH (H_2O)

were coded in R version 3.5.2 and formulated as stated in subsections 2.5.4.1, 2.5.4.2 and 2.5.4.3.

7.3.5.4.1 Solvita Soil Respiration (sol)

Syntax and equation for the reference model7.1a is as follows:

```
model7.1a.fit.lmer <- lmer (sol ~ year + base_sol17 + year*base_sol17 + (year | block) + (1 | plot2), data = SH1, REML = T).
```

$$Sol_{tij} = \beta_0 + \beta_1 \times Year_t + \beta_2 \times Base\_sol17_{ij} + \beta_3 \times Year_t \times Base\_sol17_{ij} + u_{0j} + u_{1j} \times Year_t + u_{0ij} + \varepsilon_{tij}, \quad \text{Model 7.1a}$$

where the Solvita soil respiration response at *time t* on *plot i* nested within *block j* is Sol_{tij} ;

β_0 is the intercept estimation;

β_1 is the overall fixed *year* effect;

β_2 is fixed effect associated;

with *block* – level and *plot* level covariates such as baseline Solvita soil respiration effect of season 2017 ($Base\_sol17_{ij}$);

β_3 represents a fixed effect associated with their two – way *interactions*;

u_{0j} and u_{1j} are random *block* effects associated with the intercept and *Year* slope, respectively;

u_{0ij} is the random effect associated with a *plot* nested within a *block*; and

ε_{tij} represents a residual.

Syntax and equation for the nested model7.1b is as follows:

```
model7.1b.fit.lmer <- lmer (sol ~ year + base_sol17 + (year | block) + (1 | plot2), data = SH1, REML = T).
```

$$Sol_{tij} = \beta_0 + \beta_1 \times Year_t + \beta_2 \times Base\_sol17_{ij} + u_{0j} + u_{1j} \times Year_t + u_{0ij} + \varepsilon_{tij}, \quad \text{Model 7.1b}$$

where the Solvita soil respiration response at *time t* on *plot i* nested within *block j* is Sol_{tij} ;

β_0 is the intercept estimation;

β_1 is the overall fixed *year* effect;

β_2 is fixed effect associated;

with *block* – level and *plot* level covariates such as baseline Solvita soil respiration effect of season 2017 ($Base_{sol17_{ij}}$);

u_{0j} and u_{1j} are random *block* effects associated with the intercept and *Year* slope, respectively;

u_{0ilj} is the random effect associated with a *plot* nested within a *block*; and

ε_{tij} represents a residual.

7.3.5.4.2 Soil Active C (acc)

Syntax and equation for the reference model 7.2a is as follows:

```
model7.2a.fit.lmer <- lmer (acc ~ year + base_acc17 + year*base_acc17 + (year | block) + (1 | plot2), data = SH1, REML = T).
```

$$Acc_{tij} = \beta_0 + \beta_1 \times Year_t + \beta_2 \times Base\_acc17_{ij} + \beta_3 \times Year_t \times Base\_acc17_{ij} + u_{0j} + u_{1j} \times Year_t + u_{0ilj} + \varepsilon_{tij}, \quad \text{Model 7.2a}$$

where the soil active carbon response at *time t* on *plot i* nested within *block j* is Acc_{tij} ;

β_0 is the intercept estimation;

β_1 is the overall fixed *year* effect;

β_2 is fixed effect associated;

with *block* – level and *plot* level covariates such as baseline soil active carbon effect of season 2017 ($Base\_acc17_{ij}$);

β_3 represents a fixed effect associated with their two – way *interactions*;

u_{0j} and u_{1j} are random *block* effects associated with the intercept and *Year* slope, respectively;

u_{0ilj} is the random effect associated with a *plot* nested within a *block*; and

ε_{tij} represents a residual.

Syntax and equation for the nested model 7.2b is as follows:

```
model7.2b.fit.lmer <- lmer (acc ~ year + base_acc17 + (year | block) + (1 | plot2), data = SH1, REML = T).
```

$$Acc_{tij} = \beta_0 + \beta_1 \times Year_t + \beta_2 \times Base\_acc17_{ij}$$

$$+u_{0j} + u_{1j} \times Year_t + u_{0ilj} + \varepsilon_{tij},$$

Model 7.2b

where the soil active carbon response at *time t* on *plot i* nested within *block j* is Acc_{tij} ;

β_0 is the intercept estimation;

β_1 is the overall fixed *year* effect;

β_2 is fixed effect associated;

with *block* – level and *plot* level covariates such as baseline soil active carbon effect of

season 2017 ($Base\_acc17_{ij}$);

u_{0j} and u_{1j} are random *block* effects associated with the intercept and *Year* slope, respectively;

u_{0ilj} is the random effect associated with a *plot* nested within a *block*; and

ε_{tij} represents a residual.

7.3.5.4.3 Soil pH (H₂O) (pHW)

Syntax and equation for the reference model7.3a is as follows:

```
model7.3a.fit.lmer <- lmer (pHW ~ year + base_pH17 + year*base_pH17 + (year | block) + (1 | plot2), data = SH1, REML = T).
```

$$pHW_{tij} = \beta_0 + \beta_1 \times Year_t + \beta_2 \times Base\_pHW17_{ij} + \beta_3 \times Year_t \times Base\_pHW17_{ij}$$

$$+u_{0j} + u_{1j} \times Year_t + u_{0ilj} + \varepsilon_{tij},$$

Model 7.3a

where the soil (water) response at *time t* on *plot i* nested within *block j* is pHW_{tij} ;

β_0 is the intercept estimation;

β_1 is the overall fixed *year* effect;

β_2 is fixed effect associated;

with *block* – level and *plot* level covariates such as baseline Solvita soil respiration effect of

season 2017 ($Base\_pHW17_{ij}$);

β_3 represents a fixed effect associated with their two – way *interactions*;

u_{0j} and u_{1j} are random *block* effects associated with the intercept and *Year* slope, respectively;

u_{0ilj} is the random effect associated with a *plot* nested within a *block*; and

ε_{tij} represents a residual.

Syntax and equation for the nested model 7.3b is as follows:

model7.3b.fit.lmer <- lmer (pHW ~ year + base_pH17 + (year | block) + (1 | plot2), data = SH1, REML = T).

$$pHW_{tij} = \beta_0 + \beta_1 \times Year_t + \beta_2 \times Base\_pHW17_{ij} + u_{0j} + u_{1j} \times Year_t + u_{0ij} + \varepsilon_{tij}, \quad \textbf{Model 7.3b}$$

where the soil (water) response at *time t* on *plot i* nested within *block j* is pHW_{tij} ;

β_0 is the intercept estimation;

β_1 is the overall fixed *year* effect;

β_2 is fixed effect associated;

with *block* – level and *plot* level covariates such as baseline Solvita soil respiration effect of season 2017 ($Base\_pHW17_{ij}$);

u_{0j} and u_{1j} are random *block* effects associated with the intercept and *Year* slope, respectively;

u_{0ij} is the random effect associated with a *plot* nested within a *block*; and

ε_{tij} represents a residual.

Selection and comparison of variables for best model-fitting were done preferably based on -2 log likelihood (i.e., likelihood ratio test/deviance test), Akaike's Information Criterion (AIC) and Bayesian Information Criterion (BIC) and in-part on statistical significance and /or R^2 values (Onofri et al., 2010; West et al., 2014). Model estimation for both random and fixed effects was calculated using the maximum likelihood (ML) method (Shek and Ma, 2011) and in some instances the restricted maximum likelihood method (REML).

If the likelihood ratio test is significant ($p < .05$), *model 'b'* is not kept as the final model instead *model 'a'* is refitted using REML estimation to obtain unbiased estimates of the covariance parameters. On the other hand, if the likelihood ratio test is non-significant ($p > .05$), we keep *model 'b'* as our final model and refit *model 'b'* using REML estimation to obtain unbiased estimates of the covariance parameters.

7.4 Results

7.4.1 Climate

The field experimental area lies within a moderate summer rainfall region of the eastern Free State and received an average total rainfall of 672 mm for the four-year duration of this study. Frost is experienced in cold winter months from May to August while summers are hot during the growing period. The rainfall pattern for the 2018/19 season was different from the previous two seasons, 2016/17 and 2017/18, in that the rains came in early December in 2019, while in season 2017/18 it was late November and season 2017 the rains fell in late November in the early stages of the planting period. High rainfall was also experienced from January to March 2019. The average maximum temperature of 25.7 °C was higher than the 5-year average (Table S7.2, Figure 7.1) as well as season 2016/17 and season 2017/18. The mean, maximum and minimum values of the annual temperature, rainfall, humidity, total solar radiation, daily evaporative demand, wind speed, and vapour pressure deficit from seasons 2017 (S2017), 2018 (S2018), 2019 (S2019) and the 5-year season average (S2015-2019), are presented in Figure 7.1 and Table S7.2.

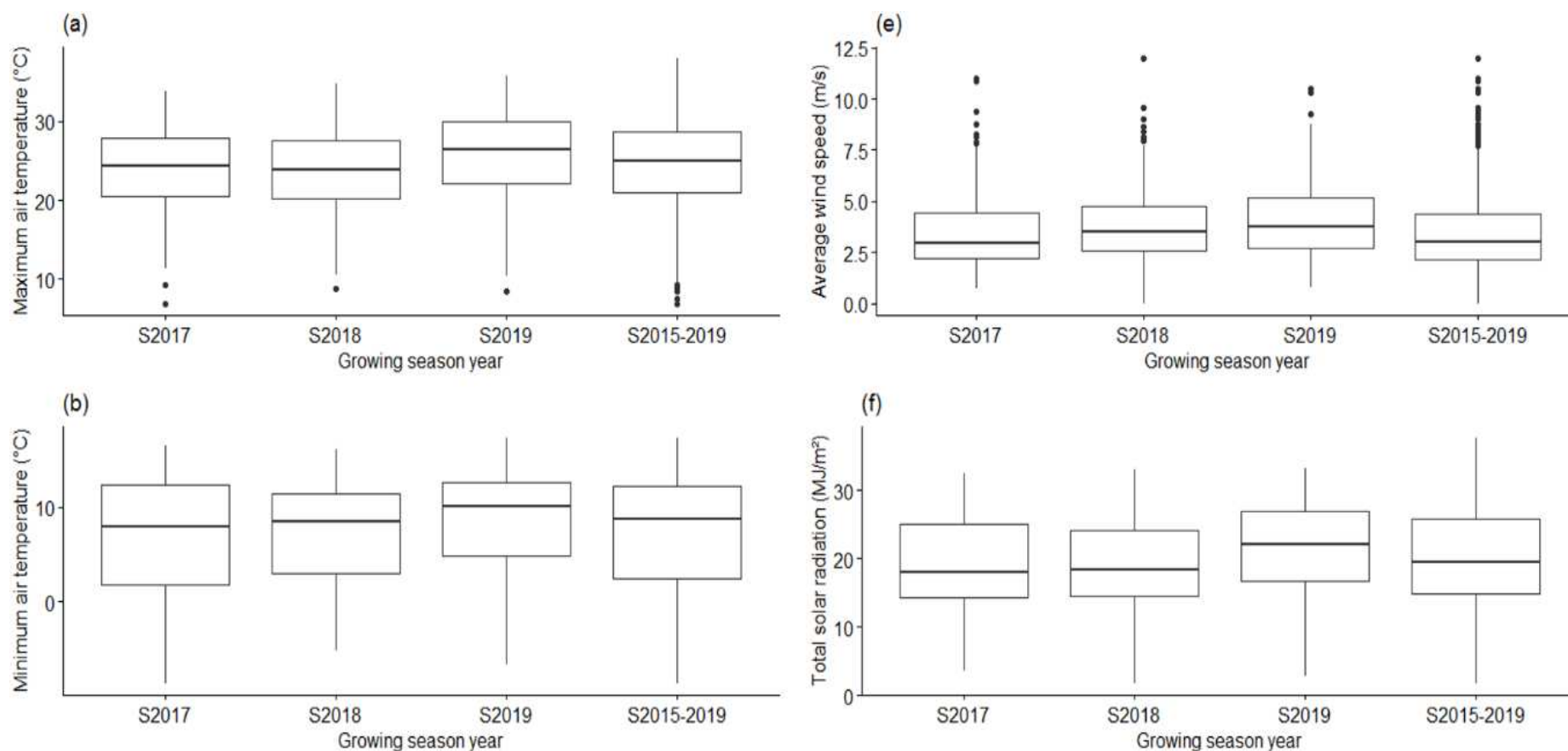


Figure 7.1 Boxplots of the seasonal variations in climate data over the growing seasons (years 2017, 2018, and 2019) in a dryland crop rotation system. (a) Maximum air temperature (°C), (b) minimum air temperature (°C), (e) average wind speed (m/s), (f) total solar radiation (MJ/m²). S2017- season 2017, S2015-2019 represents the five-year season data from 2015 to 2019. Median values are shown as bold horizontal lines across boxes, filled dots represent outliers.

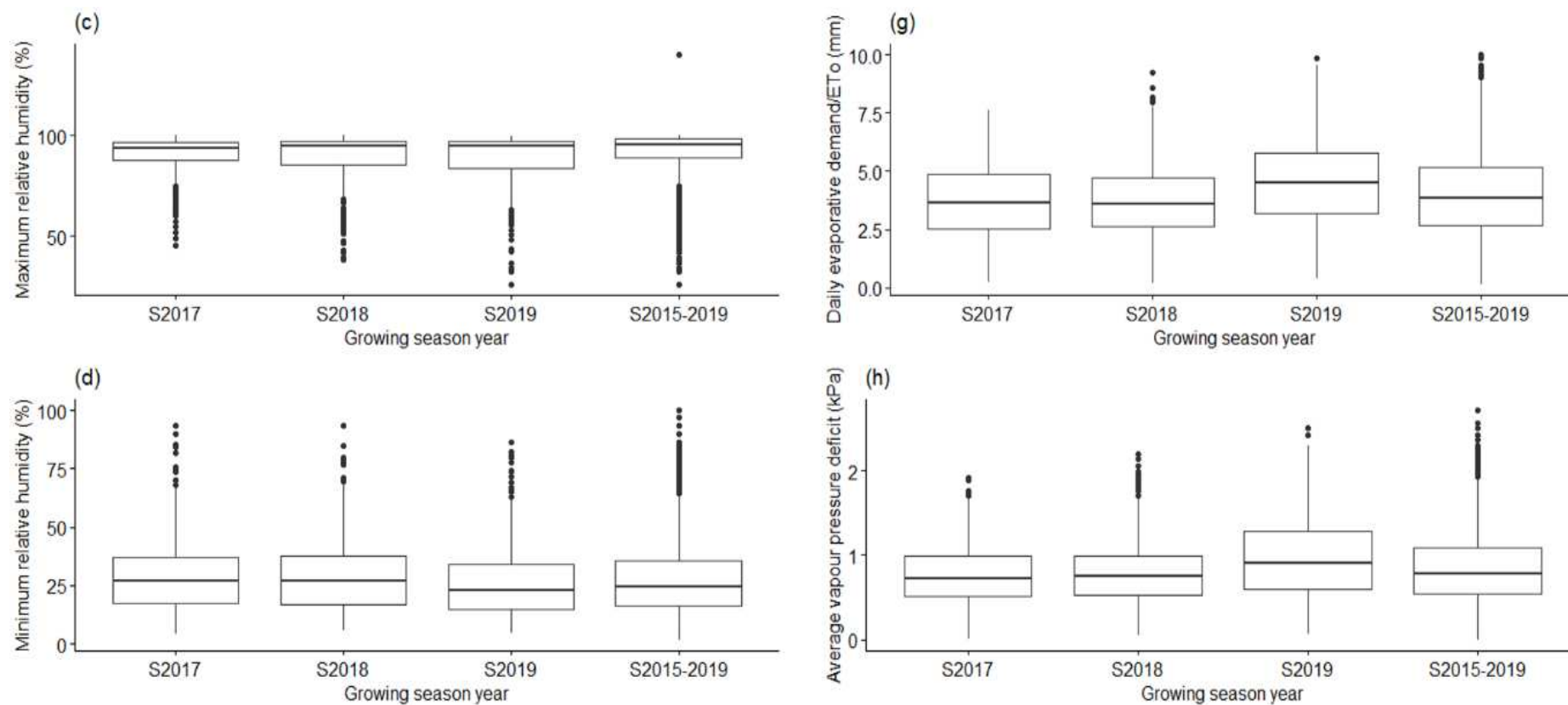


Figure 7.1 Boxplots of the seasonal variations in climate data over the growing seasons (years 2017, 2018, and 2019) in a dryland crop rotation system. (c) Maximum relative humidity (%), (d) minimum relative humidity (%), (g) daily evaporative demand/ETo (mm), and (h) average vapour pressure deficit (kPa). S2017- season 2017, S2015-2019 represents the five-year season data from 2015 to 2019. Median values are shown as bold horizontal lines across boxes, filled dots represent outliers.

7.4.2 Impact of Crop Rotation Effects on Soil Health composition

7.4.2.1 Rotation Effects on Soil Penetration Resistance, *in-situ* Bulk Density and Density

Average *in-situ* bulk density and penetration resistance measurements per crop rotation at the end of the fourth growing season are presented graphically in Figure 7.2 a-b and Figure S7.1 a-b.

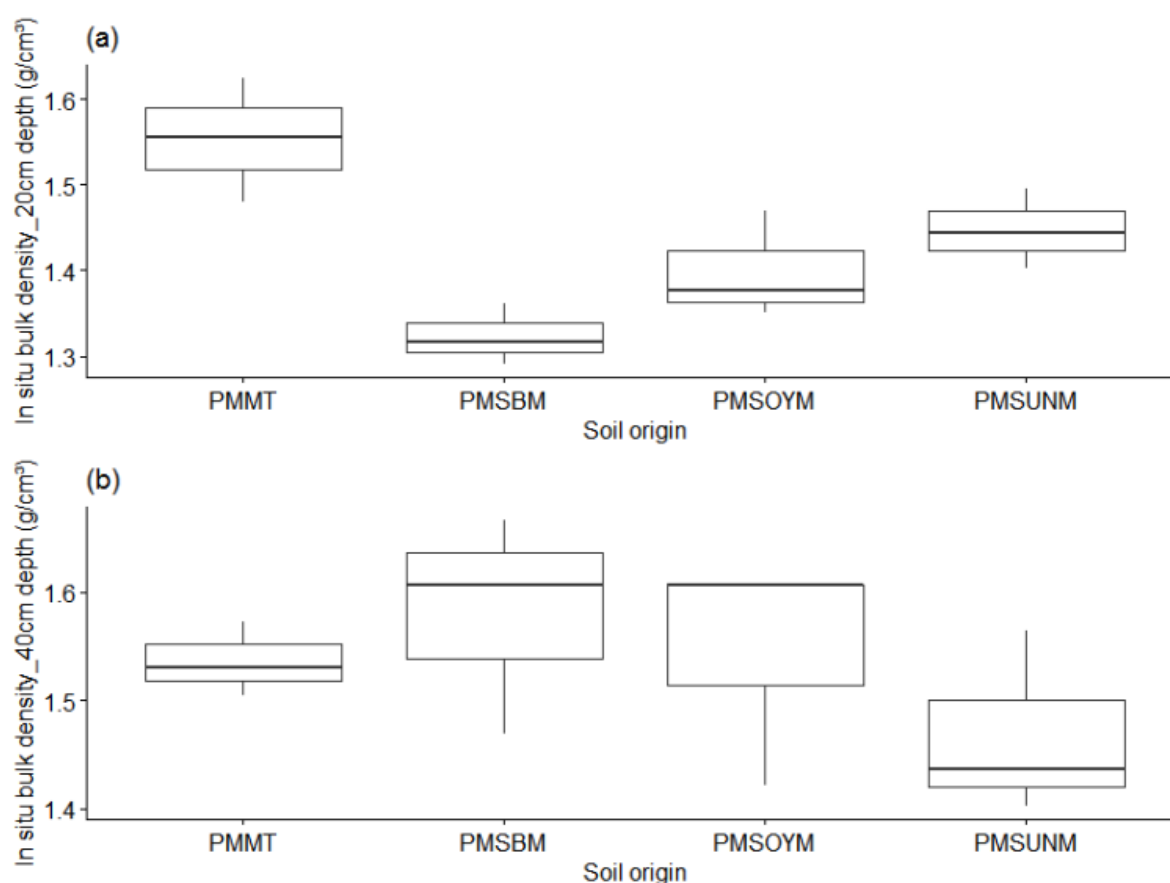


Figure 7.2 Crop sequence effects on *in-situ* bulk density (g/cm³) at (a) 20 cm and (b) 40 cm depths. The boxplots represent different bulk density ranges, as indicated by the y-axis label values represent averages across replications per treatment. PMMT – Potatoes-Maize-Maize-Teff, PMSBM – Potatoes-Maize-Sugar beans-Maize, PMSOYM – Potatoes-Maize-Soybeans-Maize, and PMSUNM – Potatoes-Maize-Sunflower-Maize rotations.

Potatoes-Maize-Sunflower-Maize rotation soil strength data suggests that this sequence resulted in fewer compact soils than other rotations with increasing soil depth (Table S7.3, Figure S7.1a). Disturbance of the soil strength in the previous year (2018 growing season) by the deep rooting traits of sunflower possibly cultivated the observed legacy of loose soil (Figure

7.2 a-b, Figure S7.1 a-b and Table S7.3). Bulk density values were typical of agriculture soils, around 1.4 g/cm³ at 20 cm depth and 1.55 g/cm³ at 40 cm depth (Brady et al., 2008). Potato-Maize-Sugar beans-Maize rotation legacy of bulk density of 1.32 g/cm³ was the ideal measurement for dryland cultivation among the rotations (Table S7.3). The soil strength (3520 kPa at 20 cm depth) and bulk density (1.55 g/cm³) of Potatoes-Maize-Maize-Teff rotation indicate a legacy of a much denser soil (Table S7.3, Figure 7.2 a-b and Figure S7.1 a-b).

7.4.2.2 Rotation Effects on Soil Nutrient concentration

Macronutrient levels were not significantly different between rotation treatments and bare field control treatment ($p > .05$, Figure 7.3) except Mg, K and P levels. One-way ANOVA post-hoc analysis using Tukey's HSD results show that soil P levels in Potatoes-Maize-Sunflower-Maize rotation (46.1 ± 2 mg/kg) was significantly higher than the Potatoes-Maize-Maize-Teff rotation (36.4 ± 1.74 mg/kg). In soil Mg legacies, the Potatoes-Maize-Maize-Teff rotation (46.4 ± 3.61 mg/kg) was significantly higher than in the Potatoes-Maize-Sugar beans-Maize rotation (37.8 ± 1.31 mg/kg). The Potatoes-Maize-Maize-Teff rotation soil K legacies (129 ± 11.2 mg/kg) was significantly higher in the Potatoes-Maize-Soybeans-Maize rotation (85.8 ± 6.73 mg/kg) ($p < .05$, Figure 7.3). Nitrate N (1.8 ± 2.1 mg/l) and ammonium (0.5 ± 0.2 mg/l) levels were on average low to marginal (Figure 7.3; FERTASA, 2016). Only levels of micronutrients Zn and B were significantly different between rotation treatments ($p < .05$, Figure 7.4). When all crop rotations were compared to the bulk field soil control, post-hoc statistical comparisons in B levels were significant while Zn differed in Potatoes-Maize-Maize-Teff vs Potatoes-Maize-Soybeans-Maize and Potatoes-Maize-Sunflower-Maize rotations ($p < .001$, Figure 7.4). Most micronutrient levels of concern to researchers, consumers and growers in the crop rotation soils were on average lower than bulk field soil levels. For example, the average soil concentration of B ranged between 0.022 - 0.17 mg/kg across the crop rotation legacies (Figure 7.4).

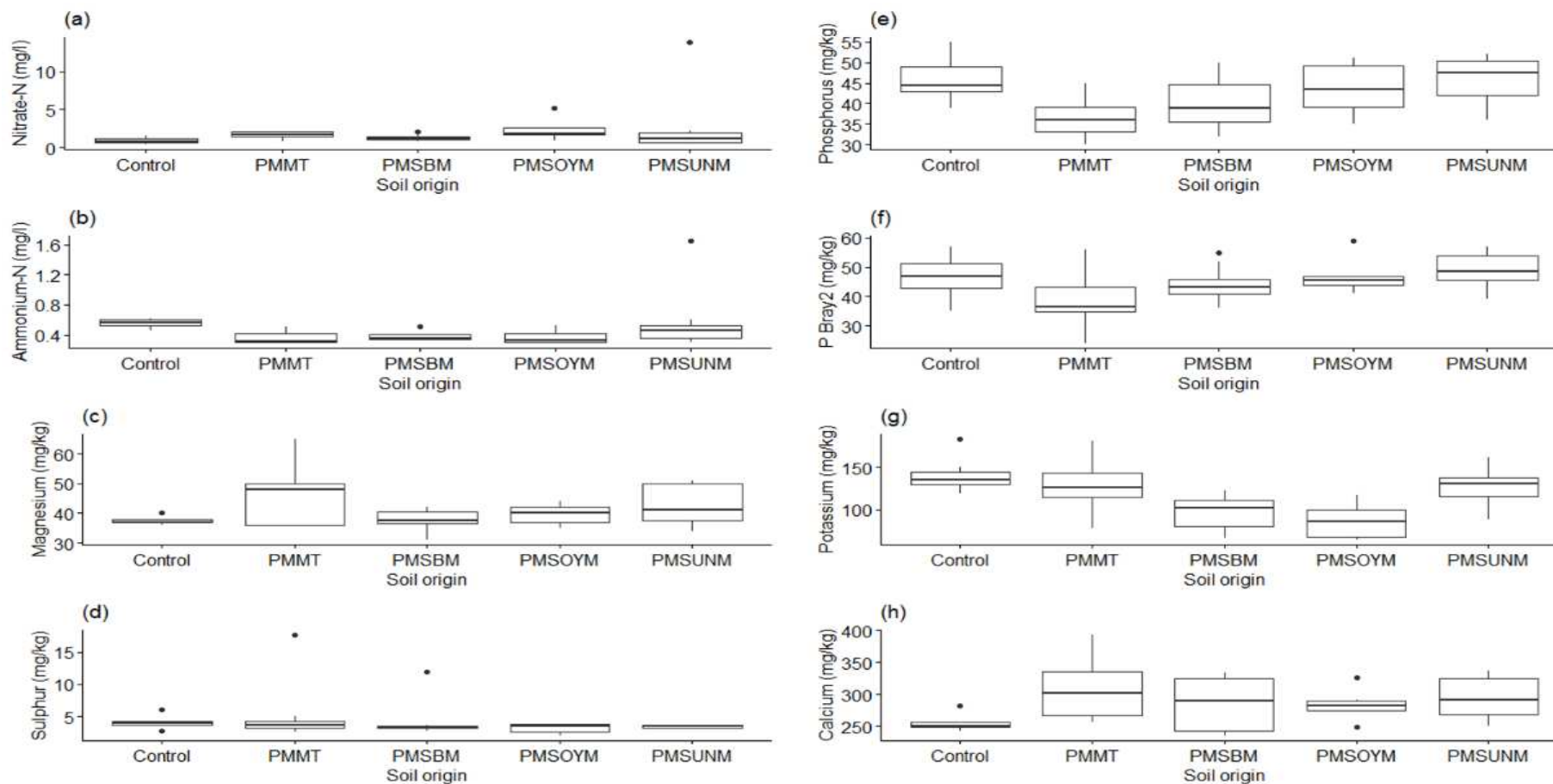


Figure 7.3 Boxplots of other soil macronutrient levels (a-h) for four rotation sequence treatments, measured at harvest in season 3 (year 2018/19). PMMT – Potatoes-Maize-Maize-Teff, PMSBM – Potatoes-Maize-Sugar beans-Maize, PMSOYM – Potatoes-Maize-Soybeans-Maize, and PMSUNM – Potatoes-Maize-Sunflower-Maize rotations. Control represents bulk field soil. Each rotation sequence treatment had eight replications and each block had four treatments. Horizontal heavy bars = median, box = lower and upper quartiles, whiskers = minimum and maximum values, filled dot = outliers.

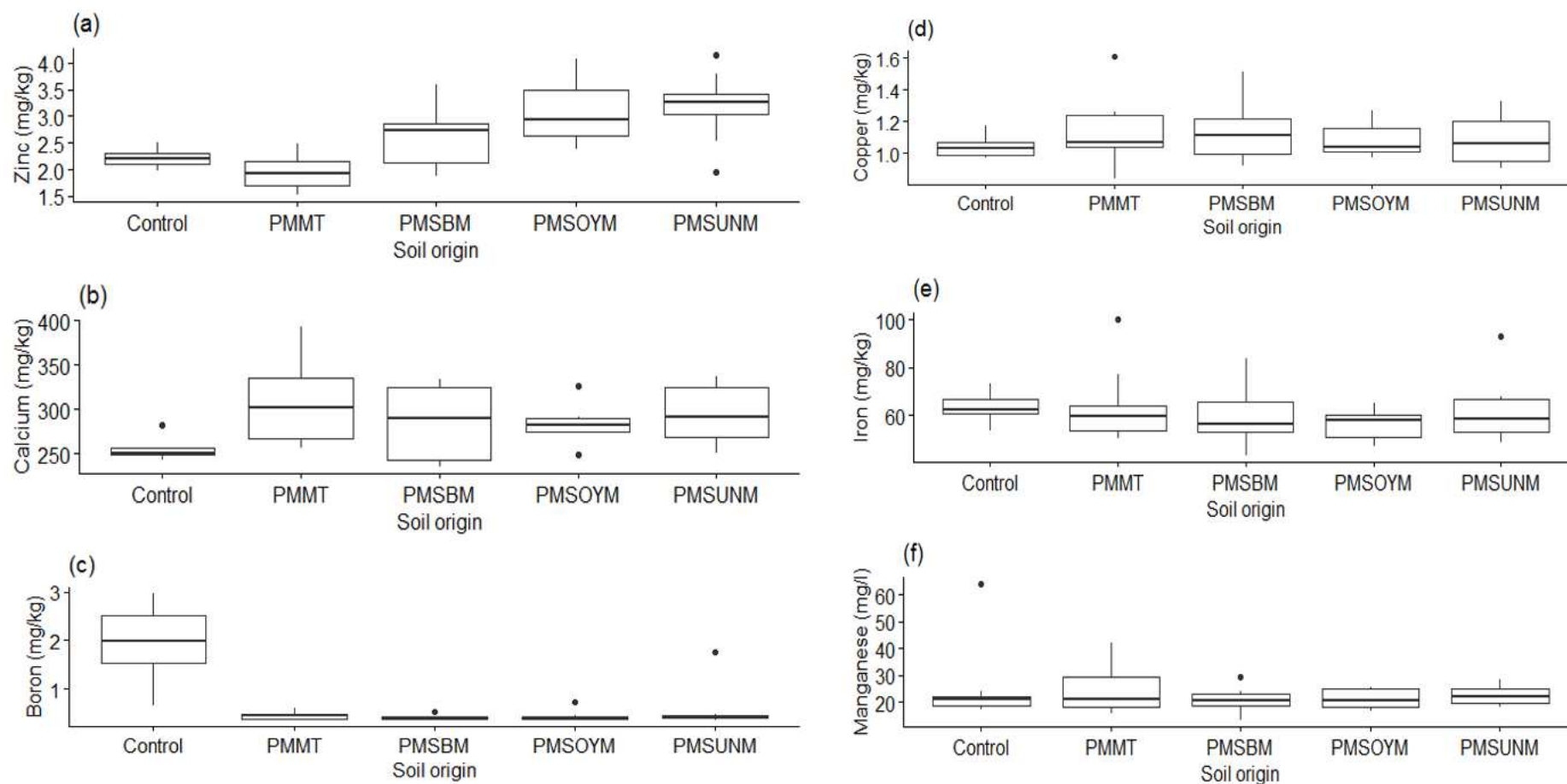


Figure 7.4 Boxplots soil micronutrient levels (a-f) for four rotation sequence treatments, measured at harvest in season 3 (year 2018/19). PMMT – Potatoes-Maize-Maize-Teff, PMSBM – Potatoes-Maize-Sugar beans-Maize, PMSOYM – Potatoes-Maize-Soybeans-Maize, and PMSUNM – Potatoes-Maize-Sunflower-Maize rotations. Control represents field bulk soil. Each rotation sequence treatment had eight replications and each block had four treatments. Horizontal heavy bars = median, box=lower and upper quartiles, whiskers=minimum and maximum values, filled dot=outliers.

Potatoes-Maize-Maize-Teff rotation (R1) had a poor legacy of most soil nutrients compared to other rotations (Figure 7.5, Table S7.4). Potatoes-Maize-Soybeans-Maize rotation (R3) had a legacy rich in ammonium-N, nitrate-N, H (BS), TEC and B. On the other hand, Potatoes-Maize-Sugar beans-Maize (R2) and Potatoes-Maize-Sunflower-Maize (R4) rotations had similar legacies of soil density, P Bray 2, Zn, P, Al and active C (Figure 7.5, Table S7.4). Figure 7.5 shows dry yields (kg/ha) of teff (R1) and maize (R2, R3 and R4) associated with soil factors. All the rotations were low in soil organic matter, Solvita CO₂ respiration burst, Mg % (BS), Mg, and Fe.

This field soil was intended for the production of potatoes in the following growing season. Therefore, the soils would require some adjustments in macro-and micronutrients reported in Figure 7.3 and Figure 7.4. In particular, Ca must be fertilized at 115 kg/ha across rotation plots, N at 80 kg/ha, and P levels at 30 kg/ha. The K levels need to be adjusted at 90 kg/ha and 55 kg/ha (for a 15 ton/ha yield potential target) in plot soils with legume legacies and the other two rotations (with sunflower or teff), respectively.

Variation in yield and soil properties is displayed using common graphical multivariate principal component analysis representations in Figure 7.5. Forty soil samples were taken before the harvest period and analysed for 28 soil properties. Principal component analysis (PCA) of results indicated different compositional responses of soil factors in soils where crops were previously grown, compared to bulk undisturbed soils (Figure 7.5). Based on the Spearman's correlation (ρ , r_s), the dry yield was strongly negatively correlated with Zn ($r_s = -0.55$, $N = 32$, $p < .001$), P ($r_s = -0.50$, $N = 32$, $p < .01$) and P Bray 2 ($r_s = -0.40$, $N = 32$, $p < .05$). Overall improvements in soil health after maize and teff crops were uniform across rotations and better than bulk soils, except for ammonium, B and soil density.

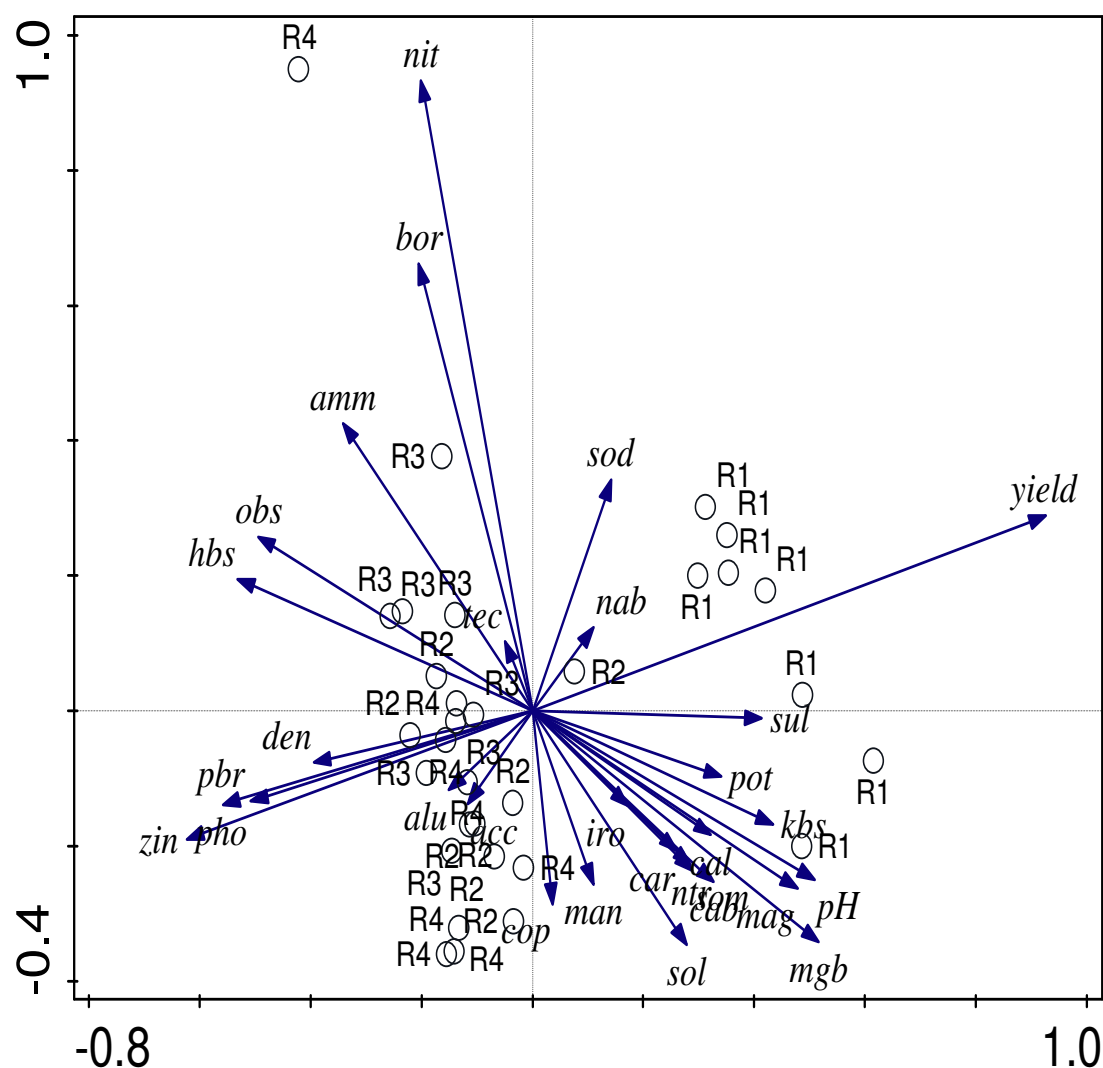


Figure 7.5 Multivariate ordination using standardized PCA for dry yields (kg/ha) of teff (R1) and maize (R2, R3 and R4) associated with soil factors in four crop rotation sequence treatments, measured at harvest in season 3 (year 2018/19). Each rotation sequence treatment had eight replications and each block had four treatments. R1 – Potatoes-Maize-Maize-Teff, R2 – Potatoes-Maize-Sugar beans-Maize, R3 – Potatoes-Maize-Soybeans-Maize, and R4 – Potatoes-Maize-Sunflower-Maize rotations.

pH - pH H ₂ O	cab - Ca% (BS)	den - Density	cal - Ca (M3)	mag - Mg (M3)
zin - Zn (M3)	sod - Na (M3)	man - Mn (M3)	pho - P (M3)	alu - Al (M3)
cab - Ca% (BS)	mgb - Mg% (BS)	nab - Na% (BS)	bor - B (M3)	hbs - H (BS)
pot - K (M3)	tec - TEC (M3)	iro - Fe (M3)	sul - S (M3)	cop - Cu (M3)
car - C Ash	acc - Active Carbon	kbs - K% (BS)	som - SOM	ntr - N Avail.
nit - NO ₃ -N(1:2)	amm - NH ₄ -N (1:2)	pbr - P Bray2	sol - Solvita CO ₂ burst	

7.4.2.3 Rotation Effects on Soil Organic Matter, Solvita Soil Respiration and Active C

For all four of the crops in the 2018 season, bacteria dominated in the rhizosphere soil, resulting in generally low overall fungal: bacterial composition ratios of 0.1 to 0.4 (unit-less). The 2018 season crop legacy effects on fungal: bacterial composition ratio in the sunflower rhizosphere were significantly different from that of the maize rhizosphere (P value = $0.015 < .05$) and control (i.e. bulk soil) (P value = $0.008 < .05$). At the end of the 2018 season, sunflower displayed the potential for increasing the fungal-bacterial composition ratio. Figure 7.6 shows the soil biological content and pH legacies in the four rotations compared to bulk soils at harvest in late July 2019.

In season 2019, the active C stocks (average 277.4 ± 52.3 mg/kg) were significantly lower in bulk soils than in all rotation soils (Figure 7.6a, $p < .05$). Soil organic matter content was generally low (0.3 ± 0.1 %) (Figure 7.6b) although there was evidence of significant differences between two rotations (Potatoes-Maize-Maize-Teff, Potatoes-Maize-Sugar beans-Maize) and bulk soil ($p < .05$). The pH (H_2O) was on average 5.6 ± 0.3 , indicating that the growing of crops promoted soil acidification uniformly across all rotations (Figure 7.6c, $p > .05$). The overall soil biological activity ranking was medium, based on the Solvita soil respiration (average of 23.8 ± 11.2 ppm) and Solvita biomass estimates (Figure 7.6d). In summary, there were no statistical differences between seasons for crop legacies in soil pH, active C and Solvita microbial respiration ($p > .05$, Figure S7.3).

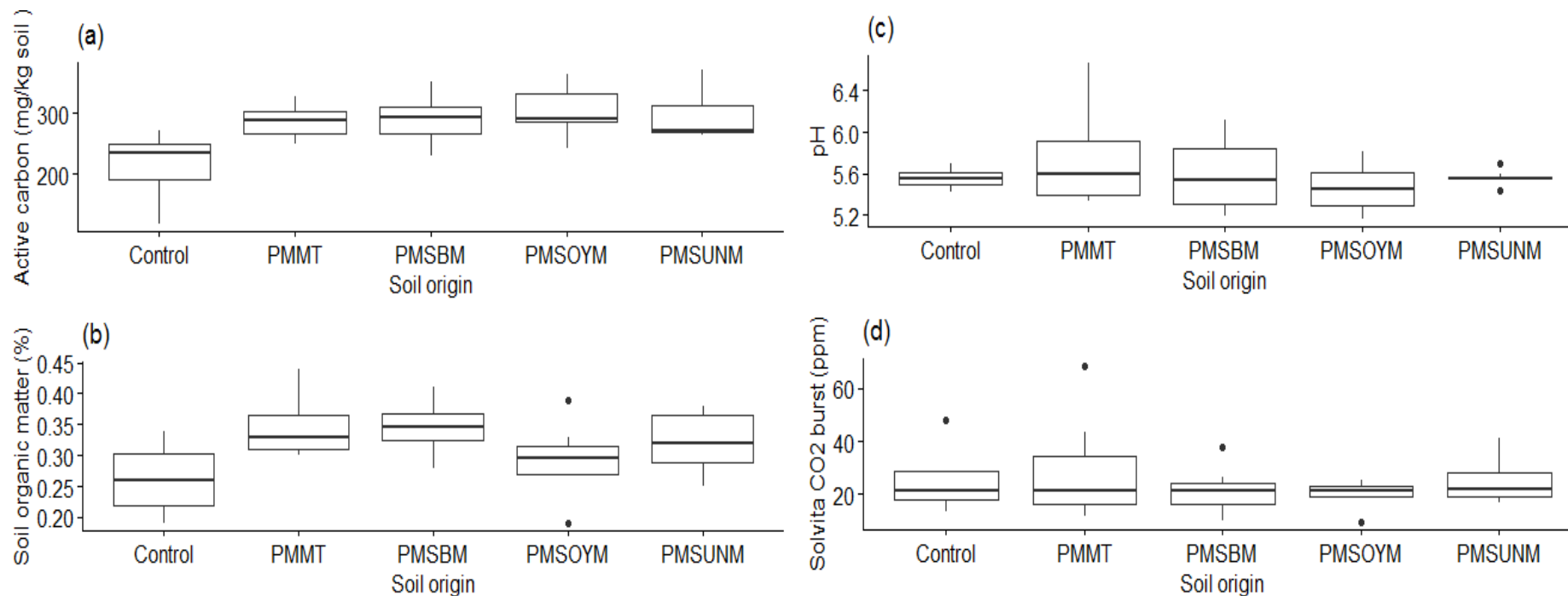


Figure 7.6 Boxplot representation of soil biological properties (a, b, and d) and pH (c) in the 32 plots under four crop rotation sequences. PMMT – Potatoes-Maize-Maize-Teff, PMSBM – Potatoes-Maize-Sugar beans-Maize, PMSOYM – Potatoes-Maize-Soybeans-Maize, and PMSUNM – Potatoes-Maize-Sunflower-Maize rotations. The control represents field bulk soil. Each rotation sequence treatment had eight replications and each block had four treatments. Horizontal heavy bars = median, box=lower and upper quartiles, whiskers=minimum and maximum values, filled dot=outliers.

7.4.4 Impact of Crop Rotation Effects on Weeds

Weed species common to the field trial were sampled using the Poisson sampling strategy. The most common weeds were *Amaranthus hybridus*, *Senecio consanguineus*, and *Taraxacum officinale*. Weeds were exhumed in each plot in 29 July 2019, using one randomly selected 1m by 1m quadrat per plot in the 2018/2019 season before crop harvesting. Sampled species were sorted into their botanical groups. The above and belowground biomass of weeds was air-dried in a hothouse and weighed separately. The weed species count (count/m²) and biomass yield (g/m²) (in Figure 7.7) responses to residual rotation treatments were significantly different using the Kruskal-Wallis analysis ($p < .05$). Post-hoc pair-wise contrasts across rotations indicate that Potatoes-Maize-Maize-Teff rotation seems to have significant differences from rotations Potatoes-Maize-Soybean-Maize, Potatoes-Maize-Sugar beans-Maize and Potatoes-Maize-Sunflower-Maize in suppressing the weed species ($p < .05$).

The relationship between the soil legacies of crop rotations and occurrence of three weed species (*A. hybridus*, *S. consanguineus*, *T. officinale*) at the end of season 2018/2019 was also investigated. The objective was to determine how the weed communities were related to soil properties at harvest. The projection of soil factors revealed that the first axis is negatively correlated with Solvita CO₂ burst, % K, % Mg, K (M3) and positively correlated with density and other factors (e.g. base saturation) (Figure 7.8).

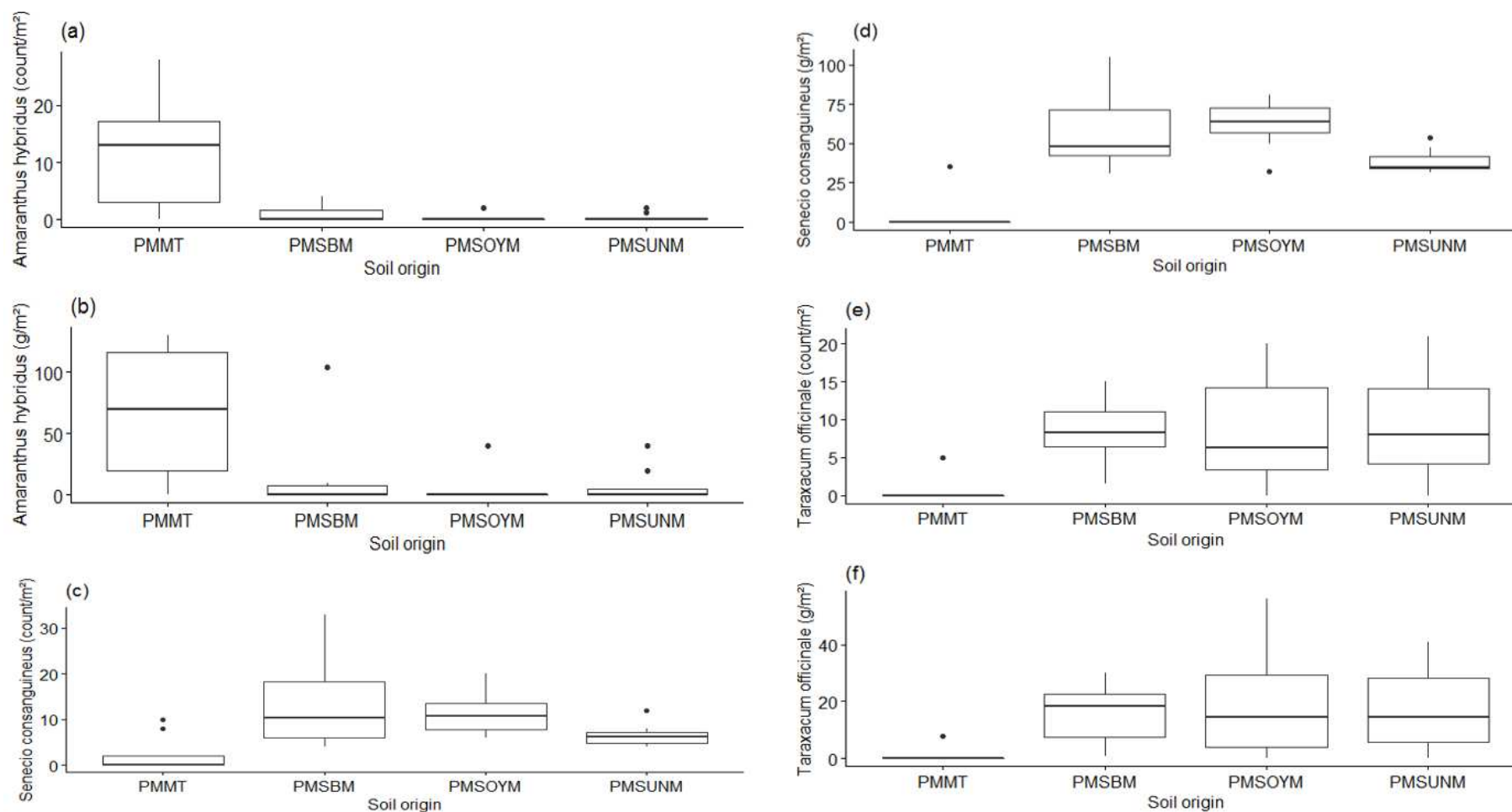


Figure 7.7 Boxplots of weeds in four rotation sequence treatments measured at harvest in season 3 (year 2018/19). PMMT – Potatoes-Maize-Maize-Teff, PMSBM – Potatoes-Maize-Sugar beans-Maize, PMSOYM – Potatoes-Maize-Soybeans-Maize, and PMSUNM – Potatoes-Maize-Sunflower-Maize rotations. Each rotation sequence treatment had eight replications. Each block had four treatments. Horizontal heavy bar = median, box=lower and upper quartiles, whiskers=minimum and maximum values, filled dot=outliers.

The biplot shown in Figure 7.8 is a multivariate analysis for weed species count (count/m²) and biomass yield (g/m²) including associated soil properties and teff and maize yields (kg/ha). Constrained ordination using canonical correspondence analysis (CCA) suggest that *S. consanguineus* (count/m²), *T. officinale* (count/m²), *S. consanguineus* (g/m²) and *T. officinale* (g/m²) prefer soils rich in P (M3), Zn (M3), NH₄-N (1:2), and H (low base saturation). On the other hand, *A. hybridus* (count/m²) and *A. hybridus* (g/m²) seem to prosper at higher S (M3), % K, Solvita CO₂ burst, % Mg, and K (M3) (Figure 7.8). This suggests that *S. consanguineus* (count/m²), *T. officinale* (count/m²), *S. consanguineus* (g/m²) and *T. officinale* (g/m²) are problematic in Potatoes-Maize-Soybean-Maize and Potatoes-Maize-Sunflower-Maize rotations where there was high Zn (M3), H (low base saturation), and NO₃-N(1:2). *Amaranthus hybridus* (g/m²), *A. hybridus* (count/m²), were present at higher populations in Potatoes-Maize-Maize-Teff, but the legacies of soil factors were not clear (Figure 7.8).

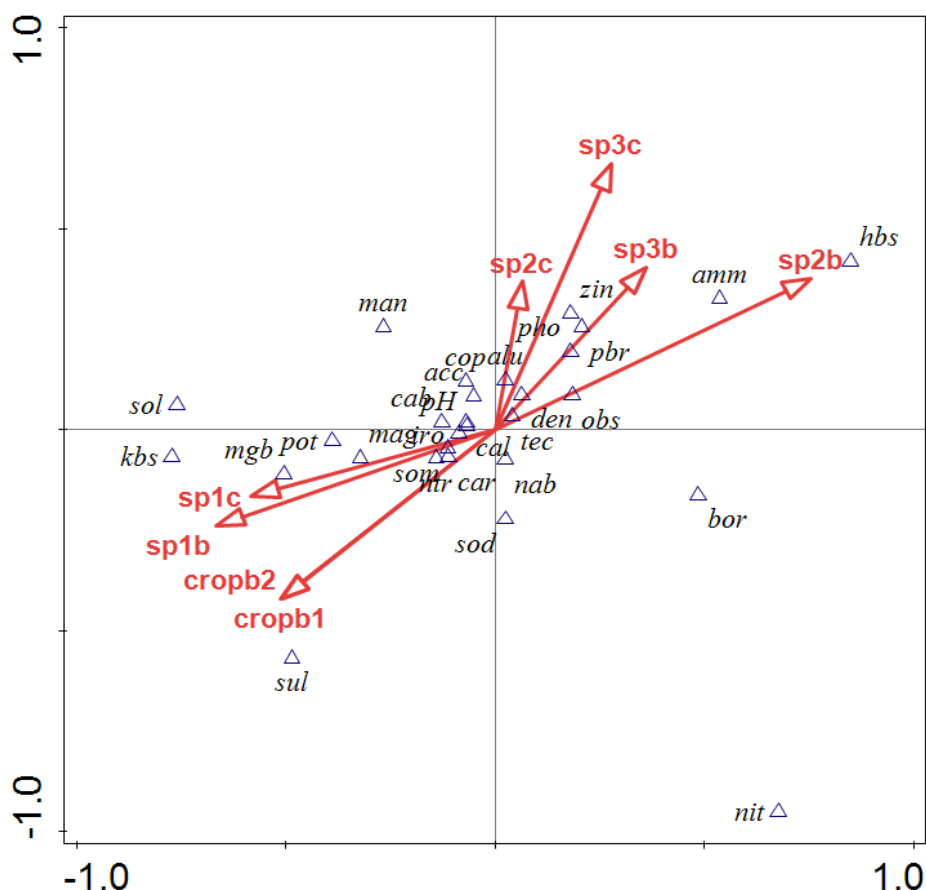


Figure 7.8 Biplot of canonical correspondence analysis (CCA) in the first two axes with soil health factors and four species (three weed species and crop) response at the harvest period. The mean weed count was sp1c (3.3 count/m²), sp2c (8.5 count/m²), Sp3c (6.8 count/m²) and the mean biomass was spb1 (23.6 g/m²), spb2 (40.8 g/m²) spb3 (13.4 g/m²). The crop yield expressed as dry weight was cropb1 (4482 kg/ha) and the 12.5% moisture adjusted yield was cropb2 (4627 kg/ha).

sp1b - <i>Amaranthus hybridus</i> g/m ²	sp2b - <i>Senecio</i> <i>consanguineus</i> g/m ²	sp3b - <i>Taraxacum</i> <i>officinale</i> g/m ²
sp1c - <i>Amaranthus hybridus</i> count/m ²	sp2c - <i>Senecio consanguineus</i> count/m ²	sp3c - <i>Taraxacum</i> <i>officinale</i> count/m ²
cropb1 - Dry Crop Yield (kg/ha)	cropb2 - Crop Yield – corrected to 12.5% moisture (kg/ha)	
pH - pH H ₂ O	pbr - P Bray2	den - Dens.
mgb - Mg% (BS)	bor - B (M3)	nab - Na% (BS)
cab - Ca% (BS)	zin - Zn (M3)	kbs - K% (BS)
amm - NH ₄ -N(1:2)	sod - Na (M3)	cal - Ca (M3)
acc - Active Carbon	iro - Fe (M3)	tec - TEC (M3)
nit - NO ₃ -N(1:2)	pot - K (M3)	cab - Ca% (BS)
		car - C Ash
		pho - P (M3)
		mag - Mg (M3)
		man - Mn (M3)
		hbs - H (BS)
		cop - Cu (M3)
		ntr - N Avail.
		sol - Solvita CO ₂ burst

Table 7.2 indicates that the value of μ (expected values) was greater than or equal to 10 for all cells in the contingency table for a goodness-of-fit using the Pearson's chi-square test. Table 7.2 shows the observed and expected values for the four crop rotations and their respective weed count legacies from a randomized complete block design with eight blocks.

Table 7.2 The 2×2 Contingency table (4 rows $\times$ 3 columns) analysis for the residual effects of crop rotations (first variable) on the final counts of weed species (second variable). The response variable was the total weed count in each crop rotation. Cells indicate observed count with expected count values in brackets.

First categorical variable (X): Crop rotations	Second categorical variable (Y): Weed species				
		W1	W2	W3	Total
	R1	138 (31.9)	26 (70.8)	4 (65.3)	168 (168)
	R2	15 (47.5)	132 (105.3)	103 (97.2)	250 (250)
	R3	4 (45)	129 (99.8)	104 (92.2)	237 (237)
	R4	8 (40.6)	79 (90.1)	127 (83.2)	214 (214)
	Total	165 (165)	366 (366)	338 (338)	869 (869)

There was an association between the weed species and crop rotations for the frequency of sampling weeds in the field trial (two-way chi-square: = 566.13, $N = 869$, $p < .001$).

In testing the goodness of fit of the model [X] [Y] using independent variables in Table 7.2 to the weeds data we concluded that $p < .0001$. For these data, our calculation of the dissimilarity index was $\hat{\Delta} = 0.2570$, implying that the model was not a good practical or agronomical fit for the data. We therefore used the Negative Binomial regression using the log-link function of the estimated dispersion parameter where the deviance was closer to 1 as shown in Table S7.5 of the Negative Binomial regression and its parameter estimates.

From a simplistic view, using Equation 7.5, the predicted weed count for R1 in terms of W1 is $\exp\{2.274 - 1.170 + 0.681\} = 60.95$. The predicted weed count for R2 in terms of W1 is $\exp\{2.274 - 1.170 + 0.189\} = 19.63$. The predicted weed count for R3 in terms of W1 is

$\exp\{2.274 - 1.170 + 0.057\} = 14.49$. The predicted weed count for R4 in terms of W1 is $\exp\{2.274 - 1.170 + 0.000\} = 12.71$.

The p -value for the Likelihood Ratio Chi-square test statistic was $P(\chi^2_5 = 5.407) = 0.368 > .05$. This meant that all crop rotation legacies had the same weed counts contradicting the descriptive and Kruskal-Wallis *post hoc* comparisons displayed in the graphical outputs (Figure 7.7 and Figure 7.8).

The log of the outcome is predicted with a linear combination of the predictors:

$$\log(\text{Weed counts}) = \begin{matrix} 0.681 \text{ for } R1 \\ 0.189 \text{ for } R2 \\ 0.057 \text{ for } R3 \\ 0.000 \text{ for } R4 \end{matrix} + \begin{matrix} -1.170 \text{ for } W1 \\ 0.035 \text{ for } W2 \\ 0.000 \text{ for } W3 \\ . \end{matrix} + (2.274) \quad (\text{Equation 7.4})$$

$$\text{Weed counts} = \exp \left\{ \begin{matrix} 0.681 \text{ for } R1 \\ 0.189 \text{ for } R2 \\ 0.057 \text{ for } R3 \\ 0.000 \text{ for } R4 \end{matrix} + \begin{matrix} -1.170 \text{ for } W1 \\ 0.035 \text{ for } W2 \\ 0.000 \text{ for } W3 \\ . \end{matrix} + (2.274) \right\} \quad (\text{Equation 7.5})$$

The assumption that the weed counts in different crop rotation legacies followed a negative binomial distribution suggests that some soil health factors caused clumping i.e. very few or very many counts at harvest (Schabenberger and Pierce, 2002).

In light of these findings, we sought to detect the spatial distribution/gradient in weed data across the field experiment. In terms of the detection of a spatial gradient in weeds data, the crop rotations were each replicated 8x to maximise the number of independent bits (true replicates) of information to strengthen the conclusion on spatial gradient in weed data responses. The spatial gradient in three weed species across a rain-fed dryland field planted with *Z. mays* (24 plots) and *E. tef* (eight plots) is displayed by the principal component analysis (PCA) in Figure 7.9 while Figure 7.10 displays weeds data plotted as the isoline values while keeping the default options used in PCA. There was a positive correlation between the *S. consanguineus* and *T. officinale* species since the angle between their arrows is sharp whereas there was negative correlation between the *A. hybridus* species and the other two species since angle was larger than 90 degrees (Figure 7.9).

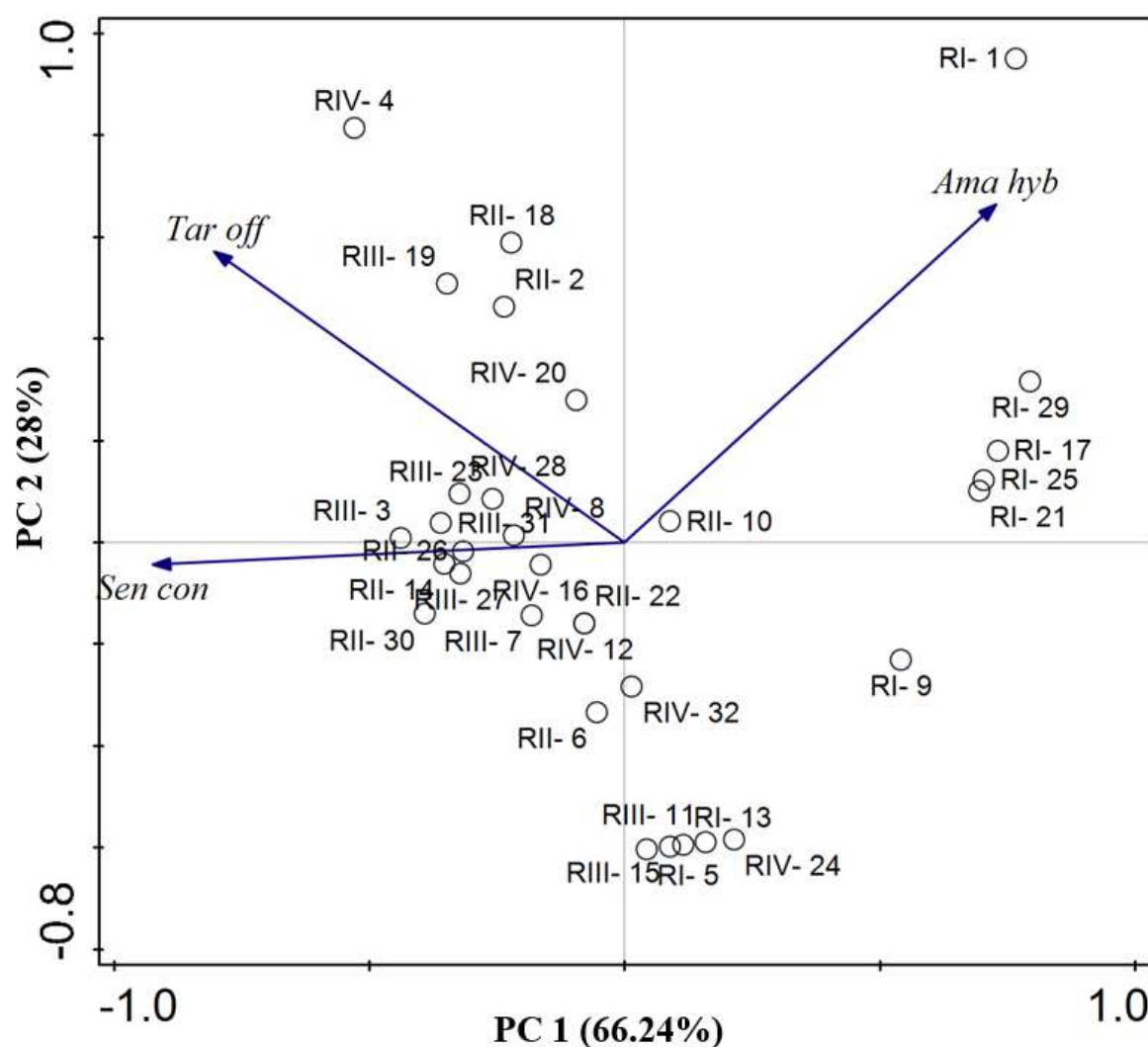


Figure 7.9 The principal component analysis biplot diagram of weed species legacies in a crop rotation field trial. Blue arrows represent three weed species (*Ama hyb* - *Amaranthus hybridus* L. subsp. *hybridus* var. *hybridus*, *Sen con* - *Senecio consanguineus* DC and *Tar off* - *Taraxacum officinale*) direction of the change while circles for weed samples from plot sites (1 to 32). Potatoes-Maize-Maize-Teff replicate plots are represented as **RI** (plots – 1, 5, 9, 13, 17, 21, 25 and 29), Potatoes-Maize-Sugar beans-Maize replicates represented as **RII** (plots – 2, 6, 10, 14, 18, 22, 26 and 30), Potatoes-Maize-Soybeans-Maize replicates represented as **RIII** (plots – 3, 7, 11, 15, 19, 23, 27 and 31) and Potatoes-Maize-Sunflower-Maize replicates represented as **RIV** (plots – 4, 8, 12, 16, 20, 24, 28 and 32).

Weed data were modelled in a similar way to the symbol plot but visualized using the loess smoother model shown in Figure 7.10. The loess smoother model explains 40 % of the variation in the PC1 axis scores using the X and Y coordinates (Figure 7.10). The main weeds

compositional trends are summarized in the field trial by using an unconstrained linear ordination of square root-transformed weed community data and these trends (represented by the case scores on the PCA axes) are shown in the X-Y diagram representing the spatial coordinates of the plots (Figure 7.10).

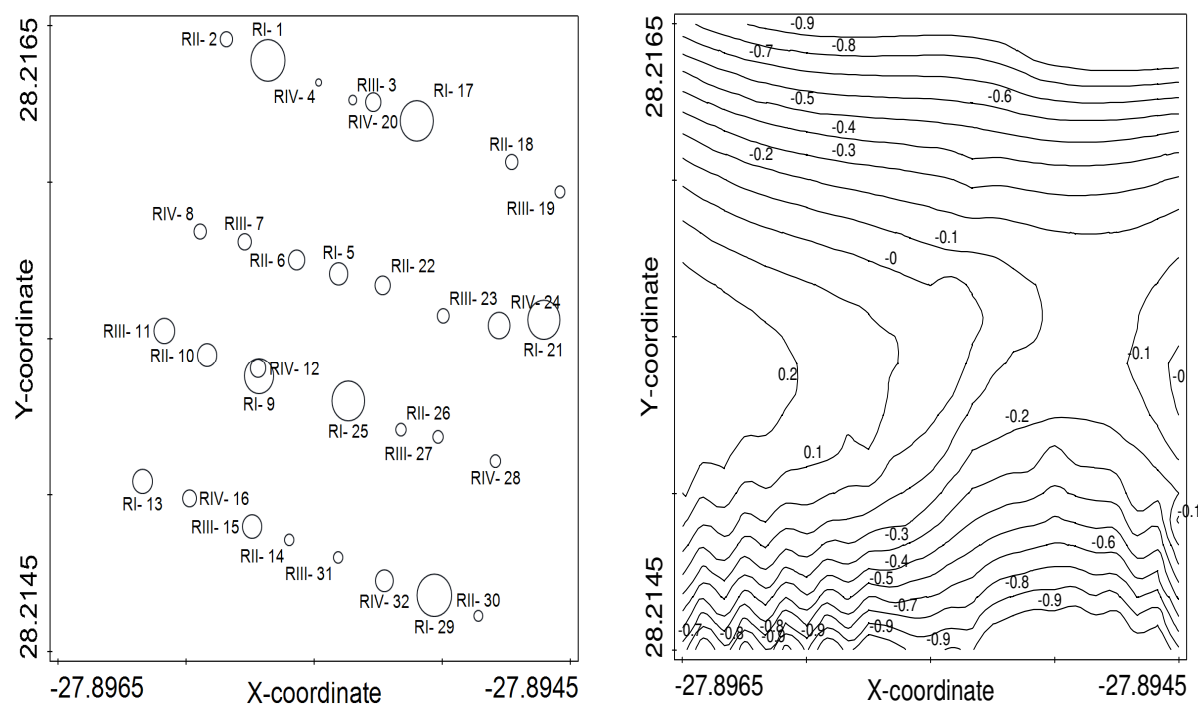


Figure 7.10 The symbol plot (left-side) and contour plot (right-side) to detect spatial gradient in weed data. Size of circle in the symbol plot is proportional to weed counts. Potatoes-Maize-Maize-Teff replicate plots are represented as **RI** (plots – 1, 5, 9, 13, 17, 21, 25 and 29), Potatoes-Maize-Sugar beans-Maize replicates represented as **RII** (plots – 2, 6, 10, 14, 18, 22, 26 and 30), Potatoes-Maize-Soybeans-Maize replicates represented as **RIII** (plots – 3, 7, 11, 15, 19, 23, 27 and 31) and Potatoes-Maize-Sunflower-Maize replicates represented as **RIV** (plots – 4, 8, 12, 16, 20, 24, 28 and 32).

The trend of compositional change in scores on PC1 increases in crop rotation treatment Potatoes-Maize-Maize-Teff while the trend of compositional change in scores on PC1 decreases in crop rotation treatments Potatoes-Maize-Sunflower-Maize and Potatoes-Maize-Soybeans-Maize (Figure 7.9 and 7.10). This was done by describing the change of the axis score values with the X and Y coordinates by a regression model.

7.4.5 Impact of Crop Rotation Effects on Grain Yields

The season 2017 and 2018 combined maize-soybean (PMSOY) legacy showed the least significant harvest index in that a total of 36 % was seed/grain yield and the remaining 64 % was straw yield ($p < .05$, Figure S7.2). The maize-sugar bean (PMSB) had the highest combined harvest index legacy of 50% i.e. 50% economic character (kg/ha) to 50% plant dry weight (kg/ha).

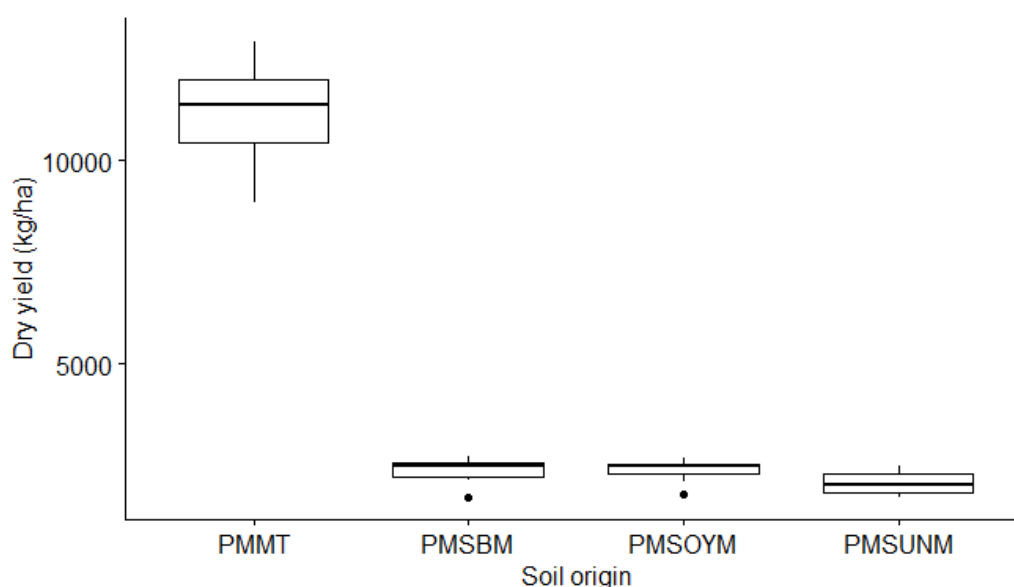


Figure 7.11 Boxplots of weeds in four rotation sequence treatments measured at harvest in season 3 (year 2018/19). PMMT – Potatoes-Maize-Maize-Teff, PMSBM – Potatoes-Maize-Sugar beans-Maize, PMSOYM – Potatoes-Maize-Soybeans-Maize, and PMSUNM – Potatoes-Maize-Sunflower-Maize rotations. Each rotation sequence treatment had eight replications. Each block had four treatments. Horizontal heavy bar = median, box=lower and upper quartiles, whiskers=minimum and maximum values, filled dot=outliers.

Dry yield of rotations with the hay crop teff (average yield Potatoes-Maize-Maize-Teff 11186 ± 485 kg/ha) and grain crop maize yields (averaging; Potatoes-Maize-Sugar beans-Maize 2343 ± 114 kg/ha, Potatoes-Maize-Soybeans-Maize 2354 ± 103 kg/ha, Potatoes-Maize-Sunflower-Maize 2047 ± 103 kg/ha) test crop are detailed in Figure 7.11. There was no statistical evidence for mean differences ($p > .05$) in grain dry yield among the maize test crop rotations using one-way analysis of variance (ANOVA). However, the maize yield averages

were less than the 5-year and 10-year average maize yield of 4360 kg/ha and 4700 kg/ha in the Free State province of South Africa, respectively (Maponya & Rampedi, 2013; USDA, 2018; Mathinya et al., 2022). Considering dryland crop rotations, sunflower showed a relative high yield compared to the other three crop species in the growing season 2018 (Figure S7.3).

7.4.6 Soil Health Indicators of short-term Rotation

There were differences in patterns of biological activity and composition in soil respiration and active C legacies, respectively, but statistics indicate that they were not significant ($p > .05$, Table S7.6). K, P and Zn were the only mineral elements that were significantly different in four cropping sequences in the final season with the highest fertility increase in first two seasons and the last two seasons ($p < .05$, Table S7.6). Neither of the soil N forms were significantly different across all seasons in the rotation sequences ($p > .05$, Table S7.6). Density was significantly different among the four crop sequences across all the three seasons ($p < .05$, Table S7.6).

Based on the Spearman's correlation (ρ , r_s), the soil pH was strongly positively correlated with soil in % Ca ($r_s = 0.90$, $N = 40$, $p < .001$, Table 7.3) and % Mg ($r_s = 0.84$, $N = 40$, $p < .001$, Table 7.3) while negatively correlated with nitrate-N ($r_s = -0.45$, $N = 40$, $p < .01$, Table 7.3). The soil active C was positively correlated with Ca ($r_s = 0.42$, $N = 40$, $p < .01$, Table 7.3), Mg ($r_s = 0.35$, $N = 40$, $p < .05$, Table 7.3) and Zn ($r_s = 0.41$, $N = 40$, $p < .01$, Table 7.4) while negatively correlated with B ($r_s = -0.50$, $N = 40$, $p < .001$, Table 7.4). The soil organic matter was positively correlated with Ca ($r_s = 0.37$, $N = 40$, $p < .05$, Table 7.3) while negatively correlated with B ($r_s = -0.48$, $N = 40$, $p < .01$, Table 7.4). The Solvita soil microbial respiration was positively correlated with soil density ($r_s = 0.38$, $N = 40$, $p < .05$, Table 7.3).

Table 7.3 Spearman's rank correlation (Spearman's rho, r_s) amongst 17 soil properties relevant to potato growers in South Africa. The strength of correlations was categorized into non-significant, significant, highly significant and very highly significant, where $p > .05$, $* p \leq .05$, $** p < .01$ and $*** p < .001$ (IBM Corp. Released, 2015), respectively.

	pH	cab	cal	nit	mgb	mag	Sol	pot	kbs	pho	den	pbr	som	sod	amm	acc	nab
pH	-																
cab	0.90***	-															
cal	0.44**	0.56***	-														
nit	-0.45**	-0.28	0.13	-													
mgb	0.84***	0.76***	0.38*	-0.29	-												
mag	0.31*	0.29	0.70***	-0.04	0.53***	-											
sol	0.29	0.21	0.03	-0.28	0.30	0.24	-										
pot	0.19	-0.09	0.12	-0.27	0.07	0.33*	0.28	-									
kbs	0.34*	0.04	-0.05	-0.33*	0.20	0.16	0.25	0.91***	-								
pho	-0.15	-0.08	0.20	-0.08	-0.05	0.32*	-0.17	0.17	-0.01	-							
den	0.07	-0.06	-0.31*	-0.43**	-0.03	-0.05	0.38*	0.35*	0.37	0.16	-						
pbr	-0.21	-0.08	0.22	0.12	-0.11	0.22	-0.09	0.07	-0.12	0.75***	-0.08	-					
som	0.04	0.14	0.37*	0.11	-0.01	0.25	0.26	0.18	0.09	-0.17	-0.23	-0.09	-				
sod	-0.10	-0.18	-0.10	0.05	-0.21	-0.12	-0.17	0.03	0.05	-0.06	0.01	0.01	-0.25	-			
amm	-0.10	-0.20	-0.33*	-0.36*	-0.21	-0.25	-0.09	0.25	0.29	0.21	0.34	0.12	-0.27	0.16	-		
acc	0.16	0.29	0.42**	0.29	0.29	0.35*	0.05	-0.23	-0.29	-0.02	-0.15	-0.02	0.24	-0.26	-0.34*	-	
nab	0.34*	0.28	-0.36*	-0.22	0.18	-0.45**	-0.01	-0.28	0.02	-0.36*	0.09	-0.29	-0.38*	0.55***	0.26	-0.18	-

pH - pH H ₂ O	pbr - P Bray2	den - Dens.	cal - Ca (M3)	mag - Mg (M3)
cab - Ca% (BS)	mgb - Mg% (BS)	kbs - K% (BS)	pho - P (M3)	nab - Na% (BS)
nit - NO ₃ -N(1:2)	amm - NH ₄ -N(1:2)	cab - Ca% (BS)	pot - K (M3)	sod - Na (M3)
acc - Active Carbon	sol - Solvita CO ₂ burst	som - SOM		

Table 7.4 Spearman's rank correlation (Spearman's rho, r_s) amongst six soil micronutrients, soil organic matter, active C, and Solvita soil microbial respiration. The strength of correlations was categorized into non-significant, significant, highly significant and very highly significant, where $p > .05$, $* p \leq .05$, $** p < .01$ and $*** p < .001$ (IBM Corp. Released, 2015), respectively.

	sol	Som	acc	Bor	cop	zin	man	iro	sod
sol	-								
som	0.17	-							
acc	0.05	0.30	-						
bor	-0.05	-0.48**	-0.50***	-					
cop	0.05	0.30	0.23	-0.20	-				
zin	-0.07	-0.04	0.41**	-0.21	0.30	-			
man	0.09	-0.06	0.08	0.33*	0.58***	0.10	-		
iro	-0.03	-0.01	-0.04	0.09	0.65***	0.26	0.54***	-	
sod	-0.12	-0.23	-0.17	0.52***	-0.29	-0.16	-0.07	0.002	-

bor - B (M3)	zin - Zn (M3)	man - Mn (M3)
sod - Na (M3)	iro - Fe (M3)	som - SOM
acc - Active Carbon	cop - Cu (M3)	sol - Solvita CO ₂ burst

7.4.7 Linear Mixed Models (LMMs) for selected Soil Health Legacies

7.4.7.1 Solvita Soil Respiration

There was not enough evidence to reject the null hypothesis for this test since the likelihood ratio test was non-significant ($p > .05$, Table S7.7). The two-way interactions involving “year” were omitted and the final *model 7.1b* was thereafter estimated using REML estimation to obtain unbiased estimates of the covariance parameters in the syntax and fitted equation (*Model 7.1b*) as follows:

```
model7.1b.fit.lmer <- lmer (sol ~ year + base_sol17 + (year | block) + (1 | plot2), data = SH1,  
REML = T).
```

$$Sol_{tij} = 36.17 + (-11.78 \times Year_t) + (2.32 \times Base\_sol17_{ij}) \quad \textbf{Model 7.1b}$$

The diagnostic plots in Figure S7.4a show that the fitted soil respiration values are independent and distributed normally and linearly with a homogeneous variance making the model ideal for approximating the changes of soil microbial respiration over the three seasons of our field experiment.

7.4.7.2 Soil active C

The likelihood ratio test was non-significant ($p = 0.9386$, Table S7.8) since there was no evidence to reject the null hypothesis for this test ($p > .05$). The two-way interactions involving “year” were excluded in the final *model 7.2b* estimated using REML estimation to obtain unbiased estimates of the covariance parameters as follows:

```
model7.2b.fit.lmer <- lmer (acc ~ year + base_acc17 + (year | block) + (1 | plot2), data = SH1,  
REML = T).
```

$$Acc_{tij} = 170.00 + (61.45 \times Year_t) + (0.0052 \times Base\_acc17_{ij}) \quad \textbf{Model 7.2b}$$

The diagnostic plots of the unstandardized and standardized residuals in Figure S7.4b showed nonlinearity, non-homogeneity of variance and lack of independence. The heterogeneity of

variation suggests that this model is less ideal for approximating the changes of soil active C over the three seasons of our field experiment.

7.4.7.3 Soil pH (H₂O)

The likelihood ratio test was not significant ($p = 0.4943$, Table S7.9), there was no evidence to reject the null hypothesis for this test ($p > .05$). The two-way interactions involving “year” were omitted in the final *model 7.3b* refitted using REML estimation to obtain unbiased estimates of the covariance parameters is as follows:

```
model7.3b.fit.lmer <- lmer (pHW ~ year + base_pHW17 + (year | block) + (1 | plot2), data = SH1, REML = T).
```

$$pHW_{tij} = 5.31 + (-0.0095 \times Year_t) + (0.048 \times Base\_pHW17_{ij}) \quad \textbf{Model 7.3b}$$

The diagnostic plots of the unstandardized and standardized residuals in Figure S7.4c indicate nonlinearity, non-homogeneity of variance and lack of independence suggesting that this model is less ideal for approximating the changes of soil pH (H₂O) over the three seasons of our field experiment.

7.5 Discussion

7.5.1 Climate

Climate data indicate that there was a high degree of total solar radiation and high rainfall at the beginning of the rainy 2019 season. Late precipitation and periodic dry spells during the maize growing season result in poor maize density stands and lower yields in continuous maize production systems (Haarhoff et al., 2020). Since sunflower tolerates drier weather and requires fewer days to reach maturity, it replaces maize in years with late rainfall to reduce potential frost risk and crop failure in the eastern regions of South Africa (Haarhoff et al., 2020). Maize rotation with sunflower in the treatment year 2017/18 had relatively high yields and fungal to bacterial PLFA composition ratios. As discussed in Chapter 4, the maize rotation with sunflower in the treatment year 2017/18 had relatively high yields and fungal to bacterial PLFA

composition ratio. Plant-soil systems encourage stronger rhizosphere interactions under increased rainfall while drought induces rhizodeposition to protect microbial biomass from moisture stresses (Zhang & Xi, 2021). Temperature can have a huge influence on soil respiration when other resources such as water and nutrients are supplied uniformly and supplemented to the optimal levels for crop growth (Luo & Zhou, 2006). Plant-soil systems encourage stronger rhizosphere interactions under increased rainfall while drought induces rhizodeposition to protect microbial biomass from moisture stresses (Zhang & Xi, 2021). Sunflower has a deeper rooting system which increase the plant's fitness for moisture and nutrient stress than maize root functional traits (Lambers et al., 2008). Also, under moisture stress, sunflower is still able to maintain fungal and rhizodeposits better than maize. Temperature can have a huge influence on soil respiration when other resources such as water and nutrients are supplied uniformly and supplemented to the optimal levels for crop growth (Luo & Zhou, 2006).

7.5.2 Rotation Effects on Soil Penetration Resistance, Density and *In-Situ* Bulk Density Legacies

The soil strength data for the Potatoes-Maize-Sunflower-Maize rotation suggest that this sequence has a legacy of less compact soils than other rotations. Disturbance of the physical properties of soil in the previous season by the deep rooting traits of sunflower possibly cultivated the observed legacy of loose soil (Jaffar et al., 1993; Nel, 2005; Fageria, 2013). On the contrary, in the Potatoes-Maize-Maize-Teff rotation plots the dense, shallow root system traits of teff dried the soil out more and resulted in high values already encountered at shallower depths (Truter et al., 2016). Penetration resistance is a product of the degree of soil consolidation and soil moisture content, which directly influence root growth (Hazelton & Murphy, 2007; Swanepoel et al., 2014) and is an indicator of soil compaction. Compacted soils, late sowing into colder soil, inadequate P, N, and Zn nutrition potentially increase the severity

of *Rhizoctonia* infection and yield loss caused by the disease (GRDC, 2008). Soils recover from degradation faster if compaction occurs before a period of high soil biological activity (Seybold et al., 1999). Apart from loosening the soil profile, the large and deeper root biomass of sunflower possibly channels or drills more carbon-rich rhizodeposits into the soil, and thus improves long-term soil carbon storage (Jansson et al., 2021).

7.5.3 Rotation Effects on Soil Nutrient concentration Legacies

The pH (H₂O) was on average 5.6 ± 0.3 , indicating that the growing of crops promoted soil acidification uniformly across all rotations. Despite the soil acidity in season 2018, sunflower performance was better than all other crops under the same climate conditions (Mathews, 2017; Mathews, 2018). Soil macronutrient legacies were not significantly different among and between rotation treatments and control bare field soils except for soil Mg, K and P levels. Potatoes-Maize-Maize-Teff rotation vs. Potatoes-Maize-Sunflower-Maize rotations were significantly different in soil P concentration, while Potatoes-Maize-Maize-Teff rotation vs. Potatoes-Maize-Sugar beans-Maize were different in soil Mg composition and Potatoes-Maize-Maize-Teff rotation vs. Potatoes-Maize-Soybeans-Maize were different in soil K levels. Teff is a shallow-rooted grass that requires at least 15 mg/kg soil of P supply (Truter et al., 2016). However, the relatively high K levels in the rotation with teff may help lower the incidence of potato disorders such as hollow heart (Harris, 1992).

Nitrate N (1.8 ± 2.1 mg/l) and ammonium (0.5 ± 0.2 mg/l) levels were on average low to marginal, these findings was similar to other field studies where nitrate N was a more abundant soluble form of N than ammonium N (Lambers et al. 2008). In the previous season 2018, soybeans tended to deplete more P and K than most other crops, while maize depleted mostly K, despite of the low biomass and grain yields produced in the specific season. Furthermore, the two legume crops, soybean and sugar bean in maize rotations, did not leave legacies of more N (as either ammonium or nitrate) from biological N-fixation in the soil profile than the

other crops, presumably due to the low pH (Graham & Vance, 2003) and in part to hail damage early in their life cycles in season 2018 (Steyn et al., 2019).

Most soil micronutrient concentrations of concern to researchers in crop rotations were on average lower than bulk field soil levels. For example, rotation treatments average of B ranged between 0.022 - 0.17 mg/kg) although the availability in major soil types is 0.1-5 mg/kg for B, 0.1-4 mg/kg for Cu, and 1-20 mg/kg for Zn (A & L Laboratories et al., 2001). The deficiency of P, B, S, and Mn in farm soils can limit the storage of carbon (Mattila et al., 2022).

In sandy soils low in organic matter and destined for potato production, the deficiencies of B manifest as potato hollow heart, where tubers develop a star-shaped cavity in the mid-region of the tuber leading to reduced potato quality (Ahmad et al., 2012). Zn assists potato plant photosynthesis and cellular growth whilst a shortage thereof in the soil leads to stunted growth of potatoes, reduction in tuber weight and count per plant (White et al., 2017). Despite these plant health implications, these micronutrients are important for human health and nutrition (Cakmak et al., 2017). Zinc deficiencies can be mitigated by adding Zn containing fertiliser (Pula/Imvula, 2020). Zn plays an important role in disease tolerance, soil application with Zn at 20 mg kg⁻¹, as soil-nutritive agent provided resistance in cluster bean seedlings against *Rhizoctonia* root rot (Wadhwa et al., 2014). Resistance to root rot by *Rhizoctonia solani* AG 8 in Bur clover increased with soil Zn legacies (Streeter et al., 2001; Gupta et al., 2017).

The Potatoes-Maize-Sunflower-Maize rotation soils had the best legacy in Zn concentration while Potatoes-Maize-Maize-Teff rotation had the lowest compared to other field rotation soils. The soil density legacies were similar for all crop rotation soil origins while, the soil NH₄-N concentration historical legacies were higher in rotation Potatoes-Maize-Sunflower-Maize than other rotation soils.

7.5.4 Rotation Effects on Soil Organic Matter, Solvita Soil Respiration and Active C Legacies

Soil organic matter content was generally low (0.3 ± 0.1 %) across rotation treatments but there were significant differences between two rotations (Potatoes-Maize-Maize-Teff, Potatoes-Maize-Sugar bean-Maize) and bulk soil. Low organic matter under drought disturbance in sandy soils leads to a decline in the relative abundance of *Verrucomicrobia* and an increase in *Acidobacteria* which is detrimental to soil microbial diversity and function (Siebielec et al., 2020). The overall soil biological activity ranking was medium, based on the Solvita soil respiration (average of 23.8 ± 11.2 ppm) and Solvita biomass estimates. The Solvita test provides an indication of soil microbial biomass and the estimates of potential release of nutrients such as N from organic matter (Stirling et al., 2016). In addition, the Solvita CO₂ test estimates the microbial activity in soil, and it is positively or highly related to soil fertility (Haney et al., 2008; Brinton, 2016). In season 2019, the biologically active C stocks (average 277.4 ± 52.3 mg/kg) were significantly lower in bulk soils than in all rotations. The active C is recommended as a rapid metric of biologically active C in soil health assessment (Stott, 2019; Karlen et al., 2021). The Potatoes-Maize-Soybeans-Maize rotation soil legacies were high in soil active-C concentration while Potatoes-Maize-Maize-Teff rotation was lowest relative to other rotation soil historical legacies. The crop rotation soil legacies for Potatoes-Maize-Maize-Teff, Potatoes-Maize-Sugar beans-Maize and Potatoes-Maize-Sunflower-Maize were highest in soil ash-C and SOM concentration while Potatoes-Maize-Soybeans-Maize rotation legacy was lowest relative to other rotation soil historical legacies.

7.5.5 Impact of Crop Rotation Effects on Weeds

The residual effects of Potatoes-Maize-Maize-Teff rotation seem to exert selection pressure on *S. consanguineus* and *T. officinale* while showing significantly reduced selection against *A. hybridus* possibly due to mutual soil quality characteristics. Légère & Stevenson (2002) suggest

that weed abundance and biomass community composition can be altered using crop rotations and other crop management practices. Furthermore, the duration of weed suppression lasts two years depending on the tillage system (Légère & Stevenson, 2002). The occurrence of weeds is explained by favourable biotic and abiotic factors and the species' dispersal mechanisms (Peterson et al., 2011). Weed species distribution is affected by abiotic factors, biotic interactions and dispersal constraints. Some weeds harbour important pathogens and diseases associated with serious crop yield losses (Jordan et al., 2016). Teasdale et al. (2004) suggests that crop rotation effects of grasses suppress weeds better than legumes do, such as in the cultural management of *A. hybridus* before planting *Z. mays*. The findings in this study suggest that problematic weeds can potentially be reduced in population density by planting teff grass or planting allelopathic crop species. Many small grains like oats and rye are allelopathic and have a strong tendency to suppress weed growth. Buckwheat and Sudan grass are also powerful weed suppressers (Sait, 2003).

Crop rotations have been used in weed suppression at different spatial and temporal scales (González-Díaz et al., 2012; Jabran et al., 2015; Gonzalez-Andujar, 2018; MacLaren et al., 2019). MacLaren et al. (2021) demonstrated that crop rotation reduced total weed abundance in the soil seed bank and dominance by one weed species. Sunflower and weeds deplete soil water levels to deeper depths and use soil water late in the growing season (Nel, 2005). This means that fewer soil water resources are available to the next crop in the rotation sequence following sunflower. Nel (2005) observed low yields in maize planted after a sunflower growing season. Dry bean and sunflower cultivation in wheat rotations increased yields by 25 and 54%, respectively, more than wheat monoculture (Nel et al., 2003). The rotational effect of sunflower on wheat was unique to that of other systems in increasing the yield and stability of the yield (Nel et al., 2003). There are small differences in maize yields between monoculture and crop rotation systems (Nel et al., 2003). Jabran et al. (2015) revealed that weeds cause a

potential yield reduction of 37%, 40% and 30% in *G. max*, *Z. mays* and *S. tuberosum*, respectively. Most weeds prosper in high nitrate-N legacies (Sait, 2003). Thus, weeding will counteract further depletion of soil moisture following the legacy of the drying-out effect of a sunflower crop (Mathews, 2021).

7.5.6 Impact of Crop Rotation Effects on Maize Grain Yield Legacies

The maize yield average was less than the 5-year average maize yield of 4360 kg/ha in the Free State province of South Africa (USDA, 2018). Maize is a C4 plant with efficient use of soil N and water resources (Huetsch & Schubert, 2017) while the legumes are C3 plants with yields that are limited by high photorespiration (Fageria, 2016). It is obvious that higher maize grain yields on plots planted with maize only in season 2016/17 compared to other two seasons (seasons 2017/18 and 2018/19) was due to optimum climatic conditions (Seybold et al., 1999). The relatively low grain yields in the test maize crop compared to the first season (season 2017) could have been due to lower total rainfall in season 2019. Furthermore, the higher than 5-year average maximum temperature in season 2019 under dryland production could have depressed crop growth by promoting respiration at the expense of photosynthesis. The harvest index value for Potatoes-Maize-Sugar beans-Maize rotation was higher than other rotations, and the stover yield increased with grain yield suggesting that farmers can produce more food and soil organic matter from increased plant litter inputs (Lorenz et al., 2009).

The high stover yielding crop rotation such as Potatoes-Maize-Soybeans-Maize has the potential of increasing plant-residue inputs, which will in turn increase soil C and N levels over time and ultimately improve soil productivity (Kelley et al., 2003; Veen et al., 2021). In addition, plant litter protects soil against the high temperature and erosion from heavy raindrops and severe dust storms (Mathews, 2021). Dryland farming can be improved by seeking alternative approaches which increase the frequency of cover crops with high plant biomass production such as carbon farming (Jansson et al., 2021; Lal, 2021).

7.5.7 Soil Health Indicators of short-term Rotation

There were pattern differences in biological composition in soil respiration and active C legacies, but the statistics indicate that they were not significant. Density, Zn, P and K were significantly different among the four crop sequences across all the three seasons. Nevertheless, Trivedi et al. (2016) proposed that soil microbial composition may indicate changes in soil health earlier than the physicochemical indicators. Dry grain yield was strongly associated with a linear decline in soil legacies for Zn, P and P Bray 2. The soil pH had a very high positive significant association with soil legacies in % Ca and % Mg while a highly significant negative association was observed with soil nitrate-N legacies. The Solvita soil microbial respiration had a significant positive association with soil density legacy. However, the soil health properties are dynamic and they vary over space and time and management (National Academies of Sciences, Engineering, and Medicine, 2021). Soil fungal diversity in agroecosystems is influenced by Mn, K, Mg and pH (Hannula et al., 2021). It is too premature to generalize key soil health indicators, however, there is need for long-term studies and monitoring of crop yield, soil respiration, active C, soil pH, off-site soil density, Zn, P and K (Van der Waals et al., 2021). Soil microbiome studies of bacterial diversity revealed that cultivated soils in the eastern Free State are richer in terms of plant growth and fitness than undisturbed soils (Dube et al., 2019).

7.5.8 Modelling of Solvita Soil Respiration, Soil Active C and Soil pH (H₂O) Legacies

Only the Solvita soil microbial respiration could be modelled using a mixed-effects based on fixed effects such as time and baseline values and random effects such as plots and blocks as repeated measurements under crop rotation perturbations (Bardgett & Caruso, 2020). Attempts by Hermans et al. (2020) to model soil health or quality scores using soil pH, nutrient compositions, bulk density, and microbial diversity data suggest that it is possible to predict the impact of land management on the soil ecosystem.

The investment in soil health at the field scale for primary productivity should lead to crop production that is commensurate with the farmer's agribusiness model and utility context (Powelson et al., 2021). For example, the UK government proposed the 2020 Agriculture Bill which is expected to support positive environmental management of farmlands (Baxter, 2021). To accelerate and scale investment in soil health there is need for affordable soil testing, incentives to farmers, legislation to dictate compliance, awareness of soil health benefits and consequences, partnerships in society, monetizing the soil health (action plans, credits, cost pricing models) to encourage a smooth transition for growers towards novel soil-health management innovations like regenerative agriculture (FAO, 2021). In addition, growers should increase the frequency of field days, tours and grower's days to promote interest in the media platforms (Mattila et al., 2022). Like all revolutions, the carbon farming proposal has challenging bottle-necks, for example, it presents farmers with the hurdle of scaling-up the poorly understood procedures and strategies for carbon storage at field levels (Mattila et al., 2022).

7.6 Conclusion

Short-term impacts of crop rotation cycles on dry grain yield of maize were similar. The four crop rotations (Potatoes-Maize-Maize-Teff, Potatoes-Maize-Sugar beans-Maize, Potatoes-Maize-Soybeans-Maize, and Potatoes-Maize-Sunflower-Maize) differed mainly in soil Mg, K and P levels. There were important differences between the Potatoes-Maize-Maize-Teff vs. Potatoes-Maize-Sugar beans-Maize rotations in legacies of soil Mg composition. The Potatoes-Maize-Maize-Teff rotation vs. Potatoes-Maize-Soybeans-Maize were different in legacies of soil K concentration. There were important differences in soil Zn and B concentration among rotations. The soil legacy in Zn concentration was superior in the Potatoes-Maize-Sunflower-Maize rotation soils and this rotation had the highest historical legacies in soil $\text{NH}_4\text{-N}$ concentration than other rotation soils. Most micronutrient levels were low and this raises

concerns for the plant health concept for human health and nutrition. All rotations maintained soil acidity legacies uniformly across the field plots. The overall soil biological activity legacy was medium based on the Solvita soil respiration and Solvita biomass estimates. The Potatoes-Maize-Sunflower-Maize rotation created a legacy of a less compact soil environment than other rotations with increasing soil depth possibly due to disturbance in the previous season by the deep rooting traits of sunflower. On the contrary, the Potatoes-Maize-Maize-Teff rotation had a legacy of compact soils. The residual crop rotation effects of Potatoes-Maize-Maize-Teff rotation seem to have a selection pressure against *S. consanguineus* and *T. officinale* species while showing significantly reduced selection against *A. hybridus* possibly due to mutual soil quality characteristics. In other words, to break the most common weeds and disease risk cycles, there is need to plant the grass teff and sunflower crop, respectively, in rotation avoiding monoculture. We suggest that the inclusion of sunflower in rotation systems under a dryland soil environment will improve yields, tap into soil nutrient and water resources in the subsoil other crops cannot access and loosen the soil for the subsequent crops in the rotation sequence. In summary, the basic rule of region-specific crop rotations for rain-fed growers in the EFS is to include legumes in their rotation to improve soil N stocks and a non-leguminous forb, such as sunflower, to increase organic stover materials. There is also an urgent need to raise soil pH and increase soil organic matter in the sandy soils of the eastern Free State under rain-fed systems in order to improve soil and plant health.

7.7 Acknowledgements

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Conflict of interest

Authors have no conflict of interest to declare.

CHAPTER 8

Crop Rotation Effects on Soil Suppressiveness against *Fusarium graminearum* and *Rhizoctonia solani* AG 2-2 IIB in Bioassays with Artificial Pathogen Inoculation under Greenhouse Conditions

8.1 Abstract

Background: Crop rotations can lead to a balance in soil physicochemical and biological composition, which in turn, improve disease suppressiveness of field soils and gains in crop yields. The soil suppressiveness against plant disease development can be assessed through bioassays using a known dose of pathogen inoculum and a susceptible bait crop under controlled greenhouse conditions.

Aims: The purpose of the study was to investigate the soil suppressiveness of field soils collected from short crop rotations. Crop rotations vary in their effects on preventing the accumulation of crop-specific root phytopathogens in the soil at the root zone of host plants, which otherwise would lead to reduced plant metrics of hosts. A soil bioassay was conducted under greenhouse conditions using field-collected soils from a conventional tillage-based dryland crop rotation trial site in the eastern Free State (EFS) province of South Africa (SA). The aim was to determine the suppressiveness of the crop rotation legacies such as soil density, pH (H₂O), P Mehlich 3, P Bray 2, K, N - NH₄, N – NO₃, Zn, active C, Solvita soil microbial respiration, soil organic matter (SOM) and C ash against root-rot pathogens *R. solani* AG 2-2 IIB and *F. graminearum*.

Methods: The *in vitro* growth assay was performed to determine the impact of the crop rotation soil legacies on colony growth measured as mycelial extension of reference *R. solani* AG 2-2

IIIB or *F. graminearum* in Petri dishes. In soil bioassays, healthy faba bean seeds were sown as baits in 1.5 L pots filled with field-collected soils inoculated with a 14-day old culture of the reference pathogen at 20 g maize meal-sand culture per kg of potting soil. The plant health metrics of faba bean were measured and recorded 30 days after sowing to estimate soil suppressiveness to plant disease.

Results: Significant suppression of *R. solani* AG 2-2 IIIB mycelial growth was observed *in vitro* for Potatoes-Maize-Sugar bean-Maize rotation treatment. The Potatoes-Maize-Sunflower-Maize rotation was the least effective treatment in the *in vitro* suppression of *R. solani* AG 2-2 IIIB mycelial growth. This result was contradictory to the findings in Chapter 6, possibly due to the inherent differences in the microclimates of the; soils *in-vitro* assays (in petri-plates, Chapter 8) and *in-planta* soil bioassays (in greenhouse pots, Chapter 6). The *R. solani* AG 2-2 IIIB was more aggressive than *F. graminearum* in causing root rot disease on faba bean as an indicator plant. The Potatoes-Maize-Sunflower-Maize rotation was significantly suppressive to *F. graminearum* ($p < .05$). However, soils from this rotation were significantly conducive to *R. solani* AG 2-2 IIIB, with associated negative effects on plant metrics ($p < .05$). The low severity of Rhizoctonia root rot disease in faba beans planted in the Potatoes-Maize-Maize-Teff soil indicated that the grass-dominated rotation cultivates suppressive soil conditions against *R. solani* AG 2-2 IIIB. The legacy in higher soil NO₃-N content in the Potatoes-Maize-Sunflower-Maize (PMSUNM) and Potatoes-Maize-Soybeans-Maize (PMSOYM) than Potatoes-Maize-Maize-Teff (PMMT) and Potatoes-Maize-Sugar beans-Maize (PMSBM) crop rotations seems to elevate the risk of *R. solani* AG 2-2 IIIB or *F. graminearum* disease severity. Despite this elevated risk of disease severity, the effects of *F. graminearum* on faba plant metrics were suppressed and less aggressive than *R. solani* AG 2-2 IIIB possibly due to differences in host plant responses to infection.

Conclusion: Soil bioassays discriminated crop rotation soil health legacies and confirmed that the PMSUNM and PMMT crop rotations reduce disease risk. These estimates indicate that the two rotations are best for soil and plant health, and significant for integrated disease management control strategies aimed at management of root rot disease in various production situations.

Key words: Crop rotation, *Rhizoctonia solani* AG 2-2 IIIB; *Fusarium graminearum*; soil suppression; soil health legacies; root rot; soil-baiting assays.

8.2 Introduction

Plant health refers to any biological or physicochemical factors occurring in and around the plant and the plant's nutrition that may harm plant form, physiology and performance in a given geographical and temporal setting (Berendsen et al., 2012; Savary et al., 2016; NSTF, 2021). Plant health or disease depends on the susceptibility of the host plant, pathogen inoculum, and soil environment favourable to pathogen disease activity (Agrios, 2015). Soil bioassays using indicator plants as bait for pathogens provide an indirect estimate of disease risk in infested field soils (Boine et al., 2014). In many cases, plant pathogens are either soil-borne or seed-borne. A soil in good health will support the growth of the bait plants better than soils of poor health (Janzen et al., 2021). The soil-baiting assay is a useful tool to rapidly estimate root rot disease risk in field soils infested with soil borne pathogens (Gatch & du Toit, 2015).

Soil borne plant pathogens such as *Rhizoctonia* and *Fusarium* spp. are saprophytes that survive on crop residues even after they are ploughed into the topsoil (Deacon, 2006). *Rhizoctonia solani* anastomosis group (AG) 2-2 IIIB is an important soil borne pathogen that causes root rot disease in cereal, sugar bean, soybean and sugar beet production systems. *Fusarium graminearum* is a major soil borne pathogen causing head- and pod blight, root- and ear rot diseases in cereals and legumes. Faba bean was selected over other plant phytometers such as *Zea mays* L., *Helianthus annuus* L., *Phaseolus vulgaris* L. and *Glycine max* L. (Merr.), because

it produces rapid visual plant responses to *Rhizoctonia* and *Fusarium* stem and root rot disease development and symptoms after 4 weeks growth in field soil and in soils under controlled conditions (Boine et al., 2014; Chang et al., 2014a; 2019). Also, commercial faba bean plants are bred for low or tannin-free making them ideal host indicator plants susceptible to soil- and seed-borne infection leading to severe root rot and seedling blight symptoms (Crépon et al., 2010). Faba bean are cultivated as low or tannin-free plants which produce more rapid plant responses to *Rhizoctonia* and *Fusarium* stem and root rot disease development and symptoms than dryland crops used in this study (Crépon et al., 2010; Boine et al., 2014; Chang et al., 2014a; 2019).

Rhizoctonia solani Kühn anastomosis groups have a wide host range (Sneh et al., 1996), poorly characterized natural infection rate for crown and root rot disease (Webb & Calderon, 2015) and a differential sensitivity to fungicides (Muzhinji et al., 2018). *Rhizoctonia* species are basidiomycete fungi that reduce crop stands and are necrotrophic in immature or compromised host plants. This seedling pathogen attacks young plants at the young shoot base just above or below the soil level (Deacon, 2006), causing damping-off characteristic of seed rots or seedling diseases associated with wet conditions. *Rhizoctonia solani* AG 2-2 IIIB, in particular, causes elephant hide on potato tubers (Gush et al., 2019), and root and crown rot in beans and sugar beet (Engelkes & Windels, 1996; Windels & Brantner, 2004; 2007; Wibberg et al., 2016). A study by Chang et al. (2017) demonstrated that soybean root-rot severity and yield loss increased with *R. solani* inoculum density.

Fusarium graminearum Schwabe is a mitosporic fungus that depresses yields and grain quality in cereals (Deacon, 2006; Wang et al., 2015), dry edible beans and soybeans (Broders et al., 2007; Deshmukh et al., 2007; Xue et al., 2007; Bilgi et al., 2011). Severe *Fusarium* disease can lead to yield loss between 30 % and 70 % (Bai & Shaner, 1994). Inoculum of *F. graminearum* builds up in field soil environments under cereal monoculture (Wang et al., 2015). Saprophytic

nutrition allows soil borne pathogenic *Fusarium* and *Rhizoctonia* species to survive and grow in the rhizosphere, before they infect host crop plants (Berendsen et al., 2012).

The hotspot of biological activity created by plant roots in the rhizosphere, aggregatusphere, detritusphere, porosphere and drilosphere represents a healthy soil (Hall et al., 2012). The rhizosphere effects include enhanced root exudation, higher microbial biomass and activity compared to bulk soil (Bardgett, 2009). The net effects of the tripartite interactions between mutualists, pathogens, and the plant, determine the fate of soil and plant health in the rhizosphere (Garbeva et al., 2004; Bais et al., 2006; Raaijmakers et al., 2009; Berendsen et al., 2012). The positive rhizosphere effects drive plant health, by improving suppressiveness of soils against pathogens in the root zone (Berendsen et al., 2012; Liu et al., 2021). Hypothetically, soil suppressiveness develops in a severe disease outbreak, due to the plant defense responses and enhanced rhizosphere effects (Berendsen et al., 2012).

The suppressiveness of soil legacies is linked to microbial diversity and influenced by soil and plant type (Garbeva et al., 2004). Soil suppressiveness is mainly a product of soil microbial activity; soil microbial respiration and high C content (Wright et al., 2021). Soil suppressiveness assessed in bioassays using artificial pathogen inoculation according to Wright et al. (2021), assumes that field-collected soils suppress pathogens in indicator bait plants under greenhouse conditions based on the following criteria:

1. The soil must prevent or suppress root-rot disease even where pathogenic *R. solani* AG 2-2 IIIB or *F. graminearum* inoculum are abundant.
2. The susceptible host indicator bait plants do not produce visible symptoms in suppressive soils despite favourable environmental conditions.

Soil suppressiveness against *R. solani* is associated with *Lysobacter* species and its extracellular enzymes (Gómez Expósito et al., 2015; Ciano et al., 2016). Suppressive soils are a neglected and underutilized resource for the integrated management of important soil

borne pathogens affecting the production of food crops (Weller et al., 2002). Suppressing soil-borne plant diseases using crop rotation management involves changing the crop sequences to create improved soil physicochemical and biological composition, which are unfavorable for the multiplication of soil-borne pathogens. González-Díaz et al. (2012) suggest that residual effects of crop rotation hypothetically control weeds and have positive below- and above-ground effects on soil health properties. Soil legacy effects from crop rotation practices have long-lasting effects, such as minimising residual crop-specific pathogen propagules and improving soil microbial diversity and activity; which are fundamental to improving crop health (Finckh, 2003; Savary et al., 2014).

Maize rotations and intercropping with faba beans promote grain yield of faba beans (Li et al., 2007; Lambers et al., 2008). Maize benefits from the phosphorus mobilization, by the organic acid and proton exudates from faba bean roots, which acidify the rhizosphere (Li et al., 2007). The impact of root rot diseases on important agricultural crops worldwide is reviewed in a publication by Williamson-Benavides & Dhingra (2021). In the current study, crop rotations were assessed for soil suppressiveness using; (1) faba beans as indicator plants to estimate seedling and root rot disease risk under greenhouse conditions, and (2) *in vitro* antagonism assays to determine the inhibition of mycelial growth of *R. solani* AG 2-2 IIIB by field soil legacy effects.

8.3 Materials and Methods

8.3.1 Experimental Setup

To determine the suppressiveness of the crop rotation legacies, soil samples from a field trial conducted in the Eastern Free State (EFS), Petrus Steyn in SA (GPS: S 27°53'45.5" E 28°12'59.8", Elevation 1705 m) under annual crop rotations for four years . The soil samples from the crop rotation systems replicated eight times, were collected and analysed for soil

suppressiveness, and content of micro- and macronutrients in the growing season designated year 2018/19.

Soil samples were collected from the four treatment plots within each replication and pooled to make composite samples plot-wise. The greenhouse bioassay experimental design was a randomized complete block design made up of eight blocks, each crop rotation treatment had eight replications and the greenhouse trial was conducted twice at the University of Pretoria (UP); once at the Hillcrest Campus (Experimental Farm Plant Pathology Tunnels, S 25°45'9.5" E 28°15'26", South Africa (SA)) and the other at the Hatfield Campus (Wheatboard Greenhouse and Labs, S 25°45'21" E 28°13'51", SA) from 29 November 2019 to 24 December 2019.

Topsoil was collected from the dryland field experiment at 0–20 cm depth using a soil auger in rows avoiding block borders, areas with a history of the disease(s), unusually eroded areas, wet or dry areas and fertilizer bands. Before using this field soil for the baiting assays, soil analysis was done, which showed that most of the selected soil properties of this arenosol grayish sandy soil (FAO, 2005) were ideal to support faba bean plant growth (Table 8.2).

In this study, field-collected rotation soils were represented as follows; PMMT – Potatoes-Maize-Maize-Teff, PMSBM – Potatoes-Maize-Sugar beans-Maize, PMSOYM – Potatoes-Maize-Soybeans-Maize, and PMSUNM – Potatoes-Maize-Sunflower-Maize. The inoculum potential of soil-borne pathogens in the different crop rotation soils was assessed by growing faba bean plants in samples of the field soil. Disease severity and plant biomass metric assessments were made through the growing season.

8.3.2 Pathogen Suppression using *in vitro* Growth Assays

The experimental layout for the *in vitro* soil suppressiveness assay was a completely randomized design made up of four crop rotations each replicated eight times (N = 8). The crop rotation treatment soil legacies for suppression of *R. solani* AG 2-2 IIIB mycelial growth were

estimated using a surface plate method. The aim of the *in vitro* growth assay was to determine the effect of the crop rotation soil legacies on mycelial growth of reference *R. solani* AG 2-2 IIIB J.G. Kühn culture strain CBS 101765.

The field soil samples used in this assay were sieved using an SABS-certified 2 mm stainless steel wire cloth sieve (Labotec Test Sieve Toetssif, Industria, South Africa). The assay for soil suppression of *R. solani* AG2-2 IIIB mycelial growth was performed as described by Ko & Ho (1983) and De Boer et al. (1998). Approximately 50 g composite field soil samples made by pooling samples from the same plot were added to 9 cm diameter-Petri dishes and evenly spread in a smooth 8 mm layer. The soils were moistened by adding 10 ml sterile distilled water and covered with a thin layer of water agar. A 7-mm-diameter potato dextrose agar (PDA) disc from the margin of the actively growing *R. solani* AG2-2 IIIB colony was inverted and placed at the centre on top of the soil-water agar surface. Control soil samples were prepared similarly by using sterilized sand soil (autoclaved twice at 121 °C for 15 minutes) instead of field soils from different rotations. The bulk soil samples from undisturbed field soil were prepared in the same way as composite rotation soils. The Petri dishes were sealed with Parafilm M® and incubated at 24 °C in darkness.

The sparsely branched colony growth of *R. solani* was measured using a transparent ruler on soil-water agar surfaces on days 2, 3 and 4 with the aid of a Zeiss stereomicroscope (WILD M7A model, Leitz, Heerbrugg, Switzerland) at a magnification of 20 X powered by a model MTR 28 transformer (0 – 7V total max 50 VA, WILD, Heerbrugg, Switzerland). The surface colony growth of the reference fungus on crop rotation soil samples was compared with that on sterile sand control treatment. The percentage of reference *R. solani* AG 2-2 IIIB growth suppression was calculated as:

$$\% \text{ Suppression} = 100 \times \left(1 - \frac{\text{Mycelial growth on crop rotation soil legacies}}{\text{Mycelial growth on conducive sterile sand soil}} \right)$$

8.3.3 Soil Bioassay of Field Soil Nutrient composition Legacies on Faba Bean Root Health

Soil bioassays were conducted by screening crop rotation soils for suppressiveness to *R. solani* AG 2-2 IIIB and *F. graminearum* using *Vicia faba* L. (faba / broad beans) plant as an indicator-baiting crop of plant disease following soil infestation with *R. solani* AG 2-2 IIIB or *F. graminearum* inoculum, as shown in Figure 8.1. Plant disease as a result of root rot (RR) and seedling blight (SB) is first noticed when upper leaves become chlorotic, plant growth becomes reduced, and as a result the whole plant damps off or dies early in the growing period (Savary et al., 2016).

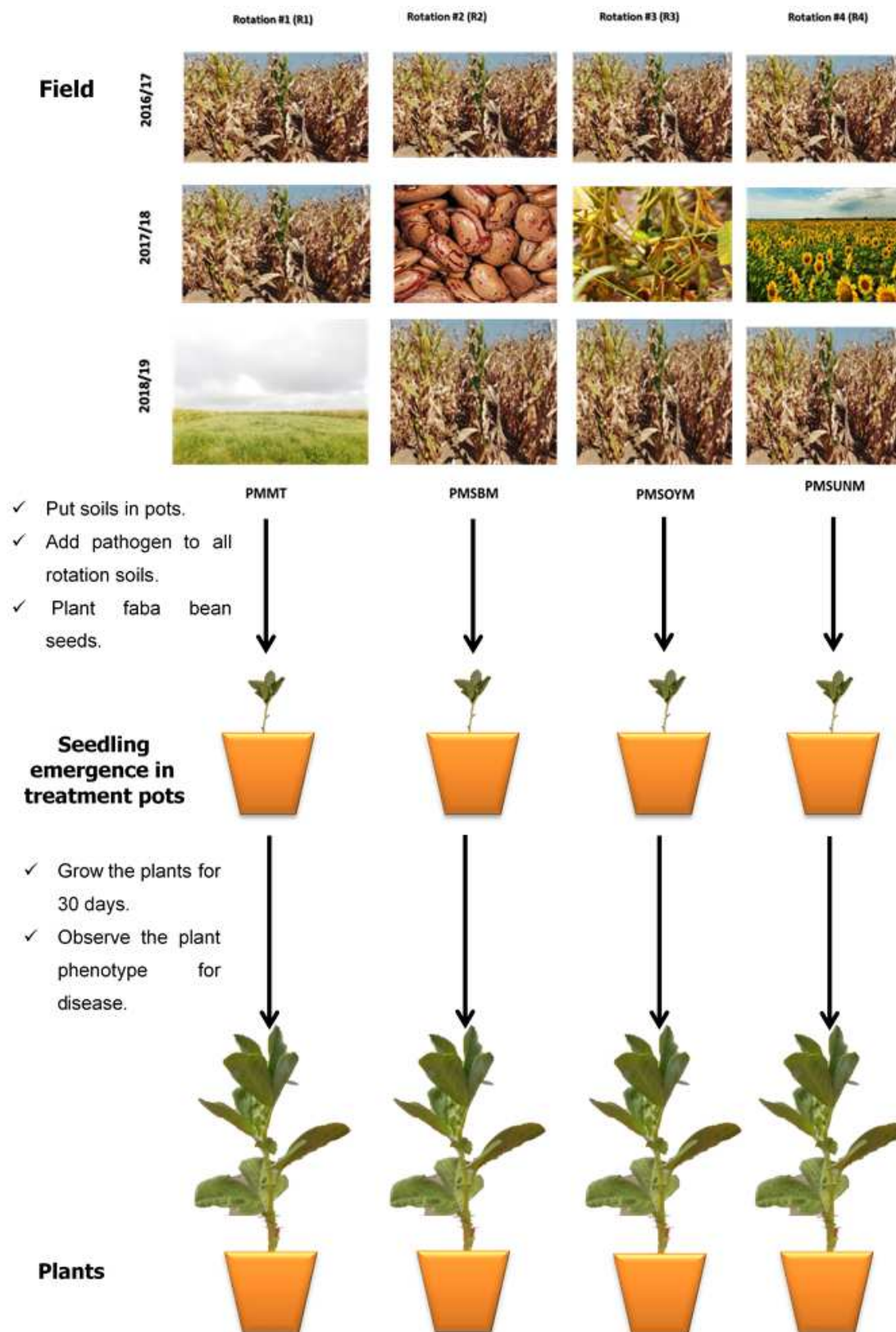


Figure 8.1 The soil bioassay estimation of soil suppressiveness by comparing among the plants grown in four different field crop rotation soils using pot experiments under greenhouse conditions. Drawing courtesy of Mr. Fungai Mushonga.

Most cultivars of faba bean are bred for low or tannin-free seeds and this makes them susceptible to soil- and seed-borne infection leading to severe root rot and seedling blight symptoms (Crépon et al., 2010). The plant is a good indicator for the manifestation of *Rhizoctonia* and *Fusarium* stem and root rot disease development and symptoms in soils under greenhouse conditions (Boine et al., 2014; Chang et al., 2014a; 2019).

Pathogenicity was estimated by comparing the number of surviving seedlings and their plant metrics among the different levels of pathogen inoculum. Successful infection and colonization of indicator faba bean root was indicated by root rot symptoms. Greenhouse climate controls were set and maintained at 25 °C maximum air temperature, 7 °C minimum air temperature, 93 % maximum relative humidity, and 27 % minimum relative humidity. Weather data including air temperature, relative humidity, and solar radiation were collected during the trial period using an Onset HOBO® data logger U12 (MacArthur Blvd).

8.3.4 Inoculum Preparation using Sand-Maize Meal Substrate

The CBS reference cultures of pathogenic *F. graminearum* and *R. solani* AG 2-2 IIIB were obtained from the Forestry and Agricultural Biotechnology Institute culture collection of the University of Pretoria. These were used throughout the experiments and cultured on potato dextrose agar (PDA) in a 9 cm Petri dish at 20 – 25 °C incubation in the dark for one week, as described by Pande et al. (2012) and Da Silva et al. (2017) for determining *Rhizoctonia* root-rot and *Fusarium* wilt disease development.

The sand: maize meal (9:1) medium stock was prepared as a mixture of 360 g sand, 40 g maize meal and 80 ml distilled water in a 500 ml Erlenmeyer conical flask. This mixture was shaken to mix and autoclaved for 15 minutes at 121 °C and this was repeated twice. The autoclaved sand-maize mixture was inoculated with 8 – 12 disks of the week-old PDA mycelia (5 mm diameter). The sand-maize meal-inoculum mix was incubated for 10 – 14 days at 23 – 25 °C in the dark and stirred every day to uniformly mix the inoculum and allow the complete

colonization of the sand-maize meal by the fungi as described by Muzhinji et al. (2018). The 14-day old culture of the reference pathogen was added to field-collected soil to determine soil suppressiveness. The disease ratings of plants at the end of the soil baiting experiment were based on a Likert scale from faba bean field studies described by Chang et al. (2014a). On this Likert scale (0 – 9) as cited in Chang et al. (2014a), the rating of 0 = seedling is well developed, no visible lesions on lower stem and roots; 1 – 2 = seedling has grown well, slight necrosis or few small lesions on lower stem and roots, total infected area < 5% (1) or < 10% (2); 3 – 4 = growth of the seedling is retarded, moderate necrosis, or small lesions scattered over the lower stem and roots, total infected area < 25% (3) or < 50% (4); 5 – 6 = seedling growth is hampered, extensive necrosis, or large lesions on lower stem and roots, total infected area < 75% (5) or $\geq$ 75% (6); 7 – 8 = seedling growth is seriously hampered, extensive necrosis, or girdling lesions on lower stem and roots, roots developed (7) or little or no roots (8); and 9 = plant dead.

8.3.5 Aggressiveness of the Reference Strains

Zea mays and *V. faba* seedlings were challenged with pathogens at low, medium and high inoculum-sand-maize meal treatment levels. The virulence of the reference strains alone or combined was assessed using *Z. mays* seedlings in pots (size 15 cm diameter and 16 cm depth) infested with a low concentration of inoculum (10 g sand-maize meal-inoculum mix per kg potting soil), medium concentration of inoculum (20 g sand-maize meal-inoculum mix per kg potting soil), high concentration of inoculum (40 g sand-maize meal-inoculum mix per kg potting soil) or non-inoculated control soil (0 g sand-maize meal-inoculum mix per kg potting soil). The inoculum culture was 14 days old at the time of infestation of potting soil.

The colonized inoculum soil was buried at 5 cm soil depth and the bottom holes of the pots were sealed to delay drainage. The pots were filled with 1.3 L of steam-sterilized sand soil and then watered to soil pot capacity for four days. Thereafter, all the pots were seeded with disinfected and hydrated *Z. mays* seeds at one seed per pot. Observations of seedling emergence

and disease incidence were done on a daily basis and recorded while the disease severity assessments were done at harvest.

The assessment of virulence of the reference strains against faba bean was done in a similar manner to the *Z. mays* experiment by preparing a series of contaminated pots by adding inoculum at low, medium and high rates prior to seeding the pots with faba bean. The pots were filled with 1.3 L of steam-sterilized sand soil, adequately watered to soil pot capacity for four days and thereafter seeded with the hydrated faba bean seeds at about 5 – 6 cm depth using one seed per pot. To adhere to Koch's postulates reisolation of *R. solani* AG 2-2IIIB and *F. graminearum* from root rot samples was done on both maize and faba bean to confirm causal agents of the disease symptoms.

8.3.6 Faba Bean as a Test Plant for Suppressiveness of Field Soil Legacies

For this study, we hypothesized that in a suppressive soil the pathogen fails to produce disease symptoms on the susceptible host (Baker & Cook, 1974; Van Elsas et al., 2006). The aim was to measure the response of *V. faba* (faba bean) cv. Espresso test plants to *R. solani* AG 2-2 IIIB and *F. graminearum* damage/rot to the roots and hypocotyl in soils from different crop rotations. Following the aggressiveness pilot results, the inoculum preparations were all done using the medium inoculum rate (14-day old culture of the reference pathogen at 20 g sand-maize meal culture per kg of potting soil) of *R. solani* AG 2-2 IIIB or *F. graminearum* alone to maximize the pathogen effects in the short-term. A similar set-up of pots (shown in Figure 8.1 and described in section 8.2.5) was repeated using field soil samples instead of steam-sterilized soil in the screening of crop rotation soils for soil suppressiveness in soil bioassays. The pots were adequately watered to soil pot capacity for four days and thereafter seeded with the hydrated faba bean seeds at about 5 – 6 cm depth using one seed per pot.

The pot experiment was a randomized complete block design with eight replications. The association of soil nutrient concentration and faba bean plant metrics (biomass (g/pot) and

length (mm)) was evaluated. Pathogenicity was estimated by comparing the number of surviving seedlings, their plant metrics and root rot ratings in the different artificially inoculated field soils. *Rhizoctonia/Fusarium* injury was rated as proposed by Chang et al. (2014a) while wilt index calculation was done according to the modified Gatch & du Doit (2015) weighted average formula:

$$\text{Disease index} = \frac{\sum(\text{severity} \times \text{number of seedlings in that rating})}{(\text{total number of emerged seedlings})}$$

8.3.7 Physiological Measurements

Seedlings were grown for 28 days and harvested at growth stage 104 (fourth node stage). The fresh weight (g/plant) of total crop biomass, aboveground and belowground biomass were measured using a laboratory weighing scale.

8.3.8 Data Processing and Statistical Analysis

Data analyses were carried out using R 3.5.2 (R Core Team, 2018), SPSS version 23 (IBM Corp. Released, 2015) and Canoco 5 version 5.11 (ter Braak & Šmilauer, 2018). Time series of climate characteristics were plotted using the “ggplot2” and “tseries” packages in R. Boxplots were drawn in SPSS version 23 for all recorded data to detect outliers in the dataset. Multivariate analysis of soil suppression and mineral nutrient composition data were visualized using principal component analysis (PCA) and non-metric multidimensional scaling (NMDS) ordination diagram of the Bray–Curtis dissimilarities, with two computed axes of soil nutrient legacies across rotation soil treatments (Canoco 5 version 5.11; ter Braak & Šmilauer, 2018).

8.3.8.1 One-Way Analysis of Variance of the % Suppression of *in vitro* Mycelial Growth

The colony mycelial growth of the reference fungus on crop rotation legacy soil samples was compared with that on conducive sand control treatments to obtain an estimate of percent suppression values. Significant differences in *R. solani* AG 2-2 IIIB *in vitro* (a) colony diameter and (b) % suppression of mycelial growth were based on analysis of variance (ANOVA) with

Tukey's honestly significant difference (Tukey HSD) test for *post hoc* multiple comparisons, conducted using SPSS version 23 (IBM Corp. Released, 2015). In this *post-hoc* test, an ANOVA F test was done to determine the significance of crop rotation treatment effects while the Tukey HSD *post-hoc* test was done to group the treatment means (Steel & Torrie, 1960) strictly for soil and plant health data (or transformed data) of equal sample sizes that meet the normality and homogeneity of variance assumptions. Overall, this *post-hoc* test provides answers to specific meaningful questions, which were after all the objectives of the study in comparing all pairs of crop rotation treatments (Ruxton and Beauchamp, 2008; Kozak & Powers, 2017).

8.3.8.2 Testing for median and mean differences in Test Plant Metrics between Four Crop Rotations using the Wilcoxon Signed Rank and Kruskal-Wallis tests, respectively.

The Wilcoxon Signed Rank test was used to determine the significance of median differences in (a) shoot length, (b) shoot biomass, (c) root biomass and (d) total biomass responses to the pathogenicity of *R. solani* AG 2-2 IIIB or *F. graminearum*. For soil baiting assay data that were normally distributed, a parametric one-way ANOVA was done, while for data violating the normality assumption, the non-parametric Kruskal-Wallis test was used to compare plant metrics per pot across each rotation.

8.3.8.3 Multivariate of Soil Composition and Test Plant Metrics responses

Multivariate analysis of soil composition legacies data for % soil suppression and plant disease index were visualized using PCA approach from Canoco 5 version 5.11 (ter Braak & Šmilauer, 2018). The PCA biplot also displayed responses of test plant and disease severity to the measured historical legacies of soil composition. The objective was to obtain an ordination diagram ranking the % soil suppression and root rot disease index data, at the same time, revealing relationships among selected crop rotations.

8.3.8.4 Correlation analyses of Soil Composition metrics with Faba Bean Root Rot Disease Index

The Spearman's correlation (ρ , r_s) coefficients were used to rank the relationships of crop rotations based on their soil composition in SPSS version 23. Furthermore, the relationships among soil composition metrics and the severity of *R. solani* AG 2-2 IIIB and *F. graminearum* root rot disease were calculated using the Spearman's correlation coefficients.

8.4 Results

8.4.1 Climate and Soil Characteristics

In this study, the climate data indicated that there was adequate total solar radiation, warm temperatures and thermal time for the normal development of faba bean plants. Faba bean plants have a low tolerance to dry hot soil conditions. Under optimum environmental conditions they take 29 days or more after planting to begin flowering (GRDC, 2008). Table 8.1 summarizes the soil analysis results of selected soil properties concentration legacies.

Comparison of soil factors indicated that Zn, P and K were significantly different among the four crop sequences across all the four seasons (as reported in Chapter 7 section 7.4.2.2, Figure 7.3 and Figure 7.4). The soil fertility recommendations (Table S8.1) guide growers in Southern Africa. These guidelines provide growers and consultants with reliable insight into the effectiveness and mismanagement of field trial experiments in Southern Africa.

Table 8.1 The soil biological and physicochemical properties of sandy (% Clay 8.94 ± 0.18 , % Silt 9.80 ± 0.24 , and % Sand 81.26 ± 0.26) topsoil collected at 0–20 cm depth from a crop rotation field trial on 29 July 2019. N = 8 composite samples. SE = standard error.

Soil factor (units), p value (s/ns)	Soil origin (Mean $\pm$ SE) [#]			
	PMMT	PMSBM	PMSOYM	PMSUNM
Density (g/cm ³), ns	1.35a $\pm$ 0.036	1.38a $\pm$ 0.009	1.39a $\pm$ 0.006	1.39a $\pm$ 0.011
pH (H ₂ O), ns	5.75a $\pm$ 0.170	5.59a $\pm$ 0.123	5.46a $\pm$ 0.078	5.56a $\pm$ 0.026
Phosphate Mehlich 3 (mg/kg), *s	36.40a $\pm$ 1.74	40.10ab $\pm$ 2.28	43.60ab $\pm$ 2.15	46.10b $\pm$ 2.00
Phosphate Bray 2 (mg/kg), *s	38.60a $\pm$ 3.29	44.30ab $\pm$ 2.23	46.50ab $\pm$ 1.93	49.10b $\pm$ 2.23
Potassium (mg/kg), **s	129.00b $\pm$ 11.2	96.90ab $\pm$ 7.83	85.80a $\pm$ 6.73	127.00b $\pm$ 7.68
Ammonium-Nitrogen (mg/l), ns	0.365a $\pm$ 0.029	0.380a $\pm$ 0.022	0.371a $\pm$ 0.031	0.588a $\pm$ 0.156
Nitrate-Nitrogen (mg/l), ns	1.64a $\pm$ 0.167	1.29a $\pm$ 0.168	2.29a $\pm$ 0.466	2.780a $\pm$ 1.60
Zinc (mg/kg), ***s	1.95a $\pm$ 0.121	2.60ab $\pm$ 0.204	3.08b $\pm$ 0.216	3.18b $\pm$ 0.242
Active-carbon (mg/kg), ns	286.00a $\pm$ 8.96	289.00a $\pm$ 13.9	304.00a $\pm$ 15.1	295.00a $\pm$ 14.2
Solvita respiration (ppm), ns	28.50a $\pm$ 6.83	21.40a $\pm$ 3.06	20.20a $\pm$ 1.74	24.60a $\pm$ 2.86
Soil Organic Matter (SOM) (%), ns	0.345a $\pm$ 0.017	0.346a $\pm$ 0.015	0.294a $\pm$ 0.020	0.320a $\pm$ 0.019
Carbon Ash (%), ns	0.199a $\pm$ 0.009	0.201a $\pm$ 0.009	0.169a $\pm$ 0.013	0.186a $\pm$ 0.010

[#]Different letters represent significant differences (s) among crop rotation combinations within a soil factor, while shared letters represent non-significant differences (ns) among soil origin combinations based on one-way ANOVA using the Tukey's HSD test statistic *post hoc* multiple comparisons.

*The significance was categorized into non-significant, significant, highly significant and very highly significant, where $p > .05$, $* p \leq .05$, $** p < .01$ and $*** p < .001$ (IBM Corp. Released, 2015), respectively.

8.4.1.1 Hillcrest Campus (Experimental Farm Plant Pathology Tunnels, S 25°45'9.5" E 28°15'26", SA)

The climate variables for greenhouse experiments conducted at the Hillcrest Campus from 29 November 2019 to 24 December 2019 are detailed in Figures S8.1. In Figure S8.1a, the calculated daily temperature ranges (12.90 to 48.77 °C) were higher than for Hatfield Campus greenhouse. In the growing phase (Figure S8.1b), relative humidity ranged from 29.03 % to 97.71 % with an average of 71.30 %, and the maximum solar intensity was 20,951.1 Lux (Figure S8.1c).

8.4.1.2 Hatfield Campus (Wheatboard Greenhouse and Labs, S 25°45'21" E 28°13'51", SA)

The climate variables for greenhouse experiments conducted at the Hatfield Campus from 29 November 2019 to 24 December 2019 are detailed in Figure S8.2. In Figure S8.2a, the calculated daily temperature ranges (17.51 to 44.44 °C) were lower than for Hillcrest Campus tunnels. In the growing phase (Figure S8.2b), relative humidity ranged from 25.27 % to 97.96 % with an average of 65.28 %, and the maximum solar intensity was 32,280.1 Lux (Figure S8.2c).

Overall, the two controlled environments at UP were similar in terms of the measured climate variables. The repeat experiment had higher plant metric values but similar patterns and trends across treatments than the first experiment. The Spearman non-parametric correlation results indicate that the faba bean plant biomass metrics of the Hatfield experiment and the Hillcrest experiment were not correlated (Table S8.2, $p > .05$). Also, there were no significant interactions between the Hatfield data × Hillcrest experiment data ($p > .05$, data not shown), as a result, data from the two trials was combined.

8.4.2 Suppression of *R. solani* AG 2-2 IIIB Mycelial Growth under *in vitro* conditions

There were no significant differences in mycelial growth inhibition among the four rotations ($p > .05$), but the rotations were significantly different from the control ($p < .05$) according to the Tukey HSD test (Figure 8.2a). However, there were similar significant differences in % suppression of mycelial growth (Figure 8.2b, $p < .05$). The findings suggest that sugar beans in rotations create soil health legacies that are relatively suppressive to *R. solani* AG 2-2 IIIB when maize is the follow-up crop (Figure 8.2).

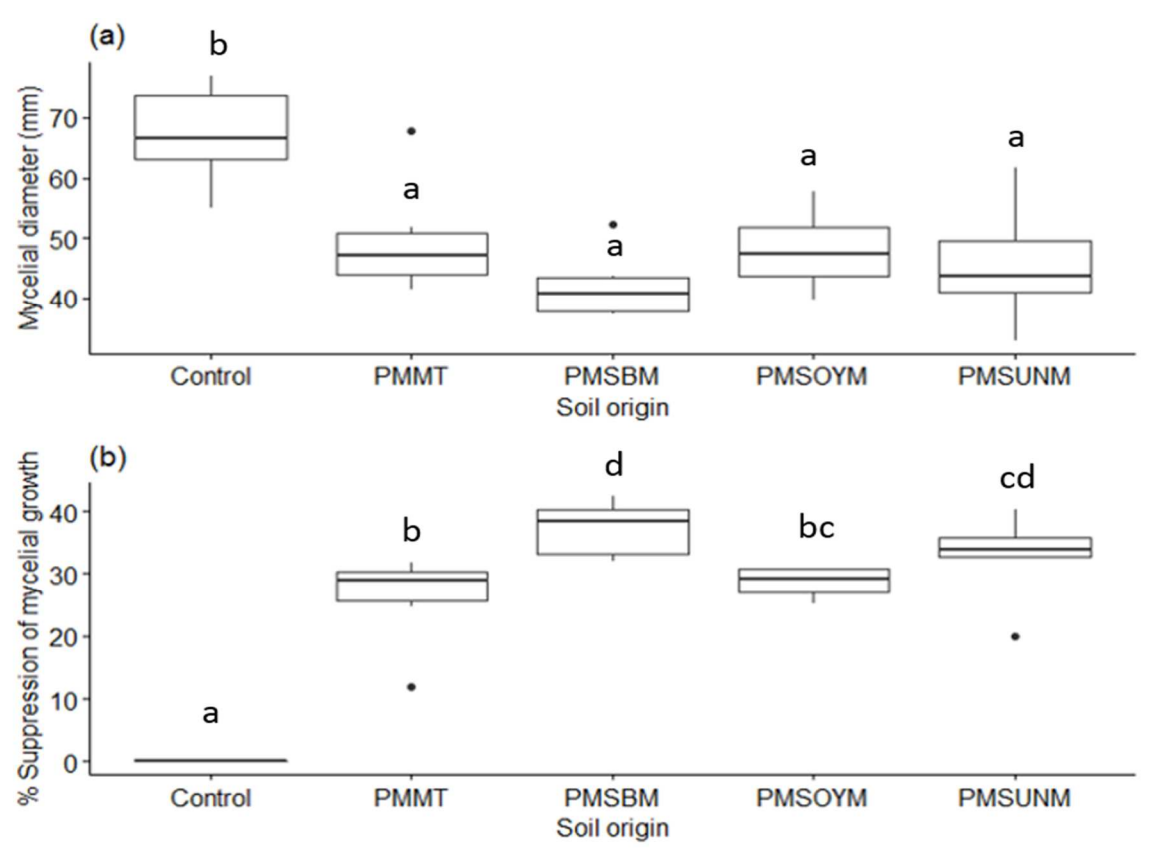


Figure 8.2 Boxplots of *in vitro* suppression (a-b) of *R. solani* AG 2-2 IIIB in soils originating from four rotation sequence treatments collected at harvest in season 4 (year 2018/19). PMMT – Potatoes-Maize-Maize-Teff, PMSBM – Potatoes-Maize-Sugar beans-Maize, PMSOYM – Potatoes-Maize-Soybeans-Maize, and PMSUNM – Potatoes-Maize-Sunflower-Maize crop rotations. Control represents sterilized sand soil. Each rotation sequence treatment had 8 replications. Each block had 4 treatments. Horizontal heavy bar = median, box=lower and upper quartiles, whiskers=minimum and maximum values, filled dot=outliers. Significant differences ($p < .05$) indicated by different letters.

The Potatoes-Maize-Sugar beans-Maize rotation seemed to cultivate legacies that inhibit the mycelial growth of *R. solani* AG 2-2 IIIB more than other crop rotations. The results suggest that commercial growers can include sugar beans in potato rotations to cultivate a disease suppressive soil against *R. solani* AG 2-2 IIIB. Baiting assays using faba beans as an indicator plant, showed significant differences in soil suppressiveness against *R. solani* AG 2-2 IIIB and *F. graminearum* among soils from different rotations ($p < .05$).

8.4.3 Preliminary tests to determine Aggressiveness and Virulence of the Pathogens

Preliminary virulence and aggressiveness of the phytopathogens was tested on maize seedlings (data not shown). The soil baiting assays used the soil transfer method to identify soil suppression (Berendsen et al., 2012; Stirling et al., 2016).

8.4.3.1 Pathogen Aggressiveness and Virulence on Faba Bean and Maize Seedlings

The inoculum potential of the isolates in causing root rot was evaluated using faba bean plants sown in arbitrary low inoculum, medium inoculum, high inoculum and zero inoculum heat treated sand soils (Figure S8.3A to S8.3B). There were significant differences ($p < .05$, Figure S8.3A to S8.3B) in faba bean shoot biomass, root biomass and total biomass between the Control, low inoculum soil for *Fusarium* (LF), medium inoculum soil for *Fusarium* (MF), high inoculum soil for *Fusarium* (HF) treatments and the low inoculum soil for *Rhizoctonia* (LR), medium inoculum soil for *Rhizoctonia* (MR), high inoculum soil for *Rhizoctonia* (HR), low inoculum soil for *Fusarium* + *Rhizoctonia* (LS), medium inoculum soil for *Fusarium* + *Rhizoctonia* (MS), high inoculum soil for *Fusarium* + *Rhizoctonia* (HS).. Among pathogen treatments, all treatments infested or co-infested with *R. solani* AG 2-2 IIIB had a higher negative impact on the metrics of the test plant than *F. graminearum* (Figure S8.3C).

Within-pathogen treatment effects on plant metrics indicate that there were no clear differences (Figure S8.3). Also, the medium inoculum soil for *Fusarium* (MF) had plant metrics that were less than those obtained in both LF and HF treatments (Figure S8.3C). The highly virulent and

aggressive pathogen treatments (LF, HF, HR, HS) showed relatively high root rot index ranging from 2.5 to 2.8 while the least virulent treatments had a root rot index value of 0.9 (Figure S8.3C).

As illustrated in Figure S8.3C, the medium inoculum soil treatments (MF, MR, MS) had similar patterns for *F. graminearum*, *R. solani* AG 2-2 IIB and coinfection ($p > .05$) in the manifestation of root rot symptoms in the test faba bean plants. Thenceforth, the arbitrary medium inoculum soils inoculum levels were selected to determine the impact of historical soil legacies on faba bean plant health.

8.4.3.2 Development of *R. solani* AG 2-2 IIB and *F. graminearum* in Faba Bean Roots

Rhizoctonia solani AG 2-2 IIB caused visible lesions on the hypocotyl and roots of young bean plants, as shown below in Figure 8.3.

Comparisons of root-rot disease severity indicated that; plants infected with *R. solani* AG 2-2 IIB had more severe symptoms such as decay, sunken cankers and girdling around the entire stem than those infected with *F. graminearum* (Figure 8.3). In severely affected plants, the lower parts of the taproot and the secondary root system were usually dead (Figure 8.3).

The moderate root rot severity rating of 3 – 4 indicated mild (medium) symptoms such as lesions on the hypocotyl and lower stem that did not extend above the soil line in both *R. solani*-infected and *F. graminearum*-infected roots. The severe (high) symptoms in the 7 – 8 root rot severity rating scale were first noticed in *R. solani*-infected young plants while root rot severities for *F. graminearum*-infected plants reached a maximum rating scale of 1 -2 whereby faba bean plants were generally stunted.



Figure 8.3 Comparison of medium/high severity of seed and root-rot symptoms caused by *R. solani* and *F. graminearum* infection of faba bean 30 days after planting. Images by Mr. Knowledge Mushonga.

8.4.4 Soil Bioassays of Crop Rotation Soil Legacy Effects on Faba Bean Plant Health

8.4.4.1 Plant Metrics

Plant metrics of the indicator faba bean plants at 30 days after planting, showed that the two pathogens significantly reduced the root biomass, shoot biomass and plant height ($p < .01$). In terms of shoot length, significant differences ($p < .01$) were observed between pathogen treatments (Figure 8.4a). In general, *R. solani* AG 2-2 IIIB was consistently more virulent in reducing plant biomass than *F. graminearum* ($p < .01$, Figure 8.4b-d).

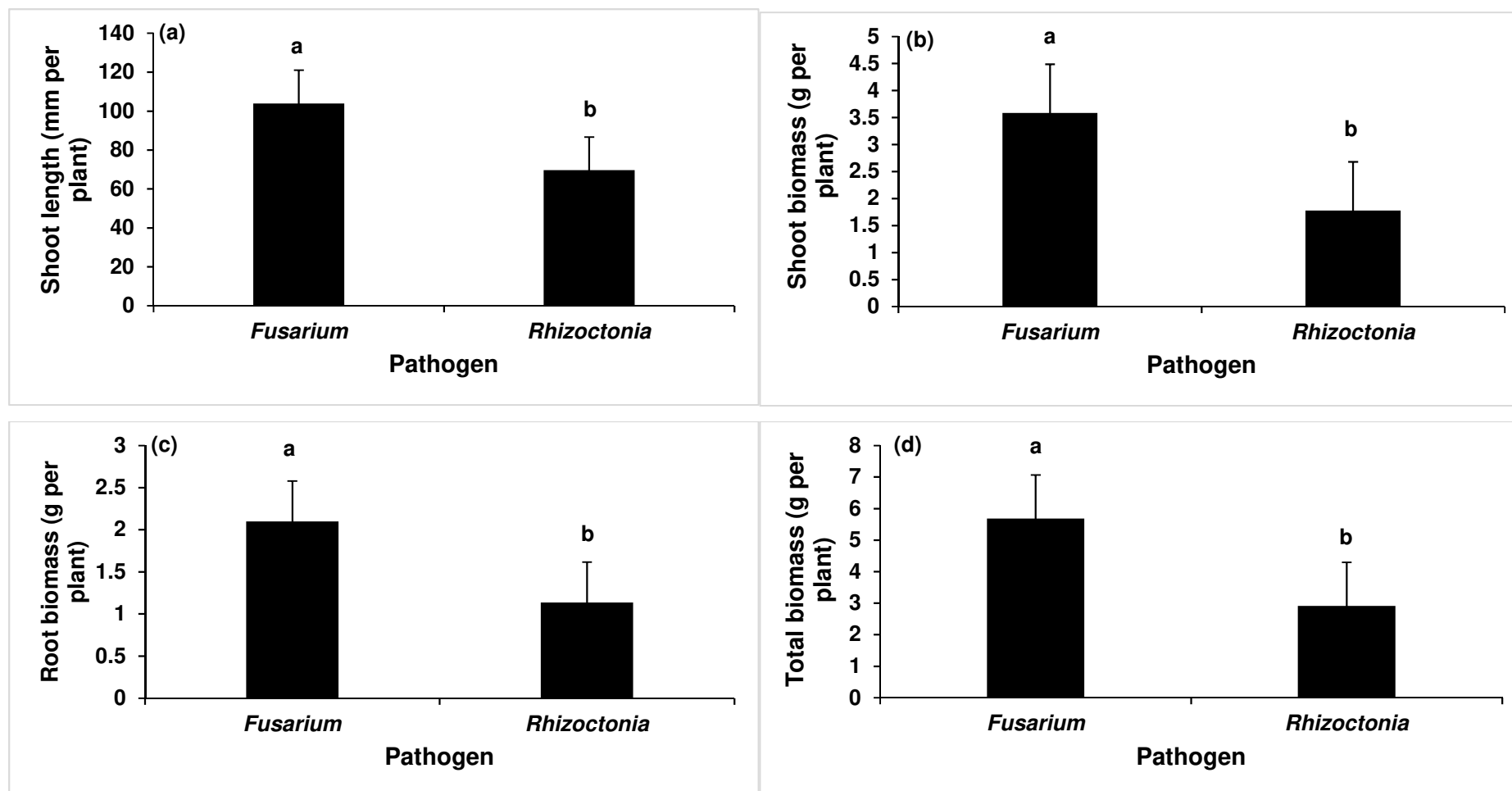


Figure 8.4 Differences in median (a) shoot length ($P = .007$), (b) shoot biomass ($P = .001$), (c) root biomass ($P = .002$) and (d) total biomass ($P = .001$) of 64 replicates responses of faba bean to *R. solani* AG 2-2 IIIB and *F. graminearum* artificial inoculum under greenhouse conditions. $N = 64$ per pathogen treatment, error bars represent standard error of the mean. Significant differences ($p < .01$) are indicated by different letters according to the Wilcoxon Signed Rank test.

8.4.4.2 Root Rot Index

The disease index was measured based on a Gatch & du Doit (2015) weighted average formula of relating the disease incidence and severity (Brown et al., 2021). The average values in root rot indices for the different soil origin and pathogens (Figure 8.5) did not show significant differences using one-way ANOVA ($p > .05$). For *Rhizoctonia* (Figure 8.5), the PMSUNM rotation had the highest average root rot index of 3.2 ± 1.1 while the PMMT rotation soil had the lowest (2 ± 0.8). On the other hand, *Fusarium* root rot index was highest at 2.5 ± 1.1 in the PMSOYM rotation and lowest (1.8 ± 0.2) in the PMSBM rotation.

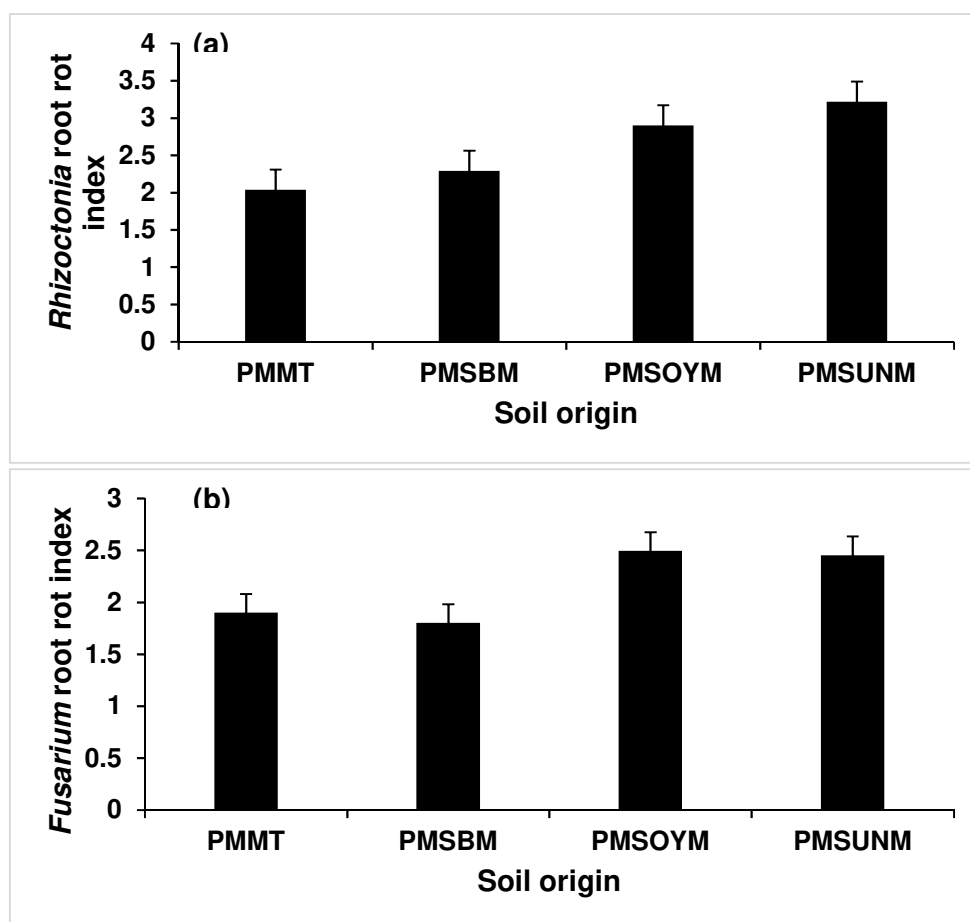


Figure 8.5 The average root rot risk in faba bean plants grown in crop rotation soils inoculated with (a) *R. solani* AG 2-2 IIIB and (b) *F. graminearum* under greenhouse conditions. PMMT – Potatoes-Maize-Maize-Teff, PMSBM – Potatoes-Maize-Sugar beans-Maize, PMSOYM – Potatoes-Maize-Soybeans-Maize, and PMSUNM – Potatoes-Maize-Sunflower-Maize rotations. Error bars represent the standard error of mean (SEM) using a total of 16 pots (8 replicates per trial site).

8.4.4.3 Plant Responses to Soil Origin and Pathogen Treatments

Plant responses to soil origin and pathogen treatment are summarized in Figure 8.6 and outlined in Tables S8.3 to S8.6. The PMMT rotation showed similar patterns in suppressing the negative effects of both pathogens on shoot length (Figure 8.6a). In the *F. graminearum* treatment, the PMSUNM rotation had on average a high shoot length (167.5 ± 10.6 mm per plant, Figure 8.6a). In *R. solani* AG 2-2 IIIB treatment, the mean shoot length was lowest, at 37.5 ± 23.5 mm per plant and 38 ± 13.9 mm per plant in the PMSBM and PMSOYM crop rotations, respectively (Figure 8.6a).

The *R. solani* AG 2-2 IIIB effects on plant shoot biomass was the lowest (1.1 ± 0.3 g per plant) in the PMSUNM rotation and highest (2.1 ± 0.8 g per plant) in the PMSOYM rotation (Figure 8.6b). In the *F. graminearum* inoculated treatment, the PMSBM rotation had an average biomass of 2.8 ± 0.9 g per plant, while the PMSUNM rotation had the highest shoot biomass of 4.3 ± 1.0 g per plant (Figure 8.6b). The PMSUNM rotation had the lowest root biomass in the *R. solani* AG 2-2 IIIB treatment (Figure 8.6c). All rotations were comparably not suppressive against reducing total biomass of the test plants (Figure 8.6d). The mean values of the plant metrics were significantly different across categories of soil origin with added *R. solani* AG 2-2 IIIB artificial inoculum ($p < .05$, Tables S8.3, S8.4, S8.5 and S8.6). For *F. graminearum*, all metrics showed significant differences across crop rotations ($p < .05$, Tables S8.3 to S8.6). The PMSUNM rotation was the best in suppression of *F. graminearum*, while the PMSBM rotation was the least suppressive against *F. graminearum* (Figure 8.6).

In the *Rhizoctonia* inoculum treatments, *post-hoc* pairwise comparison indicated significant differences in mean shoot length, shoot biomass and total biomass between the PMSUNM (higher) and PMSOYM (lower) rotations ($p < .05$, Figure 8.6, Tables S8.3 to S8.6). In the *Fusarium* treatments, there were significant mean rank differences in shoot biomass between the PMSBM and PMMT crop rotation ($p < .05$, Figure 8.6, Tables S8.3 to S8.6).

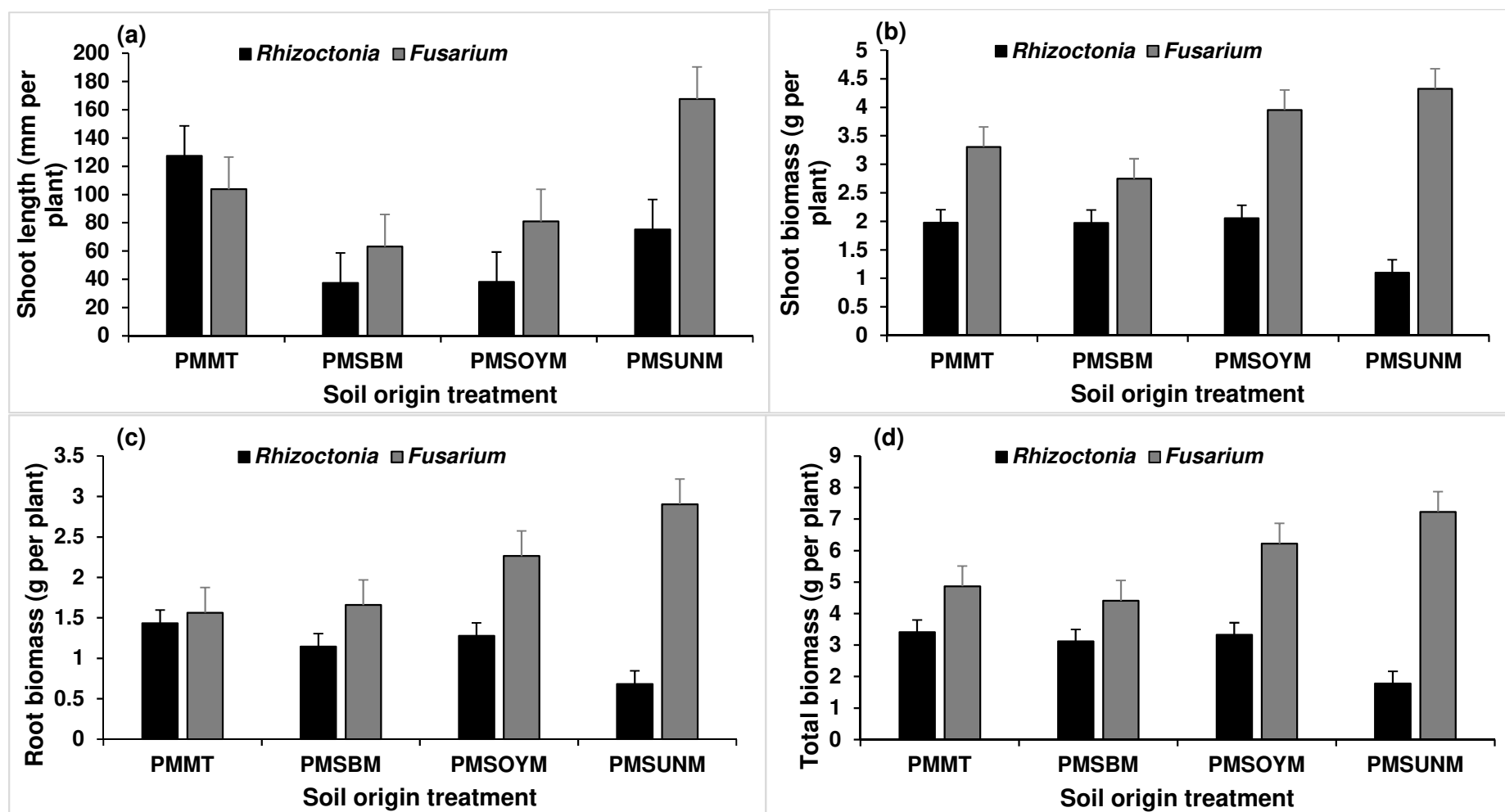


Figure 8.6 Soil baiting assay results of mean for (a) shoot length, (b) shoot biomass, (c) root biomass and (d) total biomass response $\pm$ SE for faba beans grown in different soil origin/treatments. Soils were spiked with either *R. solani* AG 2-2 IIIB or *F. graminearum* under greenhouse conditions. PMMT – Potatoes-Maize-Maize-Teff, PMSBM – Potatoes-Maize-Sugar beans-Maize, PMSOYM – Potatoes-Maize-Soybeans-Maize, and PMSUNM – Potatoes-Maize-Sunflower-Maize crop rotations. All bars represent the standard error (SE) of the mean (n=8).

8.4.4.4 Multivariate of Soil Origin Properties and Test Plant Metrics responses

The PCA (Figure 8.7) showed no relationship between crop rotation and *in vitro* mycelial growth diameter of *R. solani* AG 2-2 IIIB. All the crop rotations were correlated positively with high values of *R. solani* AG 2-2 IIIB disease index while negatively correlated with the *in vitro* % suppression of colony diameter. The PC 1 and PC 2 of the PCA described 88.19 % of the total variability of the *R. solani* AG 2-2 IIIB data.

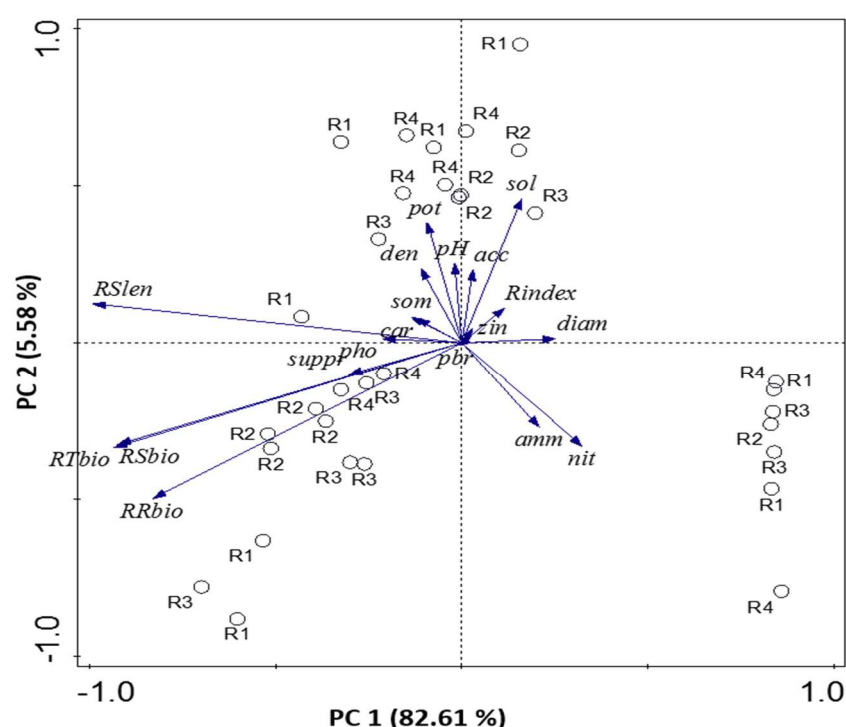


Figure 8.7 PCA of five field rotation soils (R1, R2, R3, and R4) compared and correlated with pathogen effects (root rot disease) and soil properties. Each rotation soil treatment had eight replications and each block had four treatments. R1 – Potatoes-Maize-Maize-Teff, R2 – Potatoes-Maize-Sugar beans-Maize, R3 – Potatoes-Maize-Soybeans-Maize, and R4 – Potatoes-Maize-Sunflower-Maize crop rotations.

pH - pH H ₂ O	pbr - P Bray2	car - C Ash	som – SOM
zin - Zn (M3)	amm - NH ₄ -N(1:2)	pho - P (M3)	pot - K (M3)
acc – Active-C	sol - Solvita CO ₂ burst	nit - NO ₃ -N(1:2)	den - Density
RSlen – Shoot length of faba bean		RSbio – Shoot biomass of faba bean	
Rindex – <i>Rhizoctonia</i> root rot index		RTbio – Total biomass of faba bean	
suppr – % Suppression of <i>R. solani</i> AG 2-2 IIIB		RRbio – Root biomass of faba bean	
diam – Mycelial diameter of <i>R. solani</i> AG 2-2 IIIB			

The main contributors of high values of the first axis were plant health metrics (shoot length, root, shoot and total plant biomass). The plant metrics were very close to each other indicating that there was no significant variance in the plant health data. As expected, mycelial diameter of *R. solani* AG 2-2 IIIB was negatively correlated with % suppression of *R. solani* AG 2-2 IIIB *in vitro*. Rhizoctonia root rot index was positively correlated with Solvita microbial respiration, active-C, pH and Zn while negatively correlated with NO₃-N, NH₄-N, SOM and ash-C. Most soil health metrics had variance inflation factor (VIF) values less than 3 indicating that there was no multicollinearity except for SOM and ash-C (Table S8.7, VIF > 3).

The *F. graminearum* PCA showed a very low variance in the faba bean plant data in all the crop rotations analyzed in this study (Figure 8.8). Similar to *R. solani* AG 2-2 IIIB data, the main contributors of high values of the first axis were plant health metrics (shoot length, root, shoot and total plant biomass). The plant metrics were very close to each other indicating that there was no significant variance in the *F. graminearum* plant health data (Figure 8.8). The PC 1 and PC 2 of the PCA described 87.38 % of the total variability of the *F. graminearum* data.

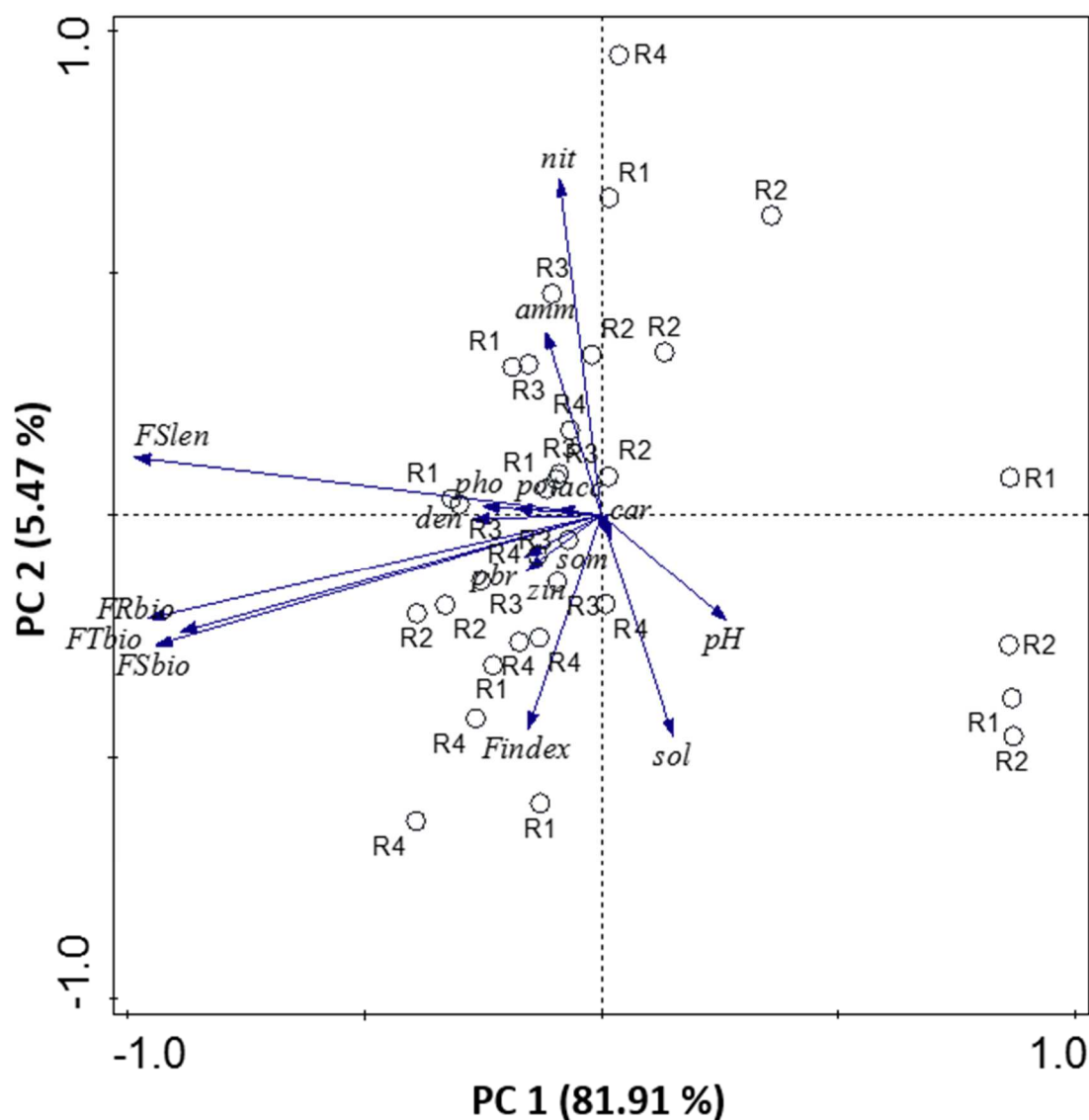


Figure 8.8 PCA of field rotation soils (R1, R2, R3, and R4) compared and correlated with *F. graminearum* pathogen effects (root rot disease) and soil properties. Each rotation soil treatment had eight replications and each block had four treatments. R1 – Potatoes-Maize-Maize-Teff, R2 – Potatoes-Maize-Sugar beans-Maize, R3 – Potatoes-Maize-Soybeans-Maize, and R4 – Potatoes-Maize-Sunflower-Maize crop rotations.

sol - Solvita CO ₂ burst	pot - K (M3)	den – Density	car - C Ash	som – SOM
amm - NH ₄ -N(1:2)	pbr - P Bray2	acc – Active-C	pho - P (M3)	zin – Zn (M3)
nit - NO ₃ -N(1:2)	pH - pH H ₂ O	Findex – <i>Fusarium</i> root rot index		
FSlen – Shoot length of faba bean		FSbio – shoot biomass of faba bean		
FRbio – Soot biomass of faba bean		FTbio – total biomass of faba bean		

8.4.4.5 Correlation Analyses of Soil Health Metrics with Faba Bean Plant Health

The twelve soil health properties composition were significantly different across the field crop rotations (Table 8.1 detailed in Section 8.4.1). The subsequent Spearman's correlations between soil factors and disease severity index (Table 8.2 and Table 8.3) did not rule out soil organic matter (SOM), NO₃-N and Ash-C, but confirmed the negative relationships observed in the PCA results (Figure 8.7 and Figure 8.8).

8.4.4.5.1 *In vitro* Growth Assays

The colony growth data of the reference *R. solani* AG 2-2 IIIB was analyzed using the Spearman's correlation coefficients to determine the associations between; the historical soil composition, *in vitro* mycelial diameter of *R. solani* AG 2-2 IIIB and *in vitro* % suppression of *R. solani* AG 2-2 IIIB. The historical legacies in soil NH₄-N, SOM and ash-C correlated positively with % soil suppression of *R. solani* AG 2-2 IIIB (Table 8.2, $p > .05$). The negative correlations of soil SOM and ash-C with mycelial colony diameter of *R. solani* AG 2-2 IIIB were highly significant ($p < .01$). Soil pH was negatively correlated with NO₃-N ($p < .05$) and weakly correlated with the root rot severity index of the two pathogens ($p > .05$) (Table 8.2 and Table 8.3). The crop rotation legacies in soil NO₃-N correlated negatively with root rot index of *R. solani* AG 2-2 IIIB (Table 8.2, $p < .05$) and *Fusarium* root rot index (Table 8.3, $p < .05$).

8.4.4.5.2 Plant Bioassays

The Spearman's correlation coefficient was used to estimate the association between the historical soil compositions and faba bean plant biomass. The crop rotation legacy in soil NO₃-N correlated negatively and significantly with both *Rhizoctonia* root rot severity index (Table 8.2, $p < .05$) and *Fusarium* root rot severity index (Table 8.3, $p < .05$).

Table 8.2 Spearman's rho correlation coefficients between soil health metrics and % suppression *in vitro* and disease index of *R. solani* AG 2-2 IIIB. The disease index was measured 26 days after planting in root rot soil bioassays using test plants grown in soil samples from the EFS field crop rotations (soil properties shown in Table 8.1). N = 32 soil samples.

	pH	P bray	Density	K	P	NO ₃ -N	NH ₄ -N	Zn	Active-C	Solvita	SOM	Ash-C	Mycelia	% S	Index
pH	—														
P bray	-0.107	—													
Density	-0.103	-0.016	—												
K	0.141	0.105	0.082	—											
P	-0.121	0.770***	0.206	0.155	—										
NO ₃ -N	-0.425*	0.098	-0.187	-0.048	-0.099	—									
NH ₄ -N	-0.118	-0.016	0.138	0.069	0.055	-0.254	—								
Zn	0.083	0.801***	0.161	-0.010	0.718***	0.028	-0.018	—							
Active-C	0.242	1.24	0.321	0.048	0.135	0.023	-0.003	0.291	—						
Solvita	0.209	-0.023	0.402*	0.252	-0.149	-0.252	-0.126	0.126	0.183	—					
SOM	-0.008	0.043	-0.089	0.473**	-0.062	-0.062	-0.017	-0.172	0.040	0.191	—				
Ash-C	-0.015	0.050	-0.092	0.484**	-0.054	-0.054	-0.011	-0.164	0.037	0.186	0.999***	—			
Mycelia	-0.064	-0.060	-0.089	-0.258	0.023	0.013	-0.093	-0.123	-0.110	-0.115	-0.537**	-0.533**	—		
% S	-0.069	0.070	-0.003	0.045	0.172	-0.199	0.335	0.138	0.139	-0.108	0.325	0.327	-0.629***	—	
Index	0.124	0.115	0.234	-0.003	0.230	-0.394*	0.223	0.269	0.266	0.217	-0.290	-0.292	-0.069	0.137	—

Note. * p < .05, ** p < .01, *** p < .001

pH - pH H ₂ O	pbr - P Bray2	den - Density	car - C Ash	som - SOM
zin - Zn (M3)	amm - NH ₄ -N(1:2)	nit - NO ₃ -N(1:2)	pho - P (M3)	pot - K (M3)
acc - Active-C	sol - Solvita CO ₂ burst	Rindex - <i>Rhizoctonia</i> root rot index		
suppr - % Suppression of <i>R. solani</i> AG 2-2 IIIB		diam - Mycelia diameter of <i>R. solani</i> AG 2-2 IIIB		

Table 8.3 Spearman's rho correlation coefficients between soil health metrics and root rot disease index of *F. graminearum*. The disease index was measured 26 days after planting in root rot soil bioassays using test plants grown in soil samples from the EFS field crop rotations (soil properties shown in Table 8.1). N = 32 soil samples.

	pH	P bray	Density	K	P	NO ₃ -N	NH ₄ -N	Zn	Active-C	Solvita	SOM	Ash-C	Index
pH	—												
P bray	-0.107	—											
Density	-0.103	-0.016	—										
K	0.141	0.105	0.082	—									
P	-0.121	0.770***	0.206	0.155	—								
NO ₃ -N	-0.425*	0.098	-0.187	-0.048	-0.099	—							
NH ₄ -N	-0.118	-0.016	0.138	0.069	0.055	-0.254	—						
Zn	0.083	0.801***	0.161	-0.010	0.718***	0.028	-0.018	—					
Active-C	0.242	0.124	0.231	0.048	0.135	0.023	-0.003	0.291	—				
Solvita	0.209	-0.023	0.402*	0.252	-0.149	-0.252	-0.126	0.126	0.183	—			
SOM	-0.008	0.043	-0.089	0.473**	-0.062	-0.062	-0.017	-0.172	0.040	0.191	—		
Ash-C	-0.015	0.050	-0.092	0.484**	-0.054	-0.054	-0.011	-0.164	0.037	0.186	0.999***	—	
Index	0.112	0.021	0.170	-0.129	0.069	-0.397*	0.111	0.143	0.299	0.117	-0.199	-0.211	—

Note. * p < .05, ** p < .01, *** p < .001

pH - pH H ₂ O	pbr - P Bray2	den - Density	car - C Ash	som - SOM
zin - Zn (M3)	amm - NH ₄ -N(1:2)	nit - NO ₃ -N(1:2)	pho - P (M3)	pot - K (M3)
acc - Active-C	sol - Solvita CO ₂ burst	Findex - <i>Fusarium</i> root rot index		

8.5 Discussion

Soil bioassays are a robust tool for predicting disease risk, particularly, root rot risk (Rollins et al., 1999; Wang et al., 2006; Deshmukh et al., 2007; Bilgi et al., 2011; Boine et al., 2014; Wang et al., 2015; Muzhinji et al., 2018) and wilt risk (Xue et al., 2007; Gatch & du Toit, 2015). The main aim of this study was to compare soil suppressiveness among four crop rotations using soil bioassays under *in vitro* and greenhouse conditions. Soil bioassays provide a good assessment tool for soil suppressiveness and risk assessment of soil borne diseases (Pal et al., 2001; Van Elsas et al., 2002; Huang et al., 2010; Chang et al., 2014a & 2014b; Larkin & Halloran, 2014; Gatch & du Toit, 2015; Chang et al., 2017; Conner et al., 2017; Hamid et al., 2017; Chang et al., 2019; Safarieskandari, 2019; Harries et al., 2020; Larkin & Brewer, 2020; Davey et al., 2021).

The daily temperatures (15 to 32 °C) and the moist sandy-soils promoted disease development. Well-drained soils, wet weather, cool to warm weather and soil temperature range of 18 °C to 29 °C favour root rot and hypocotyl rot disease epidemics of bean (Sneh et al., 1996; Horst, 2008). *Fusarium* root rot disease leads to stunted and slow growth in diseased plants, the optimal growth conditions for this pathogen to cause disease include warm temperatures of 22 to 32 °C and high moisture as well as acidic soils (Deacon, 2006). On the other hand, the optimal growth conditions for *Rhizoctonia* root rot disease include cooler temperature of 15 to 18 °C and adequate to high moisture conditions which result in more severe disease (Sneh et al., 1996; Deacon, 2006). The climate like other environmental factors is crucial to the development of root and stem rot diseases because it affects the populations of phytopathogenic fungi in the soil and in plants (Brown et al., 2021).

The findings demonstrated that the PMSBM crop rotation has soil legacies that relatively inhibit the *in vitro* colony growth of *R. solani* AG 2-2 IIIB. Contrary, sugar beans are rotated one year in three with maize because they are very susceptible to diseases under monoculture

production in South Africa (Smith, 2006). The inclusion of sugar beans in between maize plantings suppresses the pathogen, this finding suggests that the bean cultivars planted in the field trial probably carried some resistance to these pathogens (Abawi et al., 2012; Spedaletti et al., 2016) and the bean crop, its residues and litter inputs promoted microbial mutualists, rhizobial nodulators and/or competitors that improved soil suppressiveness against the root rot pathogens (Sumner & Bell, 1994; Abawi et al., 2012; Naseri & Moradi, 2015; Mushonga et al., 2020). The symbiotic relationship between the rhizosphere organisms and crop roots begins at germination, after the seedling exudes toxins to destroy pathogens while the rhizodeposits attract beneficial organisms (Jhonson, 2009; Berendsen et al., 2012). The PMSBM rotation soil indirectly influence soil population of root pathogens and their potential damage on bean indicator plants. Thus, PMSBM rotation probably improves soil health and minimize the impact of the root pathogens in the field-collected soils. Poor soil quality, low pH, and sand are associated with *R. solani* populations in soils collected from bean production fields (Sumner & Bell, 1994; Naseri & Hamadani, 2017). In the present study, the inclusion of sugar beans in maize rotations has the potential to cultivate a disease suppressive soil against *R. solani* AG 2-2 IIIB. Suppressive soils contain indicator microbes with biocontrol potential against *R. solani* (Baker, 1987; Weller et al., 2002; Berendsen et al., 2012; Jayaraman et al., 2021; Khatri, et al., 2021).

In Chapter 4 (Table 4.2), the high bacterial biomass recorded in *P. vulgaris* and *G. max* soils were likely due to the cultivation of compatible rhizobia *Rhizobium leguminosarum* and *Bradyrhizobium japonicum/Rhizobium fredii* from previous historical or natural field inoculum (Bardgett, 2009). Under greenhouse conditions in Chapter 5, *P. vulgaris* showed positive soil-feedbacks strongly associated with their own beneficial soil microbiota legacy of mutualists and decomposers that are more significant than pathogens under monoculture (Mushonga et al., 2020). Soil and plant type influence microbial diversity which in turn drives legacies of soil

suppressiveness (Garbeva et al., 2004). Soil suppressiveness is mainly a product of soil microbial activity; soil microbial respiration and high C content (De Corato, 2020a, 2020b; Wright et al., 2021). Legumes rely on microbes such as *Rhizobium* and *Cyanobacteria*, which are microbial indicators of soil health because they serve as biological control agents of some phytopathogens in legumes and non-legumes (Fageria et al., 2010; Siczek & Lipiec, 2016; Oberholster et al., 2018).

The findings on the *R. solani* AG 2-2 IIIB disease severity indicate that the soil legacy effects of the grass-dominated PMMT crop rotation were suppressive to faba bean root rot disease. This is in agreement with other studies supporting the cultural control of *Rhizoctonia* spp. using a three-year crop rotation of maize, onions, grasses or cereals (Jackson, 2021). Similarly, Larkin and Halloran (2014) recommend using Sudan grass and mustard rotations to build soil suppressiveness. In addition, teff is considered as a good nurse crop in South Africa (Smith, 2006).

All the plant metrics were significantly different across categories of soil origin, possibly due to the promotion or reduced suppression of root-rot disease activities of *R. solani* (Savary et al., 2017). The findings suggest that the legacy effects of the PMSBM and PMMT crop rotations were possibly due to suppression of the inoculum of *Rhizoctonia* and *Fusarium*. There is need for further studies on the possibility of specific disease suppression in field soils from these crop rotations through soil inoculum transfer in previously conducive soil (Wright et al., 2021).

Evidently, the PMSUNM rotation legacy effects seemed to worsen the negative effects of *R. solani* AG 2-2 IIIB on plant growth. The inclusion of deep-rooting and drought-tolerant crops like sunflower in rotations, cultivates soil legacies associated with a dry but fertile soil environment which does not alter plant-microbe competition for soil nutrients and consequently the resilience of soil (Bardgett & Caruso, 2020). On the other hand, the PMMT

and PMSBM crop rotations were generally more resilient against the negative activities of *R. solani* AG 2-2 IIIB than the PMSUNM crop rotation. Our outcomes on sunflower in a rotation were not consistent with findings in Chapter 6 where improvements in soil suppression were observed. This suggests that greenhouse rotations for sunflower provided more insights into the general patterns of interactions that occur in the field but offered little insight into the specific plant-soil feedbacks that determine reduced *in vitro* soil suppression in the field soils (Heinze et al., 2016; Forero et al., 2019). This is a different outcome compared to Chapter 6 where the PMSUNM rotation was resistant and resilient in soils conditioned under controlled environment whereas in Chapter 8, the same rotation was least effective in soils conditioned under field environment. However, the outcome in Chapter 8 under *in vitro* conditions and for the PMMT rotation were in agreement with Chapter 6 results. At the soil, site and plant level; plant soil feedbacks differ between the greenhouse- and field-conditioned soils due to the diversity of abiotic and biotic aboveground and belowground interactions in the field (Heinze et al., 2016; Forero et al., 2019; Freschet et al., 2021). In other words, plant-soil feedbacks in the greenhouse conditions may overestimate or may not be the determining drivers in field environments but may; “provide valuable insights on the nominal effects of soils on plants” (Heinze et al., 2016; Forero et al., 2019). So, the plant-soil feedbacks in our study differed in both the greenhouse- and field-conditioned soils for the PMSUNM rotation and consequently the interpretation thereof. However, the direct and indirect plant-soil feedbacks findings *in planta* (or *in vivo*) resulted in similar interpretations for greenhouse- and field-conditioned soils (Heinze et al., 2016).

The mechanisms by which biotic and abiotic factors are affected by different cropping systems remain unclear (Liu et al. 2020). However, several studies have explained the natural mechanism of soil suppression against plant diseases caused by *Fusarium*, *Rhizoctonia*, *Pythium*, and *Phytophthora* (Jambhulkar et al., 2015; Afshari and Hemmati, 2017; Khan et al.,

2019; De Corato, 2020a, 2020b). The biocontrol agent, *Pseudomonas fluorescens* LY1-8 is effective against root rot caused by *F. graminearum* in both greenhouse and field assays (Wang et al., 2015).

The positive correlations between *in vitro* % soil suppression of *R. solani* AG 2-2 IIIB and soil composition in active-C, ash-C, SOM suggest the importance of these soil properties in potentially reducing the risk of soil-borne disease. The carbon inputs have the potential for suppressing Rhizoctonia root rot (Davey et al., 2021). The high turnover of carbon leads to increased nutritional support for soil microbial biomass and activity (Stirling et al., 2016). Findings in this study contradicted other studies on the impact of high soil P concentration in reducing disease severity and root rot caused by *R. solani* AG 2-2 IIIB (Mousa and El-Sayed, 2016; Gupta et al., 2017). It is likely that the reaction with iron, calcium and aluminum including the low pH or high acid soil conditions, had a negative impact on the soil P mineral uptake (Katan, 2009; McLaughlin et al., 2011).

The pH was positively associated with disease severity possibly due to acidic soils which are conducive for mycelial growth of *R. solani* and seedling damping-off (Watanabe et al., 2011). The low soil pH problem is often solved by addition of lime (dolomitic and calcitic) and/or gypsum, which in turn, limits the severity of *Fusarium* diseases (Gatch & du Toit, 2015).

An increase in soil NO₃-N content likely reduces the disease severity of *R. solani* AG 2-2 IIIB or *F. graminearum*. This soil nitrogen form was lowest in the PMSBM crop rotation than other rotations but strongly associated with low disease severity index. On the other hand, the highest disease severity index was observed in the PMSUNM crop rotation which was also associated strongly with relatively higher soil NO₃-N composition than in the PMSBM crop rotation. An increase in soil NH₄-N composition was positively correlated with *Fusarium* root rot severity index which is also demonstrated in studies by Gatch & du Toit (2015). Growers can test these

findings by planting faba bean as test plants in-between field rows on-site, also as an early monitoring tool for root rot risks of *R. solani* AG 2-2 IIIB and *F. graminearum*.

The response of crops and the soil environment to different rotation cycles under conventional tillage systems varies, depending on the specific crop genotypes planted, as well as the soil, environmental and climatic conditions. The duration of the soil's disease suppressiveness or recovery of the observed ecosystem services following a prolonged fallow period or monoculture disturbance still remains unanswered (Berendsen et al., 2012; Janzen et al., 2021). Whether changes in microbial communities explain concurrent increases in crop productivity in diverse rotations is less documented. In addition to screening field soils for suppressiveness, an integrated disease management strategy for managing *R. solani* AG 2-2 IIIB and *F. graminearum* must also involve the breeding and screening of cultivar resistance in similar bioassays (Xue et al., 2007; Chang et al., 2017, 2019).

8.6 Conclusion

Soil bioassays are a risk prediction tool for root rot disease and symptoms caused by *R. solani* AG 2-2 IIIB and *F. graminearum* under greenhouse conditions. The bioassays of field-collected soils under greenhouse conditions can be used in demonstrating and differentiating crop rotation legacy effects on soil suppressiveness against *R. solani* AG 2-2 IIIB. This can provide an indirect estimate of disease risk by measuring root rot disease severity index and plant biomass parameters. Results from the soil bioassay suggested that the Potatoes-Maize-Sugar beans-Maize rotation cultivated legacies that inhibit the mycelial growth of *R. solani* AG 2-2 IIIB. Future studies must determine the utility of field and greenhouse soil bioassays in the screening of sugar bean cultivars in South Africa for resistance to *R. solani* AG 2-2 IIIB and intensify the understanding of the pathosystem for every situation and soil environment. In contrast to these findings, *in vitro* assays for the Potatoes-Maize-Sunflower-Maize rotation soil legacies seemed to be the least effective treatment in the suppression of *R. solani* AG 2-2 IIIB.

The high soil NO₃-N content legacy in the Potatoes-Maize-Sunflower-Maize and Potatoes-Maize-Soybeans-Maize rotations compared to the Potatoes-Maize-Maize-Teff and Potatoes-Maize-Sugar beans-Maize rotations, likely increased the risk of *R. solani* AG 2-2 IIIB or *F. graminearum* disease severity. Overall, the Potatoes-Maize-Maize-Teff and Potatoes-Maize-Sugar beans-Maize rotations are the best rotations for cultivating suppressive soil conditions against *R. solani* AG 2-2 IIIB or *F. graminearum*.

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Conflict of interest

Authors have no conflict of interest to declare.

CHAPTER 9

Impact of Region-Specific Crop Rotation Legacy Effects on Soil-Borne *Rhizoctonia solani* AG 3-PT DNA levels in a Dryland Production System

9.1 Abstract

Background: *Rhizoctonia solani* Anastomosis Group (AG) 3-potato type (PT) is a serious pathogen in potato-based cropping systems worldwide. The effects of four different rotation systems on the presence of *R. solani* AG 3-PT DNA in soil were investigated over four annual growing seasons from 2015/16 to 2018/19.

Aims: This study was designed to assess the patterns and trends of the build-up of a specific soil borne pathogen in short rotations under dryland conditions. Crop rotations greatly reduce the levels of soil borne phytopathogens under rainfed systems. Other studies have shown that rotations are effective for integrated disease management with minimum use of agrochemicals. The objective of this study was to determine the impact of the residual effects of crop rotations on DNA concentrations in the soil of *R. solani* AG 3-PT DNA concentrations in a rain-fed cropping environment in the Free State Province of South Africa.

Methods: *Rhizoctonia solani* AG 3-PT DNA levels in soil were determined using quantitative polymerase chain reaction (qPCR) method targeting the nuclear internal transcribed spacer region (ITS).

Results: Results showed that the inclusion of sunflower in a rotation system, will possibly create a legacy of a sharp increase in *R. solani* AG 3-PT population in the successive maize crop. Although known as a potato pathogen, this AG 3-PT shows cross-pathogenicity on rotational crops such as soybean and maize. Maize and teff in cropping sequences minimized

the resilience of field soils against build-up of *R. solani* AG 3-PT DNA levels in soils under dryland conditions. Furthermore, the incorporation of legumes (soybean and sugar bean) in cropping sequences appeared to reduce the soil DNA concentrations of *R. solani* AG 3-PT under dryland soil tillage conditions, suggesting that commercial growers can include legumes in crop rotations to cultivate a disease suppressive soil against *R. solani* AG 3-PT inoculum. The soybean crop rotation sequence (Potatoes-Maize-Soybean-Maize) significantly reduced *R. solani* AG 3-PT DNA concentration. The maize crop in all the rotation sequences increased the *R. solani* AG 3-PT DNA inoculum in the dryland soil environment.

Conclusion: The accurate detection and quantification of *R. solani* AG 3-PT levels in soil, and correlation thereof with disease in the crop, will enable farmers to apply effective integrated management plans for *R. solani* diseases in dryland crop rotations.

Key words: Crop rotations; dryland production; *Rhizoctonia solani* AG 3-PT; soil nutrient composition; quantitative PCR.

9.2 Introduction

Rhizoctonia solani AG 3-PT produces mild disease severity and symptoms in native prairie grass seedlings that are similar to root rot in maize, wheat and soybean (Kodati et al., 2021; Kodati et al., 2022). Maize and most grass weeds are well known hosts to *R. solani* AG 3-PT (Tsrör, 2010; McCormack et al., 2013). The weed species *Taraxacum officinale* is a confirmed host of *R. solani* AG 3-PT (Carling et al., 1986). Survival of *R. solani* 3-PT between two consecutive seasons, either on host and non-host crops as fungus propagules or on alternative primary hosts (rotational crops) and secondary hosts (weeds) are not yet established for dryland production systems in South Africa. The most common propagules of *R. solani* is from mycelium made up of runner hyphae and sclerotia present for a longer period on seed material, field soils and plant debris (Sneh et al., 1996; Deacon, 2006; Jayaraman et al., 2021). The continuous rise in input costs which have increased out of proportion compared to product price

inflation and the lower productivity due to increased disease pressure have a negative impact on the profitability of crop production, for example potatoes (Steyn et al., 2020). Of the important potato pathogens, *Rhizoctonia solani* is reported to cause losses of up to 30 % of marketable yield. Elephant hide, black scurf and stem canker, caused by *R. solani* AG 3-PT, are important diseases in potato production in South Africa (Muzhinji et al., 2014; 2015) and in many other potato growing regions globally (Woodhall et al., 2007; Yang et al., 2015; Brierley et al., 2016). Commercial production of potatoes can incur up to 50 % loss of quality due to black scurf disease caused by *R. solani* AG 3-PT (Woodhall et al., 2013; Patil et al., 2018).

Rhizoctonia solani is divided into 14 (AG 1 – AG 13) somatically incompatible groups, otherwise known as anastomosis groups (AGs) (Ajayi-Oyetunde & Bradley 2018). Also, Muzhinji et al. (2015) described 19 out of 21 binucleate *Rhizoctonia* AG groups (AG A – AG U) in the South African potato industry. *Rhizoctonia solani* AG 3-PT belongs to the multinucleate group that produces more sclerotia than the other AGs 2-1, 4, 5 and 8 on potato dextrose agar (Woodhall et al., 2008). In field soils, the pathogen inoculum of *R. solani* AG 3-PT survives and remains viable as sclerotia for at least 18 months (Ritchie et al., 2013). However, the life cycle of *R. solani* is not fully known despite having a broad host range (Wibberg et al., 2016).

The term crop rotation effect is defined as “a yield benefit that results from crop rotation even in the apparent absence of diseases or any other detectable production limitations” (Whiting & Crookston, 1993; Bennett et al., 2012; Bowles et al., 2020). The residual effects of crop rotation hypothetically control weeds and have positive below-and above ground effects on soil health properties composition (González-Díaz et al., 2012). The benefits of crop rotation legacy effects on subsequent crop yields persist between two and three years in the soil environment under conventional tillage (Zhao et al., 2020). Crop rotation in South Africa, is more effective

in reducing the risk of disease damage in cultivated sandy soils than other soils (FERTASA, 2016). In contrast, sandy soils have naturally poor soil fertility and few microsites to protect soil microbiota (Unkovich, 2014). The use of structurally and functionally diverse crop rotation programmes has enabled successful control of soil-borne pathogens through competition by increasing the population of antagonistic while decreasing that of parasitic organisms (Larkin et al., 2010; Wright et al., 2017). Studies have shown reductions in stem canker, black scurf and common scab when potato was grown in various crop rotation and tillage systems, compared to continuous potato cropping (Larkin et al., 2010).

To break the pathogen disease cycle; the host crop must be avoided in the subsequent cropping sequence, through wide rotations with non-hosts (Bennett et al., 2012; Van der Putten, 2021). Short-term mitigation of risk for soil-borne pathogen occurrence and disease development include choice of crop, cultivar, and genotype (Bilgi et al., 2011). Difficulties arise when there are few options for disease management, the pathogen has an extremely wide host range, uncharacterized natural infection rate (Webb & Calderon, 2015) or differential sensitivity to fungicides (Muzhinji et al., 2018). The AG 3-PT was recorded in 40 % of the Potato Workgroup Trials as one of the least reasons for downgrading of marketable potatoes in the season 2018/2019 (PSA, 2019). In the sixteen potato growing regions in SA, the *R. solani* AG 3-PT was recorded in 40 % of the Potato Workgroup Trials as one of the very little-to-little reasons for downgrading of marketable potatoes in the season 2018/2019 (PSA, 2019).

The accurate detection and quantification of *R. solani* AG 3-PT in soil would enable farmers to link disease incidence and severity with DNA inoculum concentration thresholds in dryland crop rotations. The quantitative polymerase chain reaction (qPCR) technique is a more accurate tool in the quantification of soil-borne pathogens than soil baiting assays, although qPCR does not consider pathogen virulence and viability (Brown et al., 2021). It is noteworthy, the qPCR results for *R. solani* across seasons, must be interpreted with caution due to within-species

genetic heterogeneity (Misawa & Kurose, 2019) and the possible emergence of new genotypes in the field (Muzhinji et al., 2016) which can lead to the underestimation of the pathogen DNA levels in the soil samples. The aim was to determine the effects of four crop rotations on *R. solani* AG 3-PT DNA concentrations in a rain-fed cropping environment in the Free State Province of South Africa.

9.3 Materials and Methods

9.3.1 Field Site Description and Sample Data Collection

The field trial was planted with potatoes in the 2015/16 season (Year 1). Potatoes in year 1 were followed by maize on all plots in 2016/17 (Year 2/ season 2017), when the soil physical, chemical and microbial observations commenced. The third year rotation crops were planted in 2017/18 (season 2018) and the 2018/19 summer growing season was year 4 (season 2019) of the four-year rotation. As detailed in Table 1 (refer to Chapter 1), the trial consisted of four four-year crop rotation systems, each replicated eight times, giving a total of 32 plots (35 m x 21.91 m in size each). The field trial was a randomized complete block design (RCBD) made up of 32 rectangular plots (35 m × 21.91 m; 766.85 m²) across a total area of 2.98 ha situated near Petrus Steyn in the Eastern Free State of South Africa (27° 53' 45.5" E 28° 12' 59.8", Elevation 1705 m). Potatoes in year 1 were followed by maize on all plots in 2016/17 (Year 2/ season 2017), when the physical, chemical and microbial observations of the relevant soils commenced. Third year rotation crops were planted in 2017/18 (season 2018) and the 2018/19 summer growing season was designated year 4 (season 2019) of the four-year rotation. In this chapter, the season 2016/17, 2017/18, 2018/19 crop rotations data will be used for analysis of the field trial (refer to Table 1.1, Chapter 1).

The soil in the field was an arenosol greyish sandy soil (81.3±1.47% sand, 9.8±1.34% silt, 8.9±1.01% clay). In the first two seasons i.e. seasons 2015/16 and 2016/17, the fields were homogenized by planting potatoes and maize, respectively. In the 2017/18 growing season, the

field was planted with eight replications of each treatment crop (maize-*Zea mays*, sugar bean-*Phaseolus vulgaris*, soybean-*Glycine max* and sunflower-*Helianthus annuus*) on 28 November 2017 following dryland conventional tillage practices of the EFS. In the growing season 2018/19, 24 plots of the 32 plots were managed uniformly and planted with the same maize cultivar, following standard practices of conventional tillage in the EFS by the same grower using the same planter on 11 December 2018 (Table S9.1). The remaining eight plots were managed uniformly and planted with the same teff variety which was broadcast at 7 kg/ha on 17 January 2019 (Table S7.1). Table S7.1 shows the crop and financial management of the four crop rotations and their respective seasons.

All soil sampling activities were georeferenced with the aid of a handheld global positioning system device (GPS) (eTrex Legend® HCx, Garmin International Inc., Olathe, Kansas, USA). Field soil sampling was done within budget constraints in a spatial diagonal belt-transect pattern covering 2 m² per plot area at physiological maturity at the R growth stage (178 days after planting). Rhizosphere soil samples were recovered by brushing off soil attached to grub-up roots from plants in a diagonal belt-transect sampling pattern and pooled to make one sample per plot on 6 June 2019. Pseudoreplication in sampling for rhizosphere health analysis was avoided by sampling only three plants per replicate/block, followed by pooling these samples into one composite replicate/block sample. The bulk soil represented a procedural control under the assumption that it was loose field soil outside the influence of plant roots, that is, the rhizosphere zone. On 29 July 2019, soil from the root zone was collected as composite samples from the topsoil 0 cm to 20 cm depth in each plot using a 5 cm diameter by 8 cm height soil core and analysed for macro-and micronutrients. In short, at the same period of every season, a total of 40 composite soil samples of bulk soil (n = 8) and rhizosphere (n = 32) were collected and pooled to make 20 soil samples (n = 20) for qPCR; rhizosphere samples for the four crop rotations were each replicated four times (n = 16) plus bulk soil samples replicated four times

(n = 4). Composite soil core samples and rhizosphere soil samples were placed in separate individual sterile zip-lock plastic bags, stored at 4 – 5 °C for soil analysis and at – 80 °C for *R. solani* AG 3-PT DNA concentration, respectively.

The maize planted was an early medium-maturity cultivar grown under dryland climate conditions. After 7 months 17 days, the maize crop was harvested from all blocks using the same combine and driver on the same day, recording on average 12 % grain moisture content after full physiological maturity on 29 July 2019. The teff crop was harvested after 27 weeks 4 days, at full physiological maturity on 29 July 2019. Yield data was collected at the end of the growing season and analysed using the Sampling for Harvest Unit technique described by Sapkota et al. (2016). Eight of the 24 plant rows of each crop per plot were harvested using a combine harvester, where after the grains were weighed and moisture content (%) per plot was determined on-site. Yields were then adjusted to 12.5% moisture content for comparison.

9.3.2 Soil Analysis

The *in situ* soil penetration resistance readings were taken in-rows, in-between rows and tractor traffic during dry conditions at 1 cm increments to a depth of 50 cm in every plot on the same month every season using a Geotron Hand Penetrometer Model P5 (Geotron Systems, Potchefstroom, South Africa) up to 5000 kPa. The soil bulk density was obtained from undisturbed soil samples collected at 20 cm and 40 cm depths following the procedures described earlier in section 3.3.4. On the other hand, soil physical, chemical and biological properties were measured using standard procedures at Nvirotek Laboratories (Pty) Ltd (ISO/IEC 17025 international standard for Testing laboratories Accredited Testing Laboratory, certified SANAS and SADCAS assessors, certified AgriLASA Fertilizer Laboratory, Unit 6, Nviro Business Hub, Ifafi, 0260, South Africa). Changes in physical and biological properties, as well as soil nutrient content over time were then assessed for each rotation system to determine both the differences and associations between rotation systems.

9.3.3 Fungal Reference Cultures and DNA Extraction

An isolate of *R. solani* AG 3-PT (GenBank accession number KJ777586) was obtained from storage cultures in Prof J. van der Waals' Healthy Soils for Healthy Crops Research Group (formerly known as the Potato Pathology Programme @UP) and its pathogenicity and virulence were characterized and confirmed by Muzhinji et al. (2015). These reference cultures were revived and sub-cultured on Potato Dextrose Agar (PDA). Genomic DNA (gDNA) of *R. solani* AG 3-PT was extracted from mycelia powder frozen in liquid nitrogen using the DNeasy® PowerSoil® Kit (QIAGEN Group, MoBio Laboratories, Inc.) and Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (ZYMO RESEARCH, Corp.) according to manufacturer's protocol, respectively. A Qubit 2.0 fluorimeter (Thermo Fisher Scientific, California, United States) instrument estimated 14.4 ng/μl quantities of reference *R. solani* AG 3-PT culture DNA template.

9.3.3.1 Verifying the validity of *R. solani* AG 3-PT PCR primers

The targeted qPCR protocol described by Woodhall et al. (2013) was validated for determining the DNA concentrations of *R. solani* AG 3-PT using conventional PCR, respectively. Specific amplification was verified by assessing the size of the PCR product by 1.5 % agarose gel electrophoresis. The validation of the *R. solani* AG 3-PT PCR primers (Table 9.1 and Table 9.2) was performed separately in a total volume of 20 μL of TaqMan-based PCR reaction mixture containing 4.0 μL of DNA template, 10.0 μL of 2x concentrated PCR Master Mix, 0.6 μL of each primer (from 10 μM stock to make 300 nM) and 4.8 μL of nuclease-free water (New England Biolabs, Inqaba Biotech). Amplification conditions consisted of 95 °C for 5 min; 35 cycles of 95 °C for 30 sec, 60 °C, 72 °C for 1 min; and a final extension at 72 °C for 10 min. The conventional PCR protocol was programmed and run using the Applied Biosystems 2720 thermal cycler (Life Technologies™ Holdings, Marsiling, Singapore). The

no-template controls included sterile nuclease-free water (New England Biolabs, Inqaba Biotech) and no template DNA.

The PCR products of the reference cultures were separated using 1.5 % gel electrophoresis prepared by dissolving 1.6875 g of SeaKem® LEA agarose powder (Lonza, Rockland, USA) in 113 ml of 1X Tris-Acetate-EDTA (TAE) buffer. A volume of 7 µl Roti Safe (ROTI®GelStain Red) was added to stain the agarose gel. A total of 12 µL of the sample was loaded i.e. 10 µL DNA + 2 µL 6X loading dye (New England Biolabs) into the wells of the stained gel and run at 100 Volts, 250 Amps for 60 min in a horizontal Bio-Rad gel tank unit (Bio-Rad Laboratories, California, USA) filled with 1X TAE buffer. The PCR amplified fragments were sized using a 100 bp Lonza DNA molecular ladder. The Roti-stained gel was then visualized to acquire the image so as to detect and quantify bands using a Gel Doc XR+ Gel documentation system (Bio-Rad Laboratories, California, USA).

9.3.3.2 Soil Pathogen DNA copies across Crop Rotations

Approximately 0.25 g rhizosphere soil was subjected to total DNA extraction with a DNeasy® PowerSoil® Kit (QIAGEN Group, MoBio Laboratories, Inc.) following the manufacturer's instructions and samples were stored at -20 °C for further use. The differences in soil DNA concentrations of *R. solani* AG 3-PT between the four-year crop rotation sequences were determined using the qPCR protocols described by Woodhall et al. (2013). The species-specific primers and probes were synthesized by Integrated DNA Technologies, Inc. (<http://www.idtdna.com>). The dual labelled fluorescent probes were labelled with 6-carboxyfluorescein (FAM) 5' modification and tetra-methylrhodamin (TAMRA) 3' modification.

A total volume of 20 µL of TaqMan-based qPCR reaction mixture containing 4.0 µL of DNA template, 10.0 µL of 2x concentrated SsoAdvanced universal probes supermix (Bio-Rad Laboratories, Hercules, CA, United States), 0.6 µL of each primer (from 10 µM stock to make

300 nM), 0.2 µL of probe (from 10 µM stock to make 100 nM), and 4.6 µL of nuclease-free water (New England Biolabs, Inqaba Biotech) was used. Amplification conditions consisted of 50 °C for 2 min, 95 °C for 10 min, 40 cycles of 95 °C for 15 sec and 60 °C for 1 min. *Rhizoctonia solani* AG 3-PT rDNA internal transcribed spacer region (ITS) (size 122 bp) was amplified successfully using TaqMan-based qPCR hydrolysis probe assay and primers listed in Table 9.1.

The no-template (negative) controls included sterile nuclease-free water (New England Biolabs, Inqaba Biotech) and no template DNA. The qPCR protocol for *R. solani* AG 3-PT was performed in a 96-well plate CFX Connect™ (Maestro Version 4.0.2325.0418) Real-time PCR Detection System (Bio-Rad).

Table 9.1 *Rhizoctonia solani* AG 3-PT specific primers and probe used in this study.

Sequence name	Description	Sequence (5′→ 3′)	Tm (°C)	Reference
AG 3-PT_F	Forward	ATGAAGAGTTTGGTTGTAGC TGGTCT	59.7	Woodhall et al. (2013) & IDT technologies Inc.
AG 3-PT_R	Reverse	TATTACAAW ^a AAATAACAAA TAAATTCCCCAA	51.5	
AG 3-PT_P	Probe	/5′-FAM/CCCTCTTTCATCCCA CACACACCTG/3′-TAMRA/	62.4	
Probe: labelled with TAMRA; Tm: Melting temperature; W ^a : ambiguous coding for A or T; Underlined bold base denotes locked nucleic acid (LNA) base.				

9.3.3.3 *Rhizoctonia solani* AG 3-PT Standard Curves Preparation

The 10-fold dilution series for *R. solani* AG 3-PT DNA was prepared with four replicates for each concentration, stored at -80 °C and thawed once before use. A log-linear regression standard curve of a six point 10-fold dilution series from 14.4 ng/µl to 0.00144 ng/µl was generated by plotting the logarithms of known concentrations of *R. solani* AG 3-PT DNA against the C_q values. PCR efficiency and coefficient of determination (R²) values were calculated using the CFX Connect™ (Maestro Version 4.0.2325.0418) Real-time PCR Detection System (Bio-Rad).

9.3.3.4 Targeted qPCR Analysis

A slope of -3.32 represents the ideal 100% amplification efficiency. The sensitivity or minimum detection limit of the assay was estimated as minimum quantity of target DNA detected when the cycle threshold was attained at 40 cycles. All reactions were run in duplicate and samples that gave positive fluorescence before no-template controls were considered positive.

9.3.4 Data Analysis

The goal of the data analysis in this study was to compare the impact of treatment legacies on soil and plant health. An RCBD layout was selected to compare the rotation crop factor as different treatments (Gibson, 2015; Dicks, 2006). All ANOVA, MANOVA and post hoc statistical analyses were done in SPSS version 23 (IBM Corp. released, 2015) and R version 3.5.2 (R.Core.Team, 2018), assuming that treatment effects were constant over the different blocks, block effects were also constant for all plots within a replication/block, and that the errors followed a normal distribution with mean of zero and variance of σ^2 . The soil nutrient data were analysed in SPSS version 23 using the Tukey's honestly significant difference (Tukey HSD) test to compare soil nutrient composition in the rhizosphere across four crop rotations. In this *post-hoc* test, an ANOVA F test was done to determine the significance of crop rotation treatment effects while the Tukey HSD *post-hoc* test was done to group the treatment means (Steel & Torrie, 1960) strictly for soil and plant health data (or transformed data) of equal sample sizes that meet the normality and homogeneity of variance assumptions. Overall, this *post-hoc* test provides answers to specific meaningful questions, which were after all the objectives of the study in comparing all pairs of crop rotation treatments (Ruxton and Beauchamp, 2008; Kozak & Powers, 2017).

The soil pathogen DNA concentration data for multivariate analysis, prior to analysis, were natural-logarithm transformed to achieve normality and homogeneity of variance (Bustin et al.,

2009; ter Braak et al., 2018). Principal component analyses (PCA) were based on a correlation matrix and done using the multivariate analysis approach from Canoco 5 version 5.11 (ter Braak et al., 2018) to display associations of soil pathogen DNA with crop rotations and selected soil factors. To rank the linear relationships between soil factor metrics, the pathogen DNA data was normalized using the natural logarithm $\ln(x + 1)$ transformation followed by computing the Pearson correlation coefficients (r) in SPSS version 23. Preliminary data analysis of soil pathogens concluded that there was no significant block variation in soil-borne pathogen DNA amounts within crop rotations ($p > .05$).

9.4 Results

9.4.1 Climate and Soil Nutrient composition data

The climate history of the field experiment is as described in Chapter 7 sections 7.4.1 and 7.4.2. Table 9.2 summarizes the soil analysis results of selected soil properties. Soil density, zinc, phosphorus and potassium were significantly different among the four crop sequences in the fourth year (season 2018/19).

Table 9.2 Biological and physicochemical properties of sandy (% Clay 8.94 ± 0.18 , % Silt 9.80 ± 0.24 , and % Sand 81.26 ± 0.26) topsoil collected at 0–20 cm depth from a crop rotation field trial at the end of season 2018/19. N = 8 composite samples. SE = standard error.

Soil factor legacy (units), p value (s/ns)	Soil origin (Mean $\pm$ SE)				
	Bulk soil	PMMT	PMSBM	PMSOYM	PMSUNM
Density (g/cm ³), *s	1.43b $\pm$ 0.014	1.35a $\pm$ 0.036	1.38ab $\pm$ 0.009	1.39ab $\pm$ 0.006	1.39ab $\pm$ 0.011
pH (H ₂ O), ns	5.55a $\pm$ 0.035	5.75a $\pm$ 0.170	5.59a $\pm$ 0.123	5.46a $\pm$ 0.078	5.56a $\pm$ 0.026
Phosphate Mehlich 3 (mg/kg), **s	45.60b $\pm$ 1.85	36.40a $\pm$ 1.74	40.10ab $\pm$ 2.28	43.60ab $\pm$ 2.15	46.10b $\pm$ 2.00
Phosphate Bray 2 (mg/kg), ns	46.50ab $\pm$ 2.81	38.60a $\pm$ 3.29	44.30ab $\pm$ 2.23	46.50ab $\pm$ 1.93	49.10b $\pm$ 2.23
Potassium (mg/kg), ***s	140.00c $\pm$ 7.30	129.00bc $\pm$ 11.2	96.90ab $\pm$ 7.83	85.80a $\pm$ 6.73	127.00bc $\pm$ 7.68
Ammonium-Nitrogen (mg/l), ns	0.556a $\pm$ 0.02	0.365a $\pm$ 0.029	0.380a $\pm$ 0.022	0.371a $\pm$ 0.031	0.588a $\pm$ 0.156
Nitrate-Nitrogen (mg/l), ns	0.909a $\pm$ 0.148	1.64a $\pm$ 0.167	1.29a $\pm$ 0.168	2.29a $\pm$ 0.466	2.780a $\pm$ 1.60
Zinc (mg/kg), ***s	2.21a $\pm$ 0.064	1.95a $\pm$ 0.121	2.60ab $\pm$ 0.204	3.08b $\pm$ 0.216	3.18b $\pm$ 0.242
Active-carbon (mg/kg), ***s	212.00a $\pm$ 20.3	286.00b $\pm$ 8.96	289.00b $\pm$ 13.9	304.00b $\pm$ 15.1	295.00b $\pm$ 14.2
Solvita respiration (ppm), ns	24.50a $\pm$ 3.87	28.50a $\pm$ 6.83	21.40a $\pm$ 3.06	20.20a $\pm$ 1.74	24.60a $\pm$ 2.86
Organic matter (%), **s	0.263a $\pm$ 0.018	0.345b $\pm$ 0.017	0.346b $\pm$ 0.015	0.294ab $\pm$ 0.020	0.320ab $\pm$ 0.019
Carbon Ash (%), **s	0.152a $\pm$ 0.011	0.199b $\pm$ 0.009	0.201b $\pm$ 0.009	0.169ab $\pm$ 0.013	0.186ab $\pm$ 0.010

^{a,b,c,d} Different letters represent significant differences (s) among crop rotation combinations within a soil factor while shared letters represent non-significant differences (ns) based on one-way ANOVA using the Tukey's HSD test statistic *post hoc* multiple comparisons where $p > .05$, $* p \leq .05$, $** p < .01$ and $*** p < .001$ (IBM Corp. Released, 2015).

9.4.2 Quantification of *R. solani* AG 3-PT across Crop Rotations

9.4.2.1 Validation of Species-specific Primers

The conventional PCR was performed to validate the specificity of the Woodhall et al. (2013) forward and reverse primers in amplifying DNA extracts from *R. solani* AG 3-PT (Muzhinji et al., 2015; 2016; Misawa & Kurose, 2019). The size of the ITS-PCR products was approximately 122 bp (Figure 9.1).

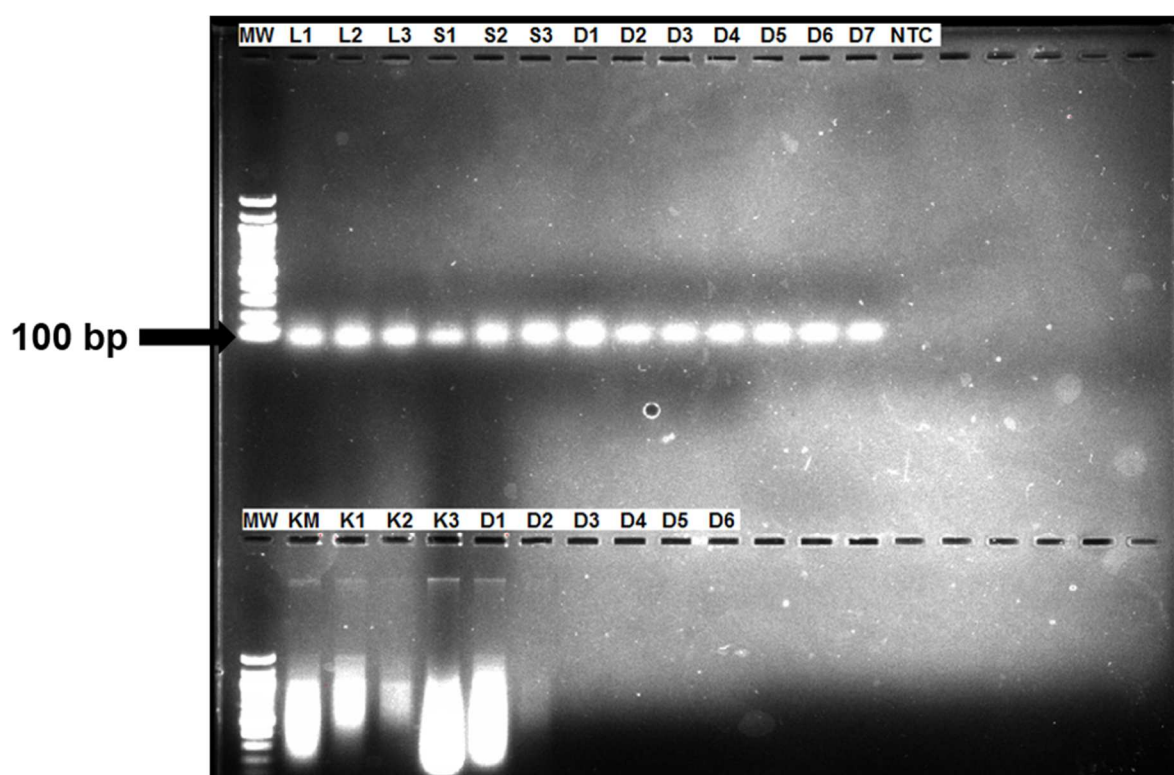


Figure 9.1 The amplicon profile (top) and genomic DNA (bottom) of *R. solani* AG 3-PT. The top wells 1 – 15; MW represented the Lonza Molecular DNA ladder/ marker 100 – 4000 bp, L1 to L3 are amplicons of technical replicates amplified using the Luna® Universal Probe qPCR Master Mix, S1 to S3 are amplicons of technical replicates amplified using SsoAdvanced universal probes supermix, D1 – 9.06 ng, D2 – 0.906 ng, D3 – 0.0906 ng, D4 – 0.00906 ng, D5 – 0.000906 ng, D6 – 0.0000906 ng, D7 – 0.0000906 ng, and NTC – 0.00 ng (negative control). The bottom wells 1 – 11 represented; MW – Lonza Molecular DNA ladder/ marker 100 – 4000 bp, KM – stock of pooled-genomic DNA of *R. solani* AG 3-PT culture, K1 to K3 are replicates of the stock genomic DNA of *R. solani* AG 3-PT culture, and D1 to D6 are 10-fold dilutions of pooled genomic DNA extraction (stock KM) of *R. solani* AG 3-PT culture.

The findings were similar to the amplicon size profile validated by Woodhall et al. (2013). The amplification reactions (top wells) were very specific with no primer-dimers as shown in Figure 9.1. In this study, Koch's postulates of the pathogenicity of the *R. solani* AG 3-PT on potatoes was confirmed in a pilot pot experiment to compare how pathogen quantity decreased as different crops were planted from one season to another season (data not shown) and in previous pathogenicity studies by Muzhinji et al. (2015; 2016).

9.4.2.2 Quantitative PCR Standard Curves

The parameters for qPCR estimations such as, efficiency (E), coefficient (R^2) and slope met the requirements for the interpretation of the data using the standard curves (Bustin et al., 2009). All the qPCR standard curves for every run had good qPCR efficiency (E) values between 90 % and 110 %, which corresponds to a slope of between -3.58 and -3.10 (<http://www.lifetechnologies.com>). The standard curves were used to estimate the soil DNA quantities (Figure S9.1 to S9.5). All the standard curves for the soil DNA data according to season, were constructed using the logarithm of the pure *R. solani* AG 3-PT DNA concentrations and cycle threshold (C_q) shown in Figure S9.1 to S9.4. For simplicity reasons, the bold letter in the abbreviation of the rotation system indicates the crop planted in that year/season, eg. PMS**S**UNM indicates that sunflower was planted in the third year or season 2017/18.

9.4.2.3 The Pathogen DNA concentration at the end of Year 1: Season 2015/16 (Potatoes on all Crop Rotations)

There were no significant differences ($p > .05$) in *R. solani* AG 3-PT DNA concentration (pg DNA per g of soil) across crop rotations at the end of the potato uniformity trial (Figure 9.2). Low soil quantities of *R. solani* AG 3-PT DNA ranging from 2.99 to 4.23 pg per g of soil were detected in the first season despite the fact that potatoes (known hosts) were grown in all field plots.

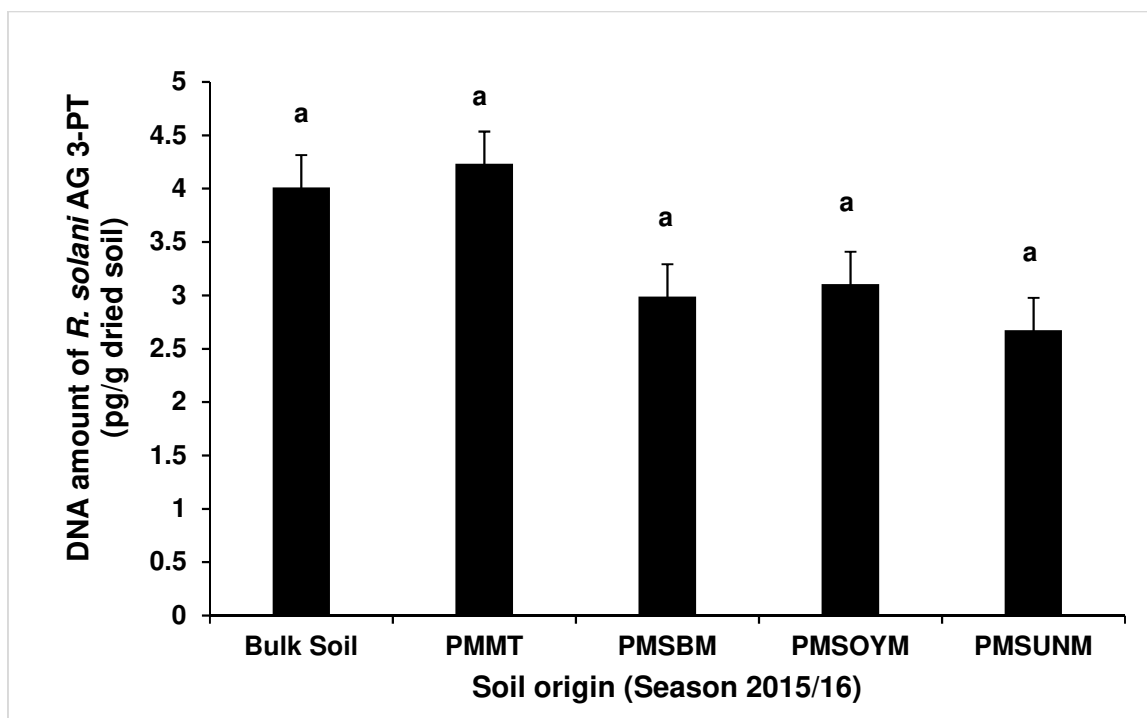


Figure 9.2 *Rhizoctonia solani* AG 3-PT DNA concentrations measured in four crop rotation sequences (PMMT, PMSBM, PMSOYM, and PMSUNM) at the end of the first growing season 2015/16 planted with potatoes. Different letters indicate significant differences ($p < .05$). Vertical error bars represent mean $\pm$ standard error ($n = 4$).

9.4.2.4 The Pathogen DNA concentration at the end of Year 2: Season 2016/17 (Maize on all Crop Rotations)

The PMMT crop rotation had the highest average quantity of *R. solani* AG 3-PT DNA at 11.04 pg DNA per g soil in the 2016/17 maize uniformity season (Figure 9.3). This was a 2.6× increase in soil DNA amounts from the previous potato uniformity season 2015/16. It is worth noting that all other plots that also contained potato followed by maize had similar levels or even lower compared to the 2015/16 season.

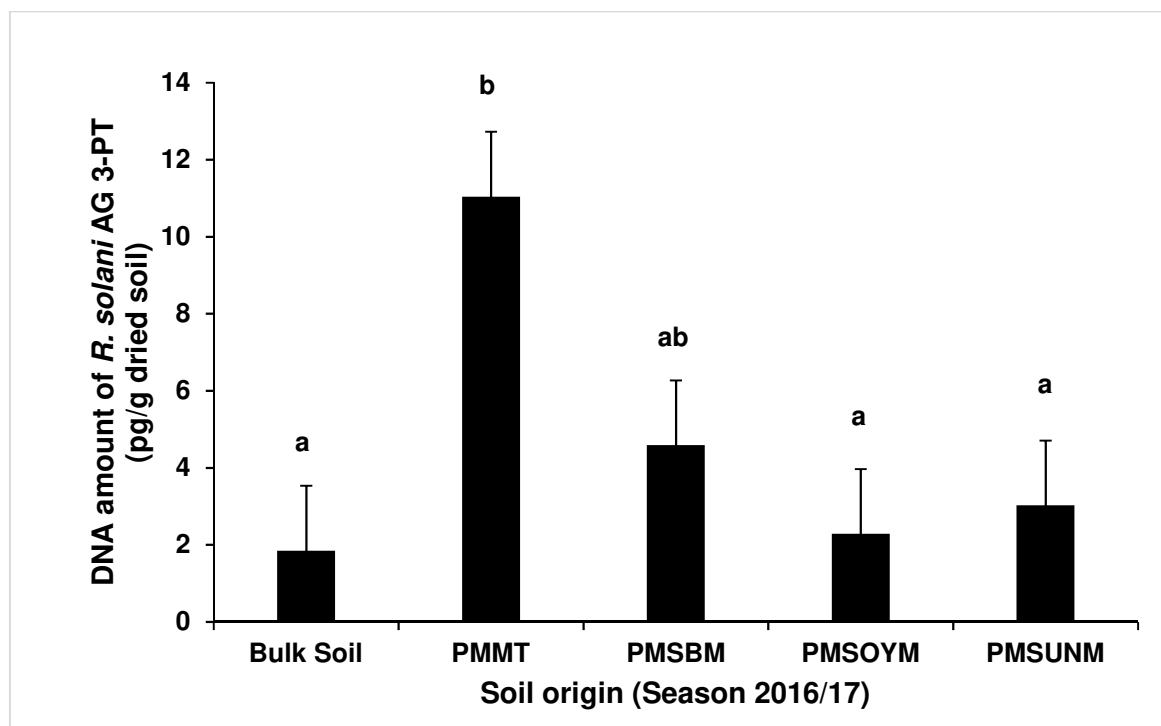


Figure 9.3 *Rhizoctonia solani* AG 3-PT DNA concentrations measured in four crop rotation sequences (PMMT, PMSBM, PMSOYM, and PMSUNM) at the end of the second growing season 2016/17 planted with maize in all plots. Different letters indicate significant differences ($p < .05$). Vertical error bars represent mean $\pm$ standard error ($n = 4$).

At the end of season 2016/17, *R. solani* AG 3-PT DNA was lowest in the PMSOYM crop rotation but high in soils with a relatively abundant soil legacy in active-C, Mg, % Mg and pH (KCl) as displayed in the PCA biplot (Figure 9.4). The PC 1 and PC 2 described 75.33 % of the total variability of the *R. solani* AG 3-PT DNA and soil analysis data. The main contributors of high values of the first axis were soil metrics in pH, density, active-C and $\text{NO}_3\text{-N}$.

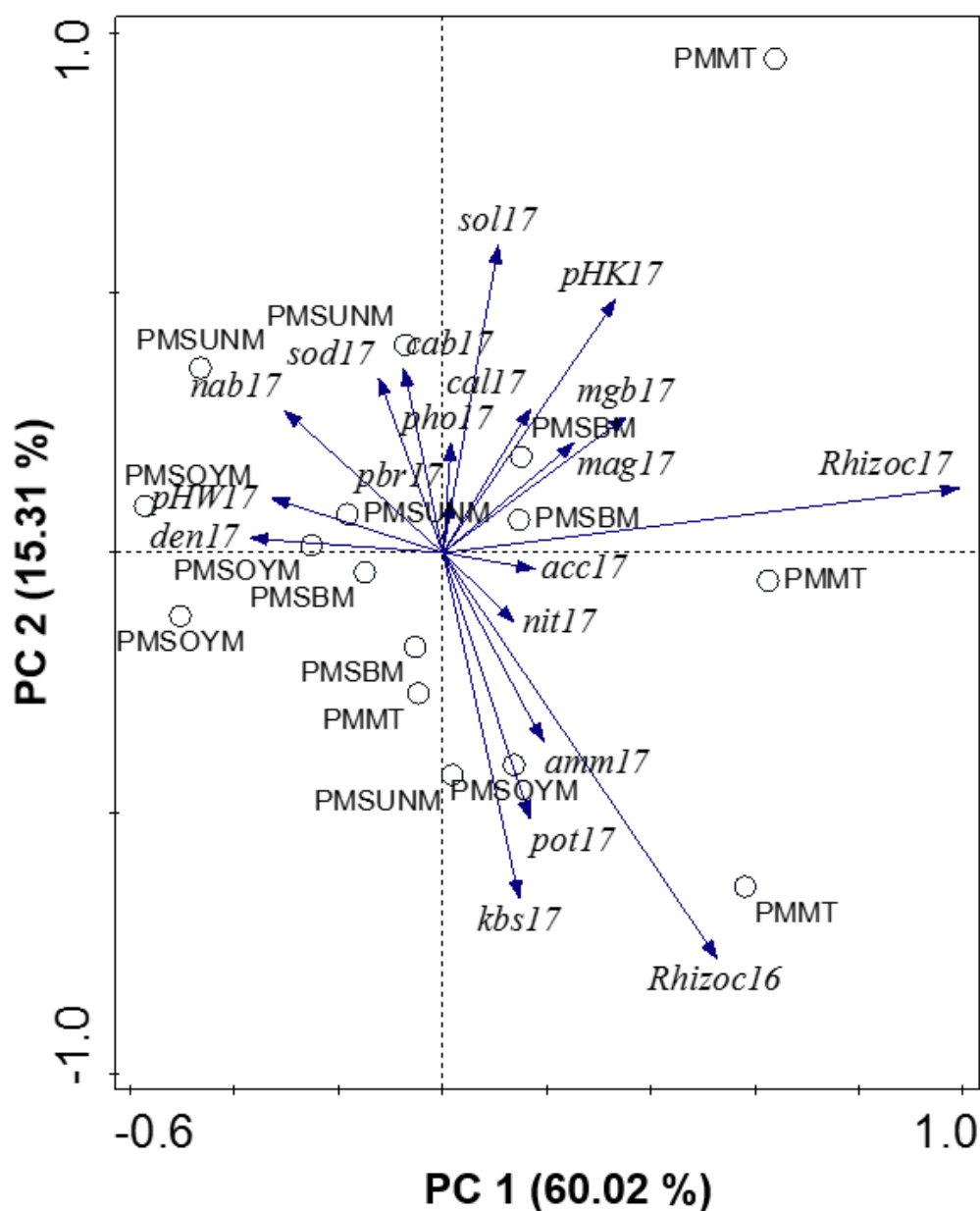


Figure 9.4 The PCA biplot of Rhizoc16 – *R. solani* AG 3-PT DNA (season 2015/16) and Rhizoc17 – *R. solani* AG 3-PT DNA (season 2016/17) associations with selected soil factors. The last two digits ‘16’ and ‘17’ in a soil factor represent season 2015/16 and season 2016/17 soil legacies, respectively. The 17 soil health properties are represented as:

acc - Active carbon (mg/kg)	pHW - pH (H ₂ O)	pHK - pH (K:Cl)
amm - 1:2 Water extract N - NH ₄ (mg/l)	cab - %Ca	cal - Ca (mg/kg)
nit - 1:2 Water extract N - NO ₃ (mg/l)	nab - %Na	sod - Na (mg/kg)
sol - Solvita CO ₂ respiration (ppm)	mgb - %Mg	mag - Mg (mg/kg)
pbr - P Bray 2 (mg/kg)	kbs - %K	pot - K (mg/kg)
den - Density (g/cm ³)	pho - P (mg/kg)	

9.4.2.5 The Pathogen DNA concentration at the end of Year 3: Season 2017/18 (Maize, Sugar Beans, Soybeans, and Sunflower in different Rotations)

The average *R. solani* AG 3-PT DNA quantities in crop rotations PMSBM (1.44 pg DNA per g of soil) and PMSUNM (1.45 pg DNA per g of soil) were lower than that in the PMMT crop rotation for the 2017/18 season (Figure 9.5). In addition, the PMSBM (sugar bean) and PMSUNM (sunflower) rotations decreased *R. solani* AG 3-PT DNA levels in the soil 3× and 2×, respectively, compared to the previous season 2016/17. These findings were contrary to the findings from Chapter 8 because in Chapter 9 (season 2017/18 results) the field conditioned soil legacies were from incomplete rotation cycles whilst the findings in Chapter 8 were based on field-conditioned soils from complete rotation cycles or treatments.

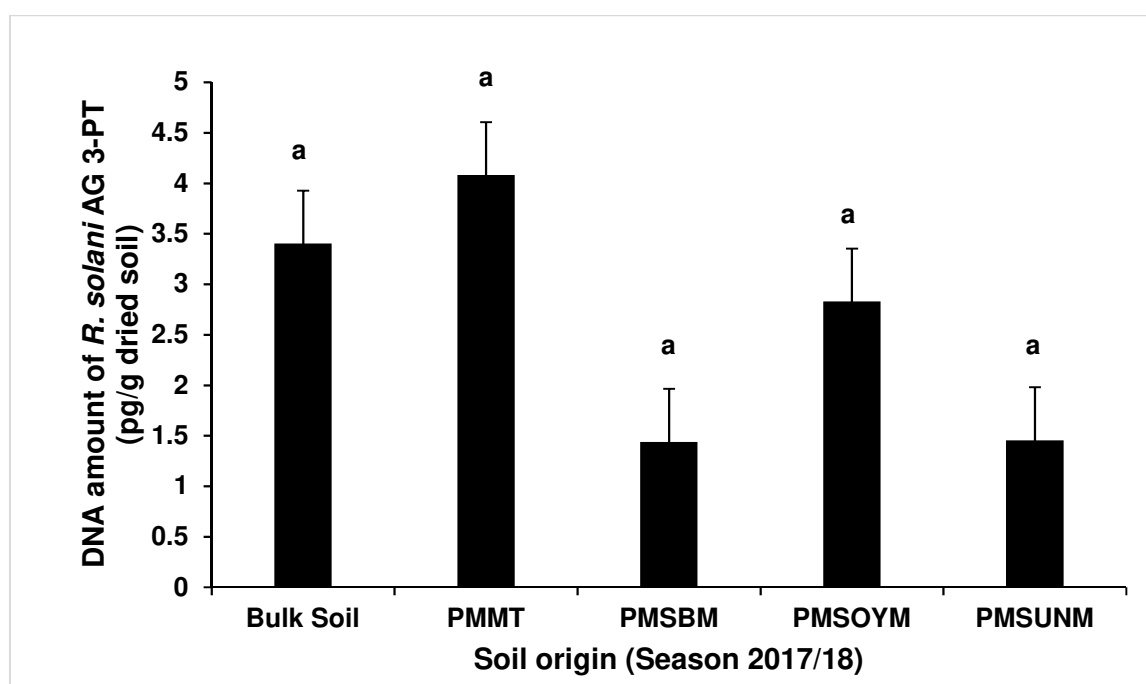


Figure 9.5 *Rhizoctonia solani* AG 3-PT DNA concentrations measured in four crop rotations; PMMT, PMSBM, PMSOYM, and PMSUNM at the end of the third growing season 2017/18 planted with maize, sugar beans, soybeans and sunflower, respectively. Different letters indicate significant differences ($p < .05$). Vertical error bars represent mean $\pm$ standard error ($n = 4$).

The clustering together of the PMSUNM and PMSBM crop rotations and their negative correlations with *R. solani* AG 3-PT DNA at the end of season 2017/18 are shown as the PCA biplot in Figure 9.6. The PC 1 and PC 2 of the PCA described 75.35 % of the total variability of the *R. solani* AG 3-PT data. The positive correlations between the *R. solani* AG 3-PT DNA season 2017/18 and the previous season's (2016/17) soil legacies in P, NO₃-N, Ca, Mg, % Mg, K and pH (KCl) are illustrated in Figure 9.6.

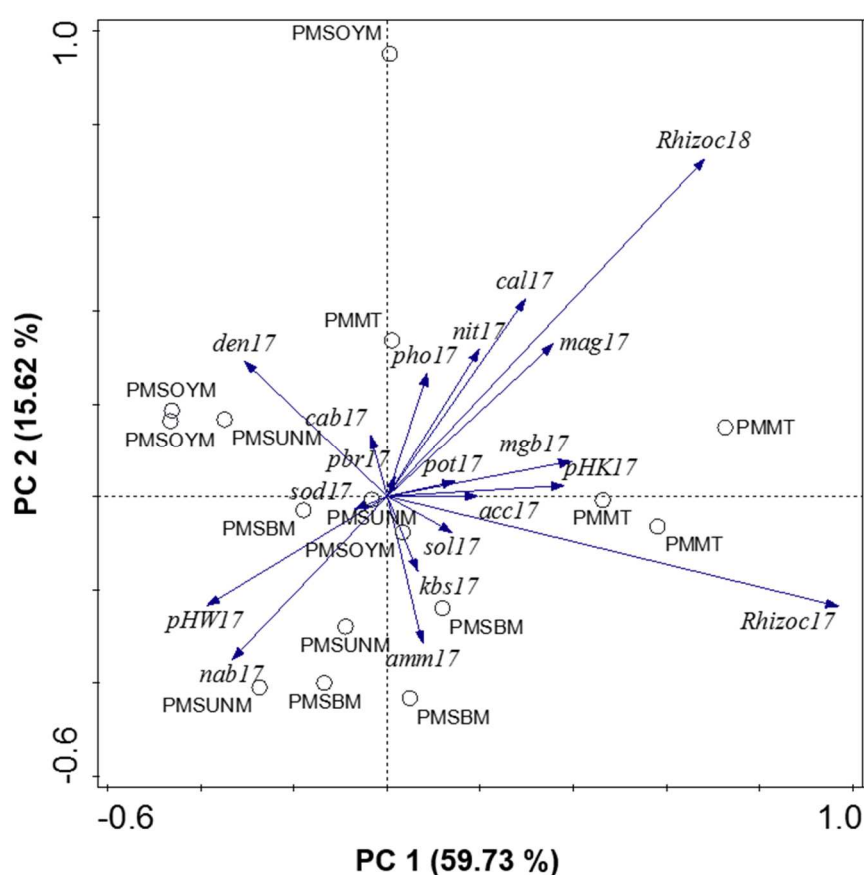


Figure 9.6 The PCA biplot of Rhizoc17 - *R. solani* AG 3-PT DNA (season 2016/17) and Rhizoc18 – *R. solani* AG 3-PT DNA (season 2017/18) associations with selected soil factors. The last two digits ‘17’ and ‘18’ in a soil factor represent season 2016/17 and season 2017/18 soil legacies, respectively. The 17 soil health properties are represented as:

acc - Active carbon (mg/kg)	pHW - pH (H ₂ O)	pHK - pH (K:Cl)
amm - 1:2 Water extract N - NH ₄ (mg/l)	cab - %Ca	cal - Ca (mg/kg)
nit - 1:2 Water extract N - NO ₃ (mg/l)	nab - %Na	sod - Na (mg/kg)
sol - Solvita CO ₂ respiration (ppm)	mgb - %Mg	mag - Mg (mg/kg)
pbr - P Bray 2 (mg/kg)	kbs - %K	pot - K (mg/kg)
den - Density (g/cm ³)	pho - P (mg/kg)	

The PC 1 and PC 2 described 68.32 % of the total variability of the *R. solani* AG 3-PT DNA data as the PCA biplot shown in Figure 9.7. The majority of the soil factors were projected along the positive PC 2 axis and had no association with *R. solani* AG 3-PT DNA. There were no associations between *R. solani* AG 3-PT DNA and % Ca or pH (H₂O) at the end of season 2017/18 (Figure 9.7). Negative associations between the *R. solani* AG 3-PT DNA with soil K, density, % K and active-C are shown as a PCA biplot in Figure 9.7. The positive association between PMMT crop rotation and *R. solani* AG 3-PT DNA along the positive values of PC 1 axis suggests conduciveness of this rotation to higher pathogen DNA than other crop rotations in the third season. This successive high loading even before alternating different crops strongly reflects that this pathogen (*R. solani* AG 3-PT) survived better in the field soils from the grass-dominated rotation. Further, it is likely that maize plant debris or litter inputs, alternative secondary hosts (weeds) and the resulting soil historical legacies not measured in this study or seasons had something to do with this observed higher load in pathogen DNA in the third season than other crop rotations. Contrary, a negative association between PMSBM crop rotation and *R. solani* AG 3-PT DNA along the negative values of PC 1 axis suggests that this rotation was suppressive to pathogen DNA.

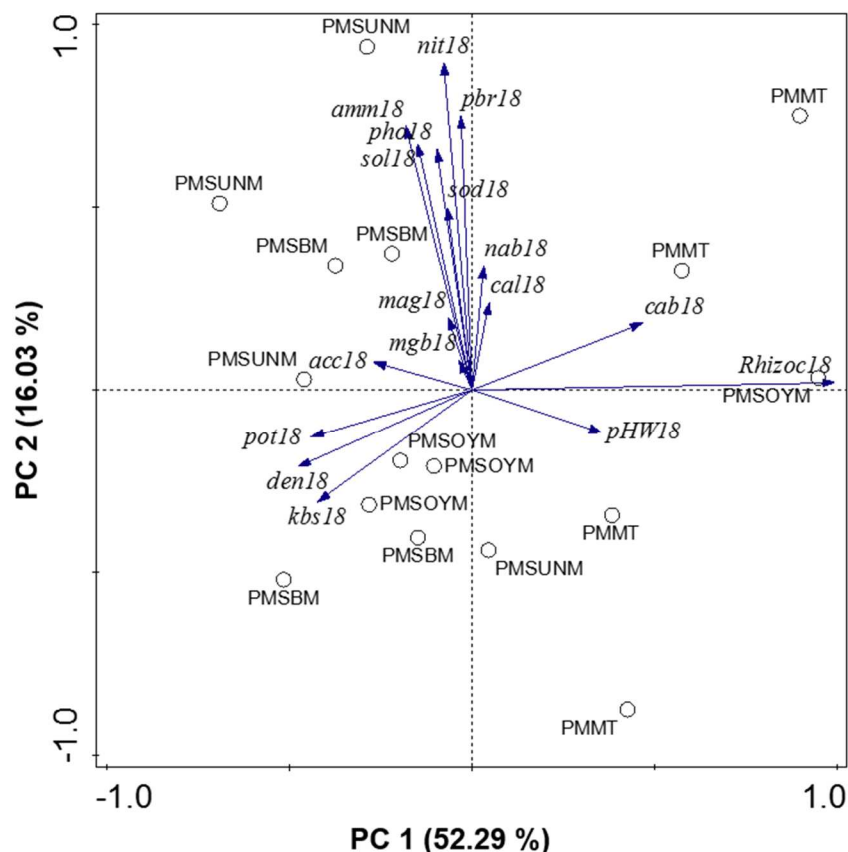


Figure 9.7 The PCA biplot of *Rhizoc18* – *R. solani* AG 3-PT DNA (season 2017/18) associations with selected soil factors. The last two digits ‘18’ in a soil factor represent season 2017/18 soil legacies. The 16 soil health properties are represented as:

acc - Active carbon (mg/kg)	pHW - pH (H ₂ O)	pho - P (mg/kg)
amm - 1:2 Water extract N - NH ₄ (mg/l)	cab - %Ca	cal - Ca (mg/kg)
nit - 1:2 Water extract N - NO ₃ (mg/l)	nab - %Na	sod - Na (mg/kg)
sol - Solvita CO ₂ respiration (ppm)	mgbl - %Mg	mag - Mg (mg/kg)
pbr - P Bray 2 (mg/kg)	kbs - %K	pot - K (mg/kg)
den - Density (g/cm ³)		

At the end of the season 2017/18, there were positive associations of *R. solani* AG 3-PT DNA, Solvita soil respiration, pH (H₂O) and enzyme activity in FDA hydrolyzed in the PCA biplot (Figure 9.8). The *R. solani* AG 3-PT DNA was negatively correlated with each of the following PLFA markers; a-C15:0, C18:1 ω 9c and C18:2 ω 6c. The *R. solani* AG 3-PT DNA had positive associations with the PMMT crop rotation and negative correlations with the PMSBM and PMSUNM crop rotation clusters at the end of season 2017/18 (Figure 9.8).

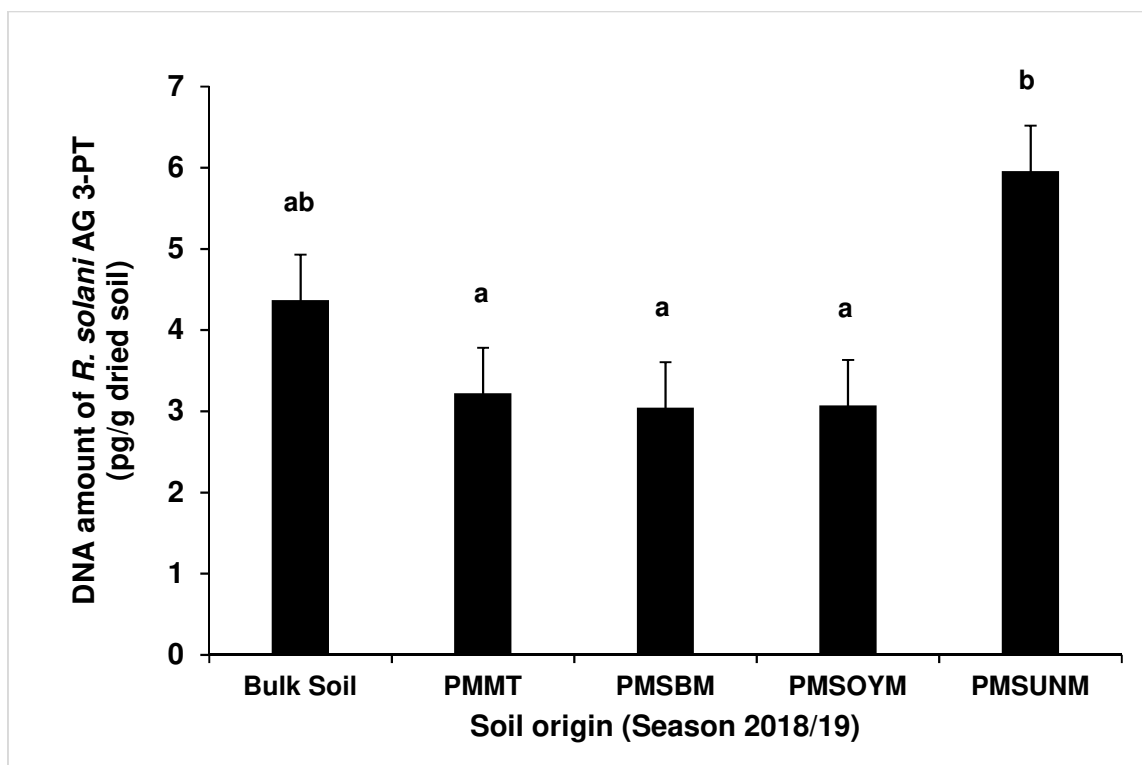


Figure 9.9 *Rhizoctonia solani* AG 3-PT DNA concentrations measured in four crop rotations (PMMT, PMSBM, PMSOYM, and PMSUNM) at the end of the fourth growing season 2018/19 planted with maize and teff. Different letters indicate significant differences ($p < .05$). Vertical error bars represent mean $\pm$ standard error ($n = 4$).

The fastest rate of increase in the *R. solani* AG 3-PT DNA occurred between season 2017/18 and season 2018/19 ($p < .01$). During this interval, the *R. solani* AG 3-PT DNA, the soil strength and bulk density of the PMMT and PMSUNM crop rotations were relatively high. The pathogen DNA content doubled in the PMSBM crop rotation between season 2017/18 and season 2018/19.

The PC 1 and PC 2 of the PCA biplot described 66.19 % of the total variability of the *R. solani* AG 3-PT data as displayed in Figure 9.10.

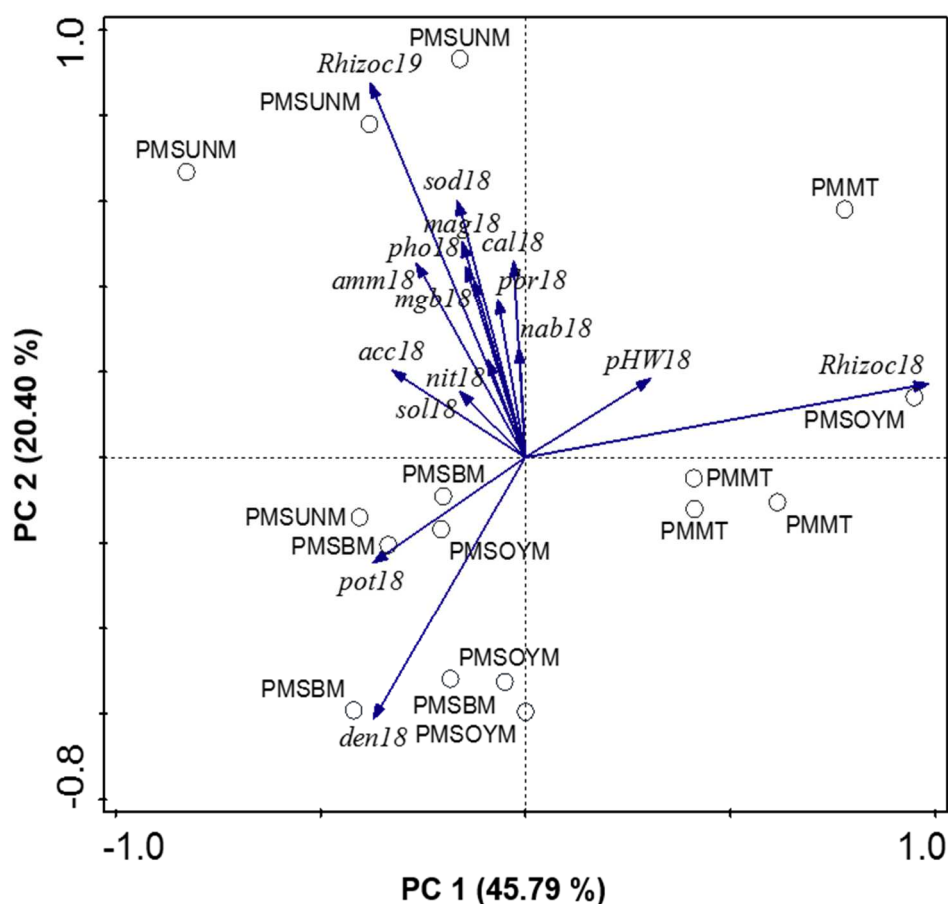


Figure 9.10 The PCA biplot of Rhizoc18 – *R. solani* AG 3-PT DNA (season 2017/18) and Rhizoc19 – *R. solani* AG 3-PT DNA (season 2018/19) associations with selected soil factors. The last two digits ‘18’ and ‘19’ in a soil factor represent season 2017/18 and season 2018/19 soil legacies, respectively. The 16 soil health properties are represented as:

acc - Active carbon (mg/kg)	pHW - pH (H ₂ O)	pho - P (mg/kg)
amm - 1:2 Water extract N - NH ₄ (mg/l)	cab - %Ca	cal - Ca (mg/kg)
nit - 1:2 Water extract N - NO ₃ (mg/l)	nab - %Na	sod - Na (mg/kg)
sol - Solvita CO ₂ respiration (ppm)	mgb - %Mg	mag - Mg (mg/kg)
pbr - P Bray 2 (mg/kg)	kbs - %K	pot - K (mg/kg)
den - Density (g/cm ³)		

At the end of the 2018/19 season, the *R. solani* AG 3-PT DNA levels were positively correlated with the high soil fertility legacies of the 2017/18 season, but were weakly negatively correlated with pH (H₂O), K and soil particle density (Figure 9.10). The *R. solani* AG 3-PT DNA level in the soil was positively associated with the PMSUNM rotation cluster at the end of season 2018/19 (Figure 9.10).

At the end of season 2018/19, the PC 1 and PC 2 described 61.40 % of the total variability of the *R. solani* AG 3-PT DNA data (Figure 9.11).

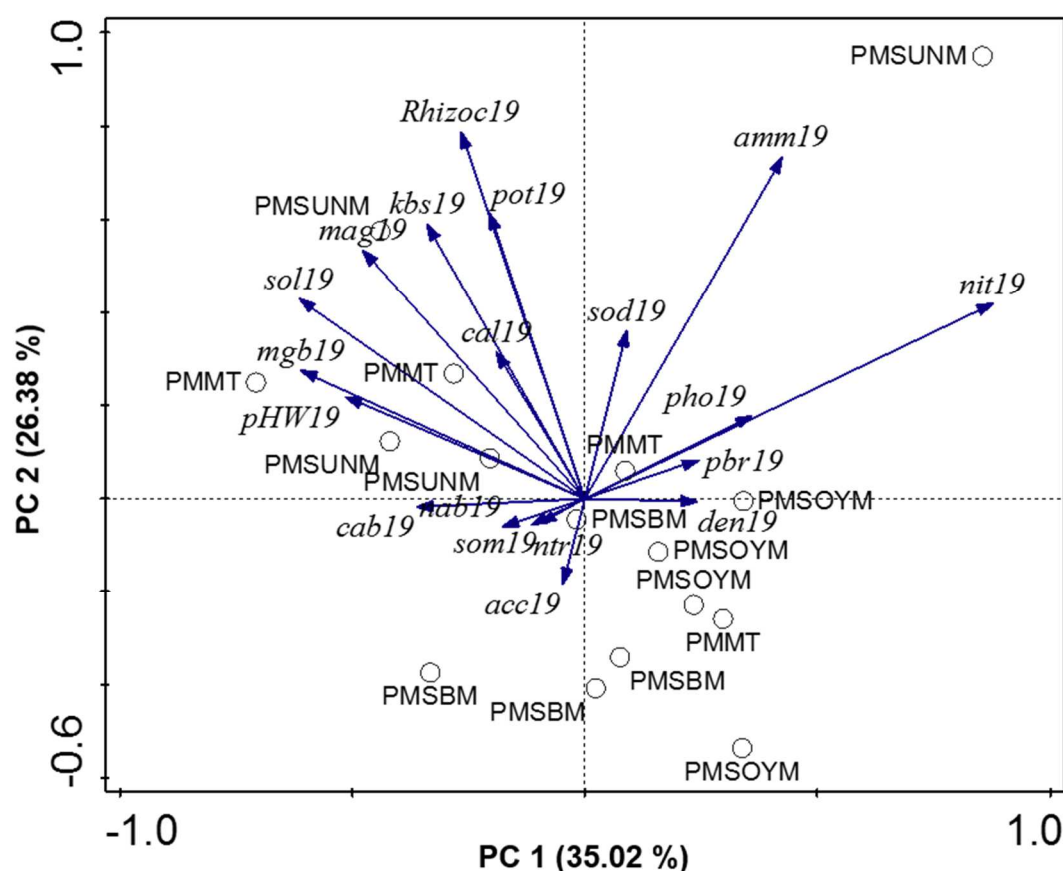


Figure 9.11 The PCA biplot of *Rhizoc19* – *R. solani* AG 3-PT DNA (season 2018/19) associations with selected soil factors. The last two digits ‘19’ in a soil factor represent season 2018/19 soil legacies. The 16 soil health properties are represented as:

acc - Active carbon (mg/kg)	pHW - pH (H ₂ O)	pho - P (mg/kg)
amm - 1:2 Water extract N - NH ₄ (mg/l)	cab - %Ca	cal - Ca (mg/kg)
nit - 1:2 Water extract N - NO ₃ (mg/l)	nab - %Na	sod - Na (mg/kg)
sol - Solvita CO ₂ respiration (ppm)	mgb - %Mg	mag - Mg (mg/kg)
pbr - P Bray 2 (mg/kg)	kbs - %K	pot - K (mg/kg)
den - Density (g/cm ³)		

In the PCA biplot (Figure 9.11), *R. solani* AG 3-PT DNA showed positive associations with soil NH₄-N, K, % K, Mg, % Mg, Solvita soil respiration, Ca, Na and pH (H₂O), and negative associations with active-C, SOM, NO₃-N, % Na and % Ca.

9.4.3 Correlation analyses of Soil Factors and Soil Pathogen DNA

The findings on seasonal data indicate that concentrations of *R. solani* AG 3-PT DNA were not associated with each other ($p > .05$, Table S9.2). There was a weak positive correlation in the pathogen DNA between the first and second year of the crop rotation ($r_{16} = 0.414$, $p > .05$, Table S9.2). Moreover, there were no significant associations between pathogen DNA and soil factors at the end of season 2106/17 and season 2017/18 ($p > .05$). At the end of season 2018/19, there was a significant positive association between pathogen DNA and $\text{NH}_4\text{-N}$ ($r_{16} = 0.514$, $p < .05$) while the other soil factors did not show significant correlations ($p > .05$).

9.5 Discussion

Pathogen DNA concentrations across Crop Rotations

The low amounts of *R. solani* AG 3-PT DNA (pg DNA per g of soil) at the end of the potato uniformity season agrees with reports by Lees et al. (2002) of low and patchy distribution of AG 3-PT in potato field soils. For practical reasons, not all the soil bioassays and microbial isolation, identification and characterization of live *R. solani* AG 3-PT culture isolates directly from field soils were investigated in this study, the real-time PCR assay step for detection directly from soil DNA extracts can be problematic. Instead, the real-time PCR step must also be combined with soil bioassays to detect and quantify AG 3-PT in both artificially inoculated field soil and naturally infested soils. Other studies have reported that the soil inoculum of *R. solani* AG 3-PT survives and remain viable as sclerotia for up to 18 months (Ritchie et al., 2013). The occurrence of soil-borne pathogens in the rhizosphere of host plants that are biotically and abiotically suitable for pathogen presence accelerates the spread of disease (Krupinsky et al., 2002; Agrois, 2005). More so, the correct choice of rotation crops, biocontrol amendments and cover crops; in combination with optimal tillage practices; can lower disease levels in the field (Deacon, 2006; Larkin & Brewer, 2020). Non-cereals are ideal break crops for *R. solani* in intensive cereal cropping (Gupta et al., 2012; Kirkegaard & Ryan, 2014;

MacBeath et al., 2015). Crop rotation effects improve crop productivity, maintain soil health and sustainable cropping systems (Wright et al., 2015).

Since all the crop rotation systems were treated similarly in season 2016/17 as a maize uniformity trial, the reason for the significantly higher *R. solani* AG 3-PT DNA by approximately 2 × to 5 × in the PMMT crop rotation compared to the other three rotations was unclear. In all four seasons, the maize and teff crops seemed to minimize the resilience of field soils against build-up of *R. solani* AG 3-PT under dryland conditions due to the fact that maize and grass weeds are hosts to *R. solani* AG 3-PT (Tsrör, 2010; McCormack et al., 2013).

The *R. solani* AG 3-PT DNA concentration was 2 × higher in the PMSUNM crop rotation than in other rotations at the end of the 2018/19 season, which may have been due to the high density of the weed *Taraxacum officinale* in this rotation (reported in Chapter 7). *Taraxacum officinale* was confirmed by Carling et al. (1986) to be a host of *R. solani* AG 3-PT. This suggests that the *R. solani* AG 3-PT survived and grew on this weed species resulting in higher DNA concentrations in the rhizosphere soil in the PMSUNM crop rotation. This weed is an alternative secondary host of *R. solani* AG 3-PT, its plant debris and the resulting soil historical legacies not measured in this study or seasons could have changed the pathogen DNA composition of this block. Since the PMMT crop rotation consisted of a maize crop for two seasons in a row, the microbial population may have not changed enough to successfully reduce the *R. solani* AG 3-PT DNA. In addition to this, maize contains a relatively high lignin content, resulting in a very slow break down with resultant lower soil organic matter to drive microbial activity. This scenario may have not promoted beneficial control agents in the soil since there may have not been enough food to sustain their population, providing the perfect opportunity for the *R. solani* AG 3-PT to survive under the PMMT crop rotation.

In an *in-vitro* pot trial conducted by McCormack et al. (2013) the amounts of *R. solani* AG 3-PT DNA in the soil grown to maize were reported to be elevated compared to the uninoculated

control suggests that maize is likely a suitable alternative host for AG 3-PT and has the potential to increase pathogen DNA in the soil in crop rotations.

The PLFA markers a-C15:0, C18:1 ω 9c and C18:2 ω 6c had a negative association with *R. solani* AG 3-PT DNA in PMSUNM rotation, suggesting the possible recruitment of *Rhizoctonia* antagonists in the sunflower rhizosphere (Collins & Norton, 2019). Indeed, it is widely accepted that a balanced microbial population accompanied by an ideal crop rotation practice will reduce pathogen inoculum in the soil (Peters et al., 2003). However, with hindsight of the spike in *R. solani* AG 3-PT DNA in the follow-up maize crop (in year 2018/19), the lower *R. solani* AG 3-PT DNA in the previous year (2017/18) of sunflower could have been due to delayed or gradual inoculum build-up in the soil (Agrios, 2005).

Further research should focus on monitoring the positive and/or negative impact of conventional tillage on the C18:2 ω 6c fungal PLFA marker and i-C16:0 Gram positive bacterial PLFA marker composition under dryland conditions. Most bacteria with biocontrol activity suppress the growth of the pathogenic *R. solani* AG 3 by producing the antibiotic pyrrolnitrin (Van Elsas et al., 2006). In other studies, members of the phylum *Actinobacteria* have shown biocontrol potential against *R. solani* (Bulgarelli et al., 2013).

The changes in Pathogen DNA across Seasons

The high rainfall experienced from January to March during the season 2018/19 and the resulting soil legacy of low pathogen DNA suggests correlative and causal relationships between AG 3-PT DNA and rainfall patterns (Gupta et al., 2010). In literature, the maximum likelihood of potato black scurf disease occurs in field-collected soil containing 1,401 pg of *R. solani* AG 3-PT DNA per g of dried soil (Sparrow et al., 2015).

Tillage practices conducted seasonally may have contributed to the spread of the weed hosts of *R. solani* AG 3-PT across the trial site, possibly explaining the similar patterns in *R. solani* AG 3-PT DNA concentrations across the rotation treatments. Tillage practice probably dispersed

the weed seeds, plant residues and litter material across the field blocks, thereby promoting the longevity of weed seeds in the soil and disrupting the soil biota antagonists of weeds (Jayaraman et al., 2021). Since ground coverage takes long to occur with maize, the alternate weed host may have been favourable to the *R. solani* AG 3-PT from the previous season for some treatments (Carling et al., 1986).

In the PMMT rotation, the $2.6 \times$ increase in the *R. solani* DNA concentrations represented the fastest rate over two seasons and likely due to soil compaction between season 2015/16 and season 2016/17 (Deacon, 2006). For rotation PMSUNM, the $4 \times$ increase of *R. solani* DNA in the soil between season 2017/18 and season 2018/19 was possibly due to host crop litter legacies (Gupta et al., 2012). Also, it is likely that the inclusion of a deep-rooting and drought-tolerant crop like sunflower in the rotation, cultivated a soil legacy associated with a dry but fertile soil environment that did not alter plant-microbe competition for soil nutrients or the resilience of soil (Bardgett & Caruso, 2020).

In the 2016/17 and 2017/18 seasons, the positive associations between PMMT crop rotation and *R. solani* AG 3-PT DNA concentration suggests the conduciveness of the rotation to this pathogen. Also, the negative associations between *R. solani* AG 3-PT DNA concentration and the PMSBM and PMSOYM rotations suggests potential suppressiveness against *R. solani* in these two seasons. In the present study, the inclusion of sunflower (PMSUNM) in season 2017/18 before subsequent planting of maize (in season 2018/19) increased the risk of a spike in the *R. solani* AG 3-PT DNA concentrations in the soil. Even so, there are no field studies to substantiate that sunflower is a break-crop for diseases caused by *Rhizoctonia* species.

The PCA biplot of soil factors and pathogen DNA at the end of season 2018/19 agreed with Pearson's correlation analysis that $\text{NH}_4\text{-N}$ was the only soil factor strongly correlated with *R. solani* AG 3-PT DNA, suggesting that this form of nitrogen is conducive to the growth of *R. solani* AG 3-PT in the soil. Also, the soil $\text{NH}_4\text{-N}$ legacy was also higher in the sunflower

rotation than the other crop rotations. This is significant, because, high $\text{NO}_3\text{-N}$ enhances disease suppressiveness while root rots of vegetables are more severe when $\text{NH}_4\text{-N}$ is applied as a fertilizer than when $\text{NO}_3\text{-N}$ form is applied (Engelhard, 1989; Jayaraman et al., 2021). Indeed, there is evidence of $\text{NH}_4\text{-N}$ fertilizers increasing the pathogen inoculum density or disease severity more than $\text{NO}_3\text{-N}$ forms (Gupta et al., 2017). However, there are inconsistent reports in literature on the impact of N because of the failure to disclose the N forms (Marschner, 2011).

In this study, the suggested finding that soil K has a positive effect on *R. solani* inoculum contradicts the antagonistic effect observed in other studies (Snyder & Ashlock, 1996; Pinheiro et al., 2011; Gupta et al., 2017). The weak negative associations of *R. solani* DNA concentration with pH and SOM at the end of season 2018/19 implies that these soil factors are antagonistic to *R. solani* (Harries et al., 2020).

In this study, the incorporation of legumes (soybean and sugar bean) in rotations PMSOYM and PMSBM appeared to reduce the soil DNA concentrations of *R. solani* AG 3-PT under dryland soil tillage conditions, suggesting that commercial growers can include legumes in dryland crop rotations to cultivate a soil suppressive against *R. solani* AG 3-PT. From current literature, the legumes are not hosts of *R. solani* AG 3-PT, but maize has been reported as a host crop (McCormack et al., 2013). A contradictory trend exist among studies because most legume lines are reported as having resistance to *R. solani* AG 3-PT and showing little or no disease damage symptoms, but also showing cross-pathogenicity disease symptoms and damaged in other studies (Sumner & Bell, 1994; Abawi et al., 2012; Naseri & Moradi, 2015; Kodati et al., 2021; Kodati et al., 2022).

We assumed that a legume like faba bean represented best the host attributes for the pathosystem in soil bioassays investigating the impact of crop rotation soil legacies on the suppression of artificially added *R. solani*, the contradictions in literature therefore had little or

nothing to do with answering the research questions of this study on soil suppressiveness as a soil health process. The decline in plant disease incidence and severity in crop rotations PMSBM and PMSOYM could also have been due to competition between pathogen, symbiotic nitrogen fixing bacteria (artificial and natural) and high fungal diversity in the rhizosphere environment (Bhardwaj et al., 2014; De Corato, 2020). Elsewhere, soil suppression of *Rhizoctonia* root rot disease was improved under controlled conditions by adding crop residues and amending the phosphorus levels in the topsoil (Davey et al., 2021).

The qPCR results for *R. solani* species across seasons in this study must be interpreted with caution due to within-species genetic heterogeneity (Misawa & Kurose, 2019) and the possible emergence of new genotypes in the field (Muzhinji et al., 2016) which may lead to false negatives or underestimation of pathogen DNA levels. Species heterogeneity may lead to false negatives and underestimations because the different pathogen variants from natural selection for more virulent variants can be detected and quantified as one microorganism or in specific probe-based assays these new and emerging species may not be detected and therefore not quantified (Bennet et al., 2011). Genetic diversity in the ITS region allowed for the identification of *R. solani* AG-3 at the strain level corresponding to potato (AG 3-PT) and tobacco (AG 3-TB) (Kuninaga et al., 1997; Kuninaga et al., 2000; Gonzalez et al., 2001). Despite the universality of ITS sequence data in phylogenetic studies of *Rhizoctonia*, they are complex and display genetic variability that minimizes their utility in some studies suggesting the need to redefine the concept of this species (Roberts, 2000; González-García et al., 2006). Therefore, it is important to demonstrate the relevance of DNA sequencing of soil-borne pathogens. This modern tool enables diagnostic services to collect huge amounts of genomic sequence data that can be used to give an absolute identification of the soil-borne pathogen present in a soil sample as well as detect variants of the same pathogen species complex (Madigan et al., 2015; Boonham et al., 2016). In addition, comparative genomic sequencing

give valuable insights into variations between phytopathogens based on their pathogenicity genes, enzymes, toxins, and other metabolites that contribute to their virulence and aggressiveness in different crops (Hou et al., 2016).

More so, to translate these findings into practical applications, there is need for more repeated measures of soil and plant health over the long-term by taking into account the farming system, climate change and soil type to accomplish wider region-specific goals of dryland agriculture (MacLaren et al., 2019; Crookston et al., 2022). Furthermore, the economic impact of different production practices should be assessed throughout the season and across the agribusiness value chain to promote sustainability by encouraging multi-stakeholder participation (Elphinstone et al., 2018; Swart, 2021).

9.6 Conclusion

The qPCR is a more accurate tool than soil baiting assays in the quantification of soil-borne pathogens, but it is a poor technique for estimating pathogen virulence and viability (Brown et al., 2021). In this study, the findings of *R. solani* AG 3-PT DNA legacies after four years indicate that the quantities were significantly reduced in the soybean (Potatoes-Maize-Soybean-Maize), sugar bean (Potatoes-Maize-Sugar bean-Maize) and teff (Potatoes-Maize-Maize-Teff) cropping sequences. The inclusion of the maize crop in all the rotation sequences increased the *R. solani* AG 3-PT DNA in the soil. Also, the higher *R. solani* AG 3-PT DNA in the year following sunflower is an indication that growers should be careful to plant sunflower in the year or two preceding potatoes. It may, however be too early to observe any changes in the other biological assessments done. Over time, we expect the situation to change and legacy effects should become more pronounced. Van der Waals et al. (2021) concluded that this study requires about fifteen years to generate conclusions on soil health and microbial composition of soil. Despite the region-specific and short-term design of the rotations, the findings from

this study will contribute towards the long-term goal of interpreting the crop rotation effects on soil and plant health, in one of the most productive agricultural provinces in SA.

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Conflict of interest

Authors have no conflict of interest to declare.

CHAPTER 10

General Discussion

10.1 Background Information

Crop losses caused by diseases, animal pests, and weeds, range from 20 to 40% of the yield that would be attained otherwise (Savary et al., 2006), depending on the production situation. Crop rotational diversity is known to influence both soil microorganisms and crop productivity, yet the specific contributions of microorganisms to rotational benefits are unknown (Brankatschk & Finkbeiner, 2015). Potato cropping systems worldwide show that the correct choice of rotation, biocontrol amendments and cover crops, in combination with optimal tillage practices, can lower disease levels in the field (Larkin & Brewer, 2020). Economically important soil-borne pathogens of potato include fungi such as *Rhizoctonia solani* (black scurf), *Helminthosporium solani* (silver scurf), *Spongospora subterranea* f. sp. *subterranea* (powdery scab), *Fusarium* spp. (Fusarium wilt), *Verticillium dahlia* and *V. alboatrum* (Verticillium wilt). The South African potato industry is also facing a growing concern regarding the increasing incidence and severity of the soft rot/blackleg disease complex caused predominantly by *Pectobacterium brasiliense* (Van Der Waals et al., 2016).

10.2 Synthesis and Theoretical Implications of Findings

The thesis demonstrates the impact of region-specific crop rotation effects on soil and plant health in a dryland system in a large-scale, region-specific and on-farm field experiment in South Africa between the years 2015 and 2019. The study attempted to integrate field research findings on soil composition, into the practical and strategic management of residual crop rotation effects in fields in the EFS dryland systems as well as in greenhouses, focusing on soil and plant health. The specific objectives of the greenhouse and field studies were as follows:

- i. To get reference data to establish predictors or indicators of soil health and to test ‘dummy’ treatment effects (Chapter 3).
- ii. To determine whether four dryland crop species namely, *Zea mays* L., *Helianthus annuus* L., *Phaseolus vulgaris* L. and *Glycine max* L. (Merr.) have specific rhizosphere microbial biomass and activities associated with the particular crop type under dryland field conditions (Chapter 4).
- iii. To determine whether different dryland crops such as *Zea mays*, *Helianthus annuus*, *Phaseolus vulgaris* and *Glycine max* have soil legacies that are related to the crop type (Chapter 5).
- iv. To determine whether plant-soil feedbacks (PSFs) can estimate the ability of crop rotation sequences to recover from (resilience) and resist (resistance) monoculture disturbance under greenhouse conditions (Chapter 6).
- v. To determine the short-term crop rotation legacy effects on selected biotic and abiotic soil conditions over three consecutive growing seasons (Chapter 7).
- vi. To determine the suppressiveness of the soil legacies against seedling root-rots caused by *R. solani* AG 2-2 IIIB and *F. graminearum* (Chapter 8).
- vii. To determine the impact of the residual effects of short-term crop rotations on the soil-borne fungus *R. solani* AG 3-PT DNA concentrations in a rain-fed cropping environment in the Eastern Free State Province of South Africa (Chapter 9).

To get a clear view of all the beneficial rotations, Table 10.1 is a summary of findings that are discussed in this section with a view of all the data. From this study, it is too premature to generalize key soil health indicators, however, there is need for long-term studies and monitoring of crop yield, soil respiration, active C, soil pH, off-site soil density, Zn, P and K (Van der Waals et al., 2021). This study requires about fifteen years of rotation cycles to generate conclusions on soil health and microbial composition of soil (Van der Waals et al.,

2021). Caution must be taken when interpreting these findings by maintaining the original goal of bringing soil and plant health findings into general use (generality) rather than sweeping statements on foretelling events (predictability). The following table (Table 10.1) summarizes which rotation(s) were best overall according to the results from the greenhouse and field trials.

Table 10.1 A summary scoring how each rotation performed across all the different tests

Chapter	R1 (PMMT)	R2 (PMSBM)	R3 (PMSOYM)	R4 (PMSUNM)
Uniformity trial is effective – Chapter 3	Uniform	Uniform	Uniform	Uniform
Compared to bulk soil – Chapter 4	Fungal biomass: 1.1x Bacterial biomass: 2.2x Enzyme activity:1.2x	Fungal biomass:1.5x Bacterial biomass: 2.1x Enzyme activity:1.3x	Fungal biomass:1.4x Bacterial biomass: 1.5x Enzyme activity:1.3x	Fungal biomass:1.7x Bacterial biomass: 2.3x Enzyme activity:1.5x
Plant performance – Chapter 5	Negative	Positive	Negative	Negative
Resilience and resistance to <i>R. solani</i> AG 2-2IIIB – Chapter 6	Less resistant & resilient	Less resistant & resilient	More resistant & resilient	More resistant & resilient
Soil health – Chapter 7	Raise soil pH and increase soil organic matter and micronutrients. Ideal break crop for weeds.	Raise soil pH and increase soil organic matter and micronutrients	Raise soil pH and increase soil organic matter and micronutrients	Raise soil pH and increase soil organic matter and micronutrients. Ideal break crop for weeds.
Suppression of <i>R. solani</i> AG 2-2 IIIB mycelial growth – Chapter 8	<i>In vitro</i> : moderate effective <i>In vivo</i> : most suppressive	<i>In vitro</i> : most effective <i>In vivo</i> : moderate suppressive	<i>In vitro</i> : moderate effective <i>In vivo</i> : moderate suppressive	<i>In vitro</i> : least effective <i>In vivo</i> : most suppressive
Build-up of levels of <i>R. solani</i> AG 3-PT DNA – Chapter 9	Least resilient	Most resilient	Most resilient	Least resilient

The baseline soil health factors were measured in the first and second seasons using mainly the maize uniformity field trial findings described in **Chapter 3**. Soil chemical, physical and microbial data collection showed that all plots in this trial were relatively uniform in the 2016/17 season. Field soil texture was described in Chapters 3 to 9 as a sandy soil made up of 8.94 % clay, 9.80 % silt and 81.26% sand. The soil texture has the greatest influence on multiple soil health scores and indicators in some soil health testing frameworks, for example, the comprehensive assessment of soil health (CASH), the soil management assessment framework (SMAF), and the Haney soil health tool (HSHT) (Crookston et al., 2022). The findings showed that soil *F. graminearum* DNA concentrations among and within the studied four crop rotations were significantly reduced. Diseases caused by *Fusarium graminearum* can lead to yield losses between 30 and 70 % in most cultivated cereals and soybeans (Bai & Shaner, 1994). Soil fertility, high microbial diversity and composition are important in the controlling pathogenic *Fusarium* species (Zou, 2015).

The phospholipid fatty acids analysis (PLFA) technique was used to estimate soil microbial community composition described in **Chapter 4**. The ecosystem role of the soil microbial community composition is considered superior to microbial diversity, particularly on cycling soil C (Domeignoz-Horta et al., 2021). The PLFA data findings in **Chapter 4** (season 2016/17), indicated that there were clear differences in microbial population compositions according to crop type, with bacteria dominating over fungi. Intensive agriculture encourages dominance of bacteria energy channels and biomass composition rather than fungal energy channels (Bardgett & Caruso, 2020). The high bacterial biomass composition relative to fungal biomass; indicates the hindrance of fungi and their potential roles in soil C cycling (Domeignoz-Horta et al., 2021).

The sunflower rhizosphere compared to that of other dryland crops supported a greater community of total fungal PLFA markers, fungal to bacterial PLFA ratio, C18:2 ω 6c fungal

PLFA marker and i-C16:0 Gram positive bacterial PLFA marker. This high fungal to bacterial biomass composition ratio in a drought-tolerant crop like sunflower, agrees with findings that drying-out the soil environment is associated with higher fungal communities than bacterial communities (Bardgett & Caruso, 2020). The C18:2 ω 6c fungal PLFA marker and i-C16:0 Gram positive bacterial PLFA marker are potential biological indicators for estimating fungal to bacterial PLFA ratios in dryland conditions in the short term from 1 to 3 years (Stott, 2019). So, these markers are ideal biological indicators for estimating and monitoring active microbial community structure in disturbed soil conditions over time (Zaeem et al., 2019; Pérez-Guzmán et al., 2021). In South Africa, the sunflower rhizosphere soils were found to be rich in *Sphingomonas* and *Rhizobium* taxa which are potential biological inoculants for crop growth and plant health (Oberholster et al., 2018). However, the identification of the taxonomy of markers requires application of soil microbial ecology techniques such as stable isotope labelling and single-cell genomics (Madigan et al., 2015). The identification of taxa or markers must be calibrated by comparing and validating them based on sensitivity, accessibility and applicability (Keesstra & Mol, 2011). Thenceforth, growers would be able to monitor soil health changes as well as minimize the disturbance to ecological balance between the beneficial microbial markers and the harmful ones; and thereby reduce the abuse of the soil using chemical pest control (Cadet et al., 2002).

Greenhouse studies using plant-soil feedback findings (**Chapters 5 and 6**), suggest that *Phaseolus vulgaris* (sugar bean) rotations promote their own beneficial soil microbiota. Higher resilience to pathogens associated with *H. annuus* monoculture, in pot experiments, was closely related to the inclusion of *P. vulgaris* in *H. annuus* crop rotations. Under greenhouse conditions, the frequency of sunflower in rotation sequences increases fungal relative to bacterial biomass (Mushonga et al., 2020). These outcomes of soil health are still debatable

although a mechanistic understanding of plant-soil systems would stimulate learning cycles in soil health research (Janzen et al., 2021).

Greenhouse studies on plant-soil feedbacks using the same field soil collected after the maize uniformity trial in the year 2017 confirmed this observation on indirect feedback effects of crop rotations (Mushonga et al., 2020). These findings indicated that the soil health of *H. annuus* rotations in three-year cycles is driven by legacies and changes in plant decomposer communities, which are crucial for soil improvements in arable systems (Clocchiatti et al., 2020, Ning et al., 2021), in particular, soil fertility (Ning et al., 2021). We recommend growers to shift from maize and soybeans to crops such as sunflower and sugar beans to cultivate enzymatic and microbial-rich soils under rain-fed conditions in the EFS province of SA. The growers need to be careful in growing sunflower frequently in rotation with maize, this is important for the sustainability of the cropping system, because sunflower increased pathogen DNA and decreased soil resilience and recovery in subsequent planting of maize. Before including sunflower in rotations, Debaeke et al. (2021) cautions farmers to consider the impact of sunflower on the; subsequent crop yields, pest challenges, cost of production, market value of the rotation crop, and the total crop productivity of every crop in the rotation system. The findings in our study suggest that the best option for growers would be to plant sunflower in other agroecological cropping systems and avoid frequent rotation with maize to prevent the stocking up of fungal diseases inoculum under rain-fed conditions. The effects of changes in soil microbial biomass and enzymatic activities on soil pathogen DNA and resilience are not fully understood (Bennet et al., 2011). The higher observed levels of microbial biomass and enzymatic activities in the rotations could also be attributed to the effects of the absence of pesticides (Sena et al., 2002). If some important soil health metrics were omitted in our study, then the dryland farmers need to determine the presence or absence of pesticides and herbicides in soils as a way of confirming and validating these findings before implementation at the field

level. The potential indicators for soil resilience include gains in the soil microbial activity, plant nutrient uptake, SOM content, soil microbial composition and biomass, Solvita soil respiration and soil pH (Seybold et al., 1999; GRDC, 2008). However, thresholds for these soil properties will need to be evaluated, in particular, for region-specific field experiments in the long-term (Seybold et al., 1999).

The results from field experiments in **Chapter 7** of this thesis highlight that at the end of the rotation cycle, *Eragrostis tef* (teff) could significantly reduce the populations and density of *Senecio consanguineus* and *Taraxacum officinale* weed species. Studies have shown that crop rotation creates a soil environment favouring crops over weeds (Sait, 2003). The yields of the maize test crop in the final season of the rotations were very similar although the highest estimate was obtained under the Potatoes-Maize-Soybeans-Maize treatment. The results presented in **Chapter 7** indicated that this yield increase could have been due to soybean (*G. max*) litter inputs, which was similar to findings in studies done elsewhere (Huffman, 2021; Bowles et al., 2022). This maize yield result supports the idea from overseas studies that this rotation will most likely increase sustainable maize profitability (Zaeem et al., 2019; Bowles et al., 2020; Zaeem et al., 2021). In addition, most farmers prefer legumes in crop rotations because they improve yields in the subsequent crops by creating high legacies of biologically fixed nitrogen (Fageria, 2016; Kleijn et al., 2019). Soil analysis results on soil nitrogen legacies were confounded by the different N fertilization regime for crop rotations in both seasons 2017/18 and 2018/19 under conventional tillage of the EFS (Table S9.1). The hypothesis was that legumes in a rotation would improve maize yields. After comparing the maize yield differences between season 2016/17 and season 2018/19, the PMSBM and PMSOYM rotations yielded 5% and 9% higher than PMSUM rotation, respectively. The percent maize yield differences logically suggest that legumes supply more N to the top soil layer than sunflower

and improve relative decline in maize yield growth rate in the successive season (Ditzler et al., 2021).

Soil health initiatives use organic matter and nutrient availability as possible soil health indicators (Cardoso et al., 2013) for practical soil management in region-specific crop rotations. This field experiment (in **Chapter 7**) showed that the soil organic matter (SOM) levels after the four seasons were generally low at 0.3 ± 0.1 % and typical of sandy soils (FERTASA, 2016; Crookston et al., 2022). The SOM is the natural bioreactor for biogeochemical cycles by maintaining the biotic and abiotic reactions linked to ecosystem functioning and services (Timmis & Ramos, 2021). Only two crop rotations (Potatoes-Maize-Maize-Teff, Potatoes-Maize-Sugar beans-Maize) had SOM composition legacies that were significantly different from the bulk soil ($p < .05$). The present findings support other findings that grasses increase SOM and soil active C through providing biomass cover and high litter inputs (Pérez-Guzmán et al., 2021). However, the role of legumes in providing plant cover associated with the increase in soil C (Bowles et al., 2022) contradicts findings by Pérez-Guzmán et al. (2021).

Although the soil active carbon stocks during the four seasons was still low and typical of sandy soils, it increased at the end of the rotation cycle by an estimated 64 % for the Potatoes-Maize-Maize-Teff and Potatoes-Maize-Sugar bean-Maize treatment, 68 % for Potatoes-Maize-Sunflower-Maize, and 92 % for Potatoes-Maize-Soybean-Maize crop rotation treatments. Thus, these findings (described in **Chapter 7**) indicated that the soil active C composition is a rapid indicator of soil health changes while SOM is a key indicator of soil health (Pérez-Guzmán et al., 2021; Crookston et al., 2022).

The Solvita soil microbial respiration represents a good predictor of appropriate N requirements through measuring soil microbial metabolic activity in the short-term (Crookston et al., 2022). Measurements of the soil microbial respiration during the four seasons were generally low and typical of sandy soils (Brinton, 2016). However, the soil microbial

respiration for the four crop rotation sequences in the final cropping season increased; approximately 101 % for Potatoes-Maize-Sugar bean-Maize, 340 % for Potatoes-Maize-Soybean-Maize, 401 % for Potatoes-Maize-Sunflower-Maize, and 470 % for Potatoes-Maize-Maize-Teff. The grass-dominated rotation generally cultivated soil legacies that have high bacterial composition due to good litter quality and high quantity litter from grasses, including lower rates of N leaching (Pérez-Guzmán et al., 2021). The long-term crop rotation effect on subsequent soybean yield could be explained by soil and root-associated microbiomes and soil health indicators (Neupane et al., 2021). Soybean rotations with cereal crops such as maize increase soil carbon and nitrogen legacies (Kelly et al., 2003).

The impact of soil acidity dynamics is difficult to interpret because the magnitude of the same pH units have a different impact on soil properties and functions (Deacon, 2006). The findings showed that the soil acidity in these sandy soils changed slightly in all crop rotations. Overall soil pH results indicated that the Potatoes-Maize-Maize-Teff cropping sequence was the most highly resilient crop rotation cycle against soil acidity in the short-term while the least resilient was the Potatoes-Maize-Soybean-Maize rotation. In South Africa, soil acidity is an endemic problem in crop production (FSSA, 2007; FERTASA, 2016). There is a need for research to focus on crop rotations that minimize soil acidity and associated costs. However, for potato production the acidic soils are ideal for the management of common scab (FERTASA, 2016).

In **Chapter 8**, the results from *in vitro* assays suggest that soil legacy effects at the end of the Potatoes-Maize-Sugar bean-Maize rotation treatment, significantly suppressed *R. solani* AG 2-2 IIIB mycelial growth, while the Potatoes-Maize-Sunflower-Maize soils were the least effective in suppression of *R. solani* AG 2-2 IIIB mycelial growth. Understanding the role of microbial diversity in the control or suppression of soil-borne pathogens through quantification of functional markers in soil bioassays would allow farmers to plan environmentally-friendly

control strategies which support the biocontrol potential and fertility of the soil environment (Cheeke et al., 2012; Elphinstone et al., 2018; Jayaraman et al., 2021).

Further findings from the pot experiments in **Chapter 8**, showed that field-collected Potatoes-Maize-Sunflower-Maize soils were suppressive to *F. graminearum* but conducive to *R. solani* AG 2-2 IIIB. The low severity of Rhizoctonia root rot disease in broad beans planted in the field-collected Potatoes-Maize-Maize-Teff soil indicate that this grass-dominated rotation cultivates suppressive soil conditions against *R. solani* AG 2-2 IIIB. The assessment and management of disease risk, requires a large number of field studies to validate soil bioassays in predicting disease risk and impacts on crop yield (Gatch & du Toit, 2015). So, to confirm the greenhouse soil bioassays, they need to be repeated in field trials under multiple dryland conditions.

In **Chapter 9**, studies on crop rotation effects on soil DNA concentration of phytopathogens focused on *R. solani* AG 3-PT. This chapter applied the quantitative PCR method to follow the temporal changes of DNA concentration of *R. solani* AG 3-PT in a dryland soil environment under different crop rotation sequences. The findings indicated that, after four years, the Potatoes-Maize-Soybean-Maize crop rotation sequence significantly reduced *R. solani* AG 3-PT DNA concentrations in the soil compared to Potatoes-Maize-Sunflower-Maize rotation. A tropical pasture grass (teff) in maize crop rotations minimized the resilience of field soils against build-up of *R. solani* AG 3-PT DNA. In South Africa elephant hide, black scurf and stem canker caused by *R. solani* AG 3-PT are important diseases in potato production (Muzhinji et al., 2014; 2015; 2016).

Results from our four year study confirm that crop rotation effects influence the rhizosphere environment by either reducing or increasing disease incidence or severity (De Corato, 2020a; 2020b).

10.3 Future Work

Management practices to reduce the impact of disease caused by soil borne pathogens such as *R. solani* and *F. graminearum* can be improved through cover cropping, reducing tillage, managing nutrient amendments, and improving soil health (Stern et al., 1991; Strauss, 2017; Dusengemungu, 2021). At the same time, the rate of organic matter decomposition must be assessed using community physiological profiling that will provide information on the status of the soil microbial community metabolism (Garland et al., 2010). Future studies must investigate the benefits of cover crops and conservation agriculture to soil and plant health under dryland conditions. However, there is need to be cautious about adoption of conservation agriculture in drylands because it is limited by inherent challenges of low plant available water in sandy soils and soil crusting after precipitation (Trytsman, 2017). Elsewhere, studies have proven that ecological interventions can minimize soil acidity and compaction while increasing soil organic matter, thereby improving the soil microbiome essential for plant health.

Future work must also focus on procedures for culture-dependent and culture-independent analyses of soil microbial communities and functions using field-sampled soil to isolate soil borne pathogens and identify microbial indicators against disease for the integrated management of crop diseases which influence crop yield and quality in dryland production systems. There is need for more efforts in SA to formulate or adopt a soil health scoring framework aimed at the grower level. Already, researchers through the soil health workshop hosted by ZZ2 laboratories in Polokwane SA have emphasized the need for a holistic and region-specific soil health assessment (McLeod et al., 2012). Also, Slippers (2020) highlights the urgent need for a resilient national plant health management network that will add knowledge on how society contributes to the spread of plant diseases.

10.4 Concluding Remarks

This study has indicated that crop rotation effects on soil and plant health in a dryland production system, in particular, the Potatoes-Maize-Soybean-Maize rotation sequence demonstrate the potential of reducing *R. solani* AG 3-PT DNA concentration in the soil. In South Africa, this finding will assist farmers in the integrated management of *R. solani* AG 3-PT; the most predominant and difficult to control of the *R. solani* AGs across the sixteen potato growing regions. Acidic conditions and low soil organic matter composition in collected field soils are typical of cultivated sandy soils in South Africa and the studied cropping sequences illustrate their negative impacts on an eastern Free State dryland production system. The C18:2 ω 6c fungal PLFA marker and i-C16:0 Gram positive bacterial PLFA marker compositions were sensitive to crop rotations. These lipid markers are potential biological indicators for estimating fungal to bacterial PLFA ratios in dryland conditions in the short term (1 to 3 years). The soil bioassays demonstrated that infested soils in greenhouse conditions can discriminate dryland rotation soil legacies and provide an indirect estimate of disease risk in a short period of time, by measuring the root rot disease severity index and plant biomass parameters of bait plants.

10.5 Publications, Symposium papers and Industry Reports

- Steyn, J.M., Van der Waals, J.E., Sibiya, M., **Mushonga, K.**, & de Wet, A. (2022). *Finding the best cropping sequences for the Eastern Free State - Part 1. On Farm Level - Production Systems. SA Graan| Grain August 2022, 85-86.* <https://www.sagrainmag.co.za>. (Accessed 22/09/2022).
- Steyn, J.M., Van der Waals, J.E., Sibiya, M., **Mushonga, K.**, & de Wet, A. (2022). *Finding the best cropping sequences for the Eastern Free State - Part 2. On Farm Level - Production Systems. SA Graan| Grain September 2022, 108-109.* <https://www.sagrainmag.co.za>. (Accessed 22/09/2022).

- Steyn, J.M., Van der Waals, J.E., Swart, W.J., Sibiya, M., **Mushonga, K.**, & de Wet, A. (2022). In *Development of region-specific crop rotation programmes for the Eastern Free State*, Artappelnavorsingsimposium-Potato Research Symposium Abstract Book, Mykonos Langebaan, Cape Town, South Africa, 19–21, Jul 2022. (Available from the first author by request).
- Van der Waals, J.E., **Mushonga, K.**, Sibiya, M., de Wet, A., Swart, W.J., & Steyn, J.M. (2021). In *Development of region-specific crop rotation programmes for the Eastern Free State*, Virtual Presentation–Potatoes South Africa, South Africa, September 2021. (Available from the first author by request).
- Steyn, J.M., Van der Waals, J.E., Sibiya, M., **Mushonga, K.**, & de Wet, A. (2021). *Grain SA Progress Annual report 2021*: Development of region-specific crop rotation programmes for the Eastern Free State. (Available from the first author by request).
- Steyn, J. M., Van der Waals, J.E., Sibiya, M., & **Mushonga, K.** (2021). *PSA Annual report 2020/21*: Development of region-specific crop rotation programmes for the Eastern Free State. (Available from the first author by request).
- **Mushonga, K.**, Steyn, J.M., Swart, W.J., & van der Waals, J.E. (2020). Plant–soil feedback responses of four dryland crop species under greenhouse conditions. *Plant-Environment Interactions 1*, 181–195. <https://doi.org/10.1002/pei3.10035>.
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- Steyn, J.M., Van der Waals, J.E., Cowan, D., & **Mushonga, K.** (2019). In *Development of region-specific crop rotation programmes for the Eastern Free State*, Artappelnavorsingsmposium-Potato Research Symposium Abstract Book, Klein Kariba near Bela-bela (Warmbad), Limpopo, South Africa, 23-25, Jul 2019. (Available from the first author by request).
- **Mushonga, K.**, Steyn, J.M., Cowan, D., Swart, W.J., & Van der Waals, J.E. (2019). Crop rotation effects on soil and plant health in a dryland production system in South Africa. *Department of Plant and Soil Sciences Postgraduate Symposium Abstract book*, Symposium held on 18th September 2019 at Future Africa, University of Pretoria, South Africa. (Available from the first author by request).
- Steyn, J. M., Van der Waals, J.E., Cowan, D., & **Mushonga, K.** (2019). *PSA Annual report 2018/19*: Development of region-specific crop rotation programmes for the Eastern Free State. (Available from the first author by request).
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SUPPLEMENTARY MATERIALS

Supplementary Material for Chapter 3

Supplementary Figures

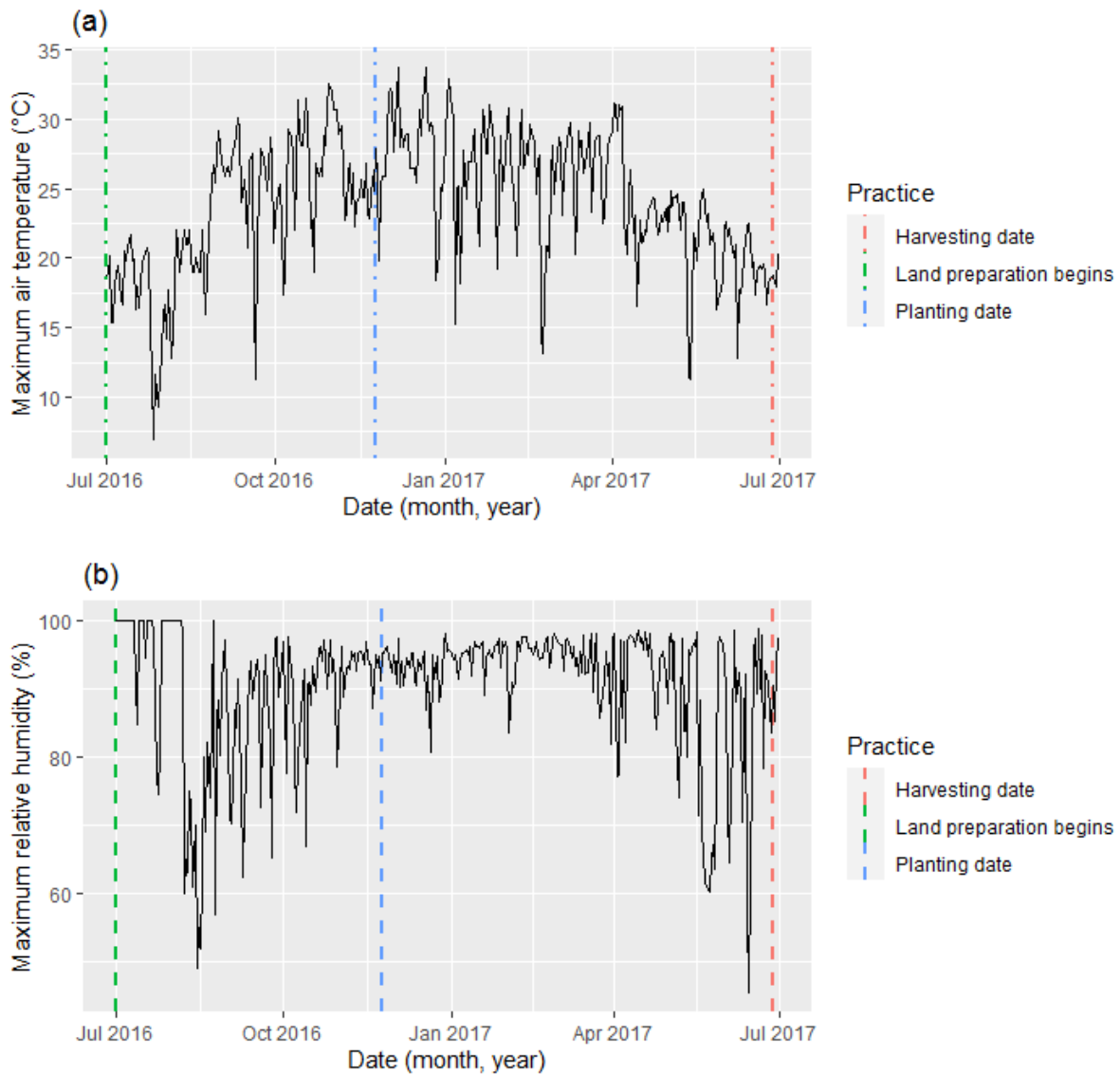


Figure S3.1A Climate conditions in the EFS field experiment. (a) Maximum air temperature (°C) and (b) maximum relative humidity (%), from planting to harvesting. Planting date – 26/11/2016 and harvesting date – 27/07/2017.

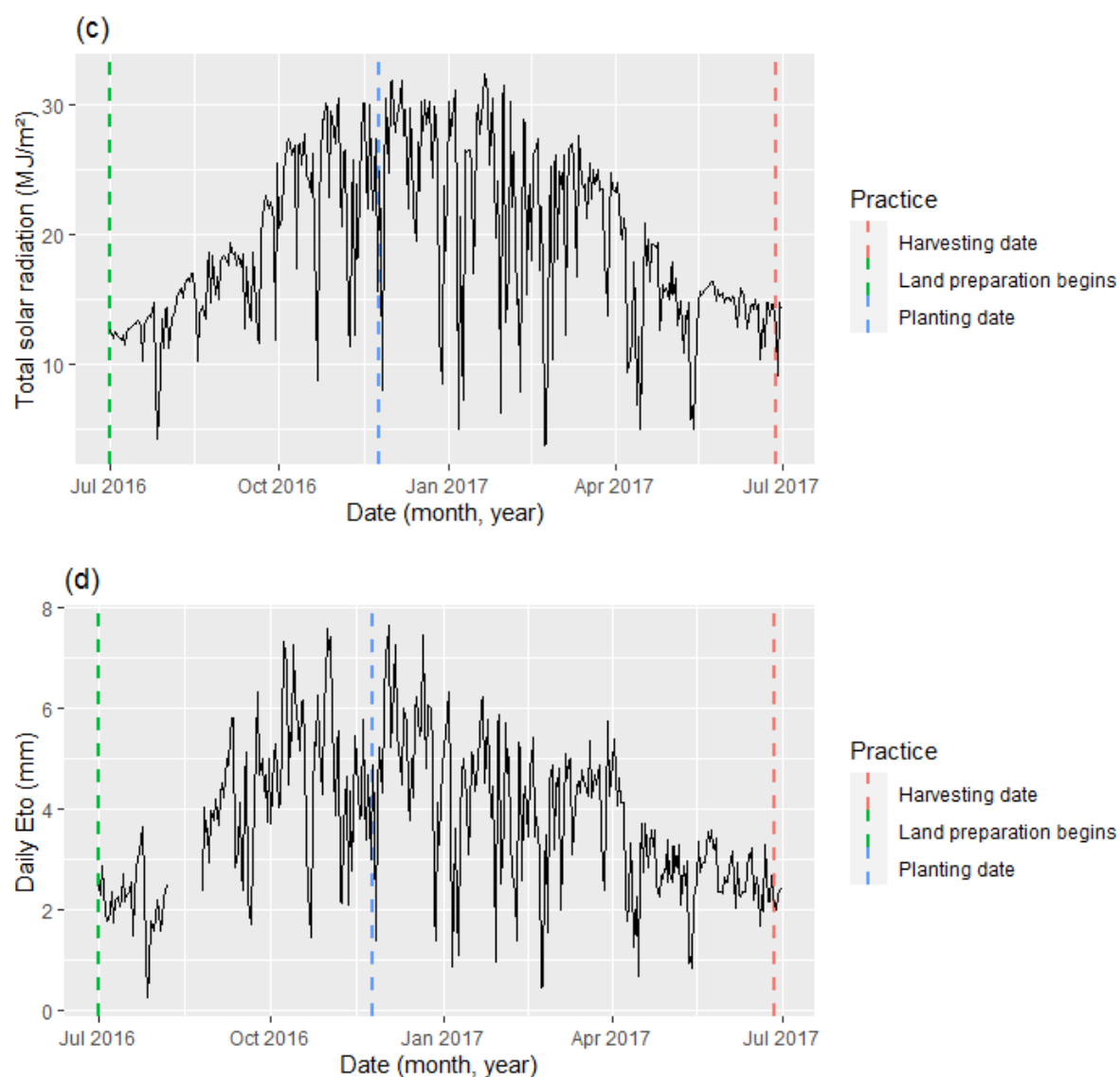


Figure S3.1B Climate conditions in the EFS field experiment. (c) Total solar radiation (MJ/m^2) and (d) evaporative demand (Eto), from planting to harvesting. Planting date – 26/11/2016 and harvesting date – 27/07/2017.

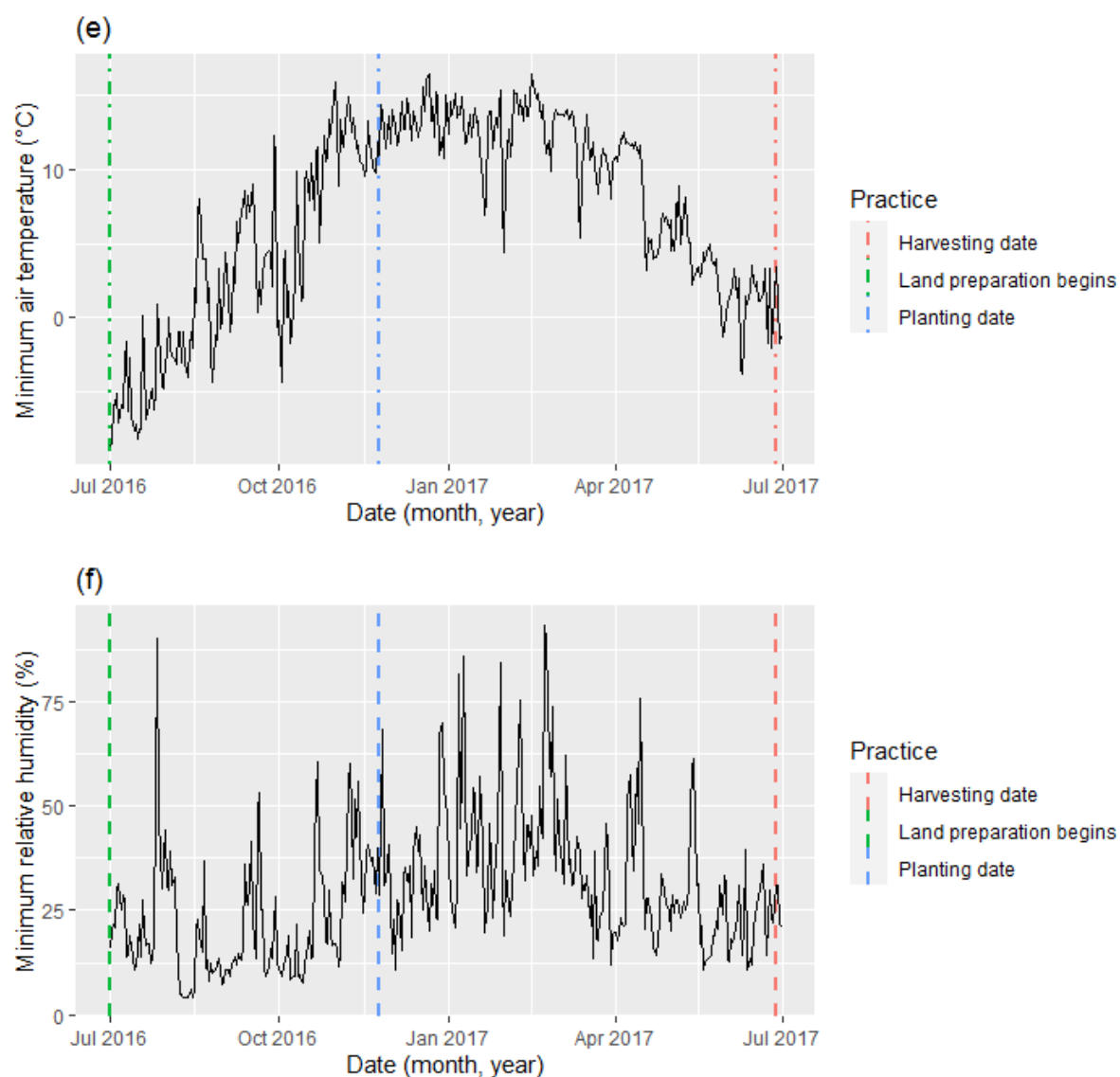


Figure S3.1C Climate conditions in the EFS field experiment. (e) Minimum air temperature (°C) and (f) minimum relative humidity (%), from planting to harvesting. Planting date – 26/11/2016 and harvesting date – 27/07/2017.

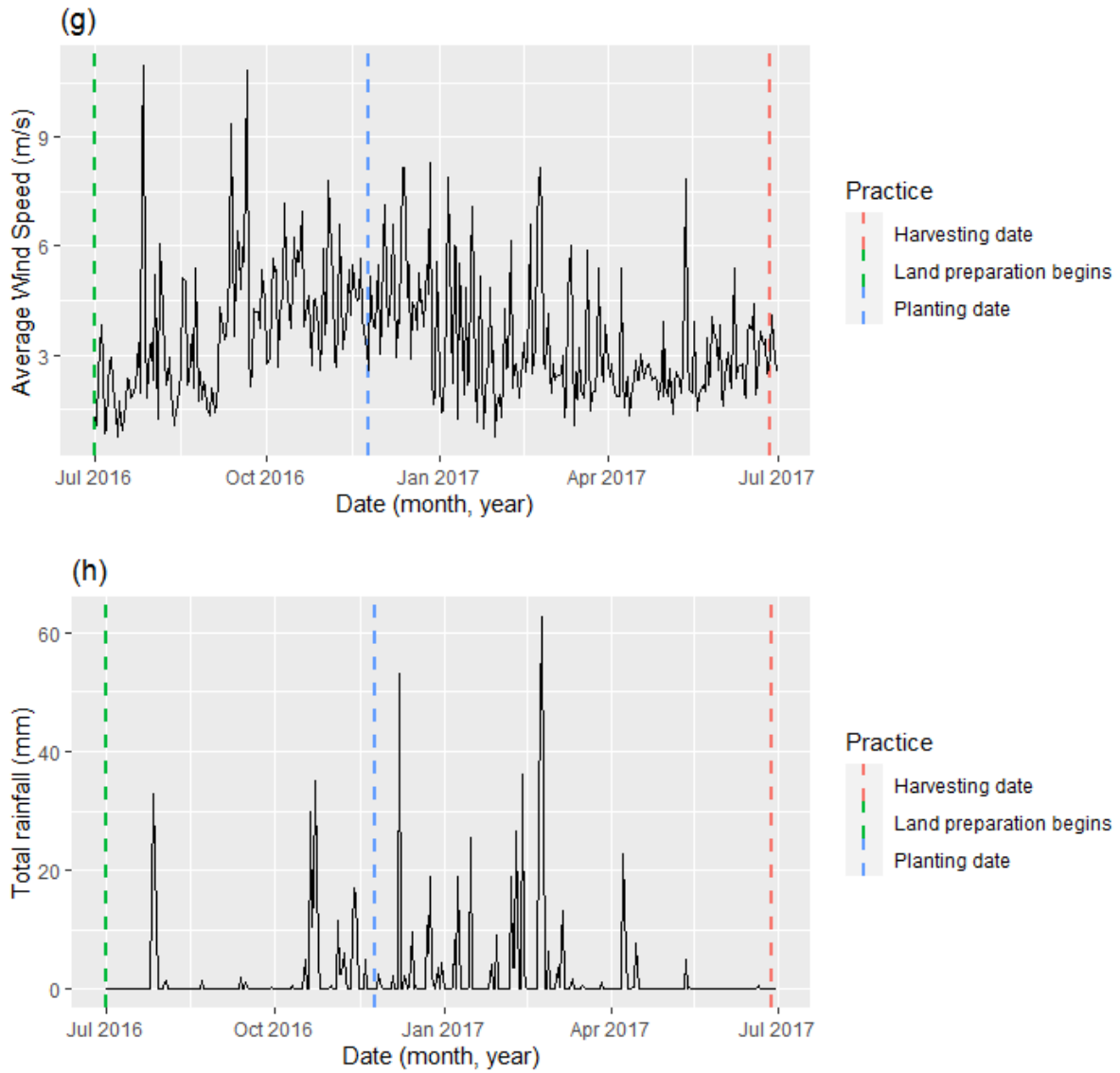


Figure S3.1D Climate conditions in the EFS field experiment. (g) Average wind speed (m/s) and (h) total rainfall (mm), from planting to harvesting. Planting date – 26/11/2016 and harvesting date – 27/07/2017.

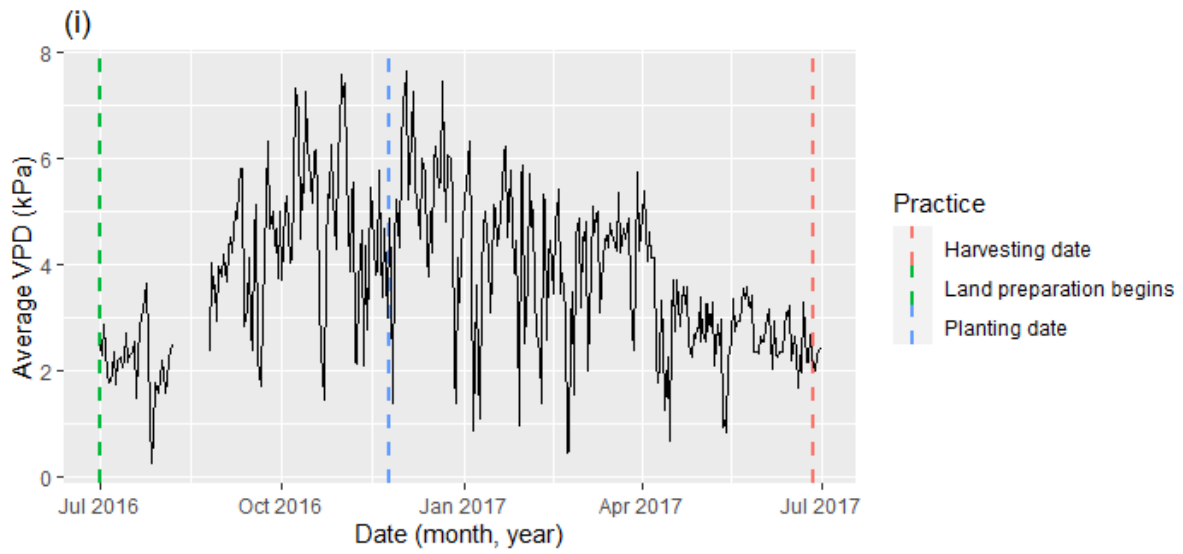


Figure S3.1E Climate conditions in the EFS field experiment. (i) Average vapour pressure deficit (kPa) from planting to harvesting. Planting date – 26/11/2016 and harvesting date – 27/07/2017.

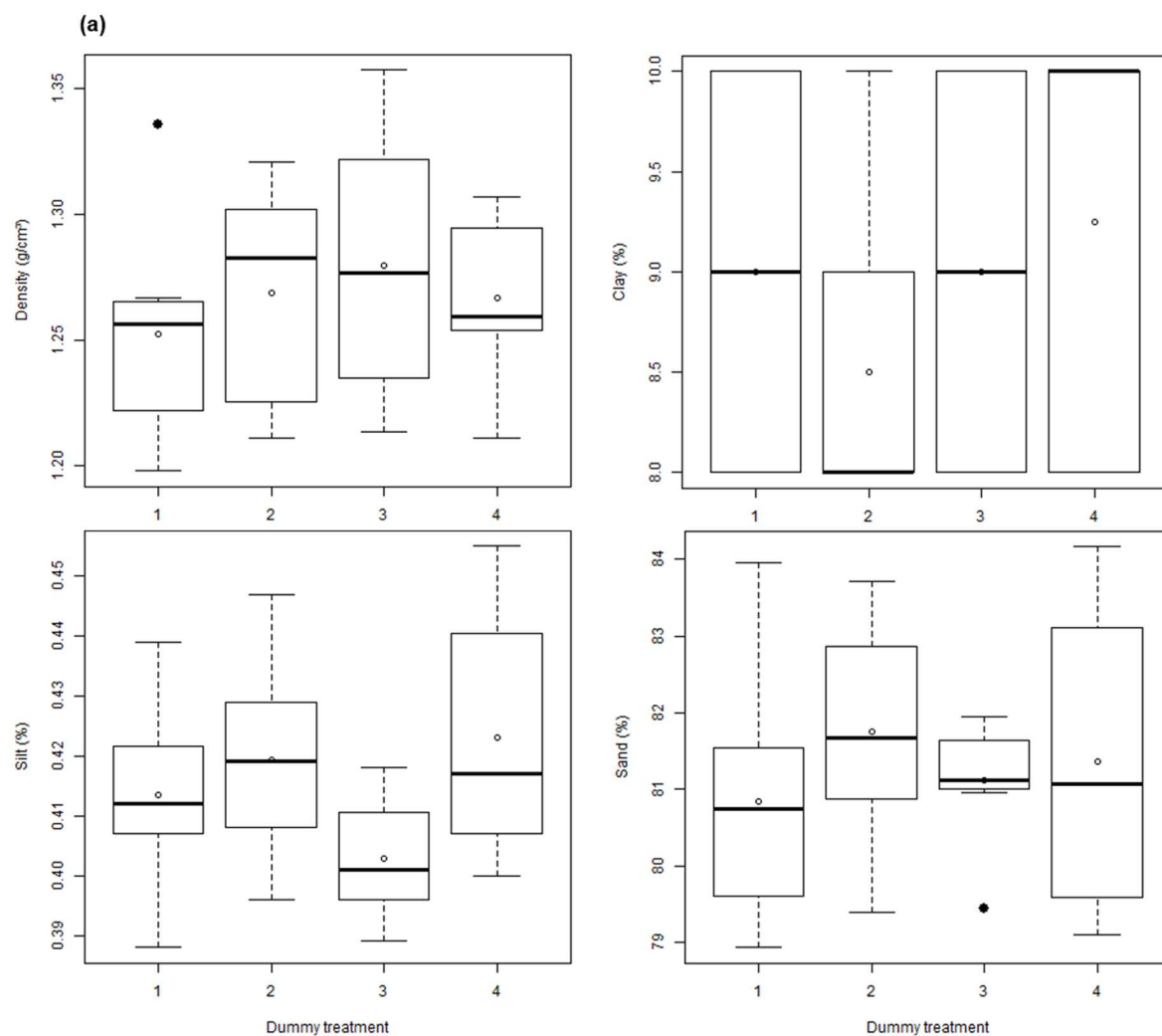


Figure S3.2A The box and whisker plots illustrating uniformity in soil physical properties across dummy treatments of the randomized complete block designed field in a dryland system of South Africa. Each dummy treatment had 8 replications. Horizontal heavy bar = median, box=lower and upper quartiles, whiskers=minimum and maximum values, open dot=mean, filled dot=outliers.

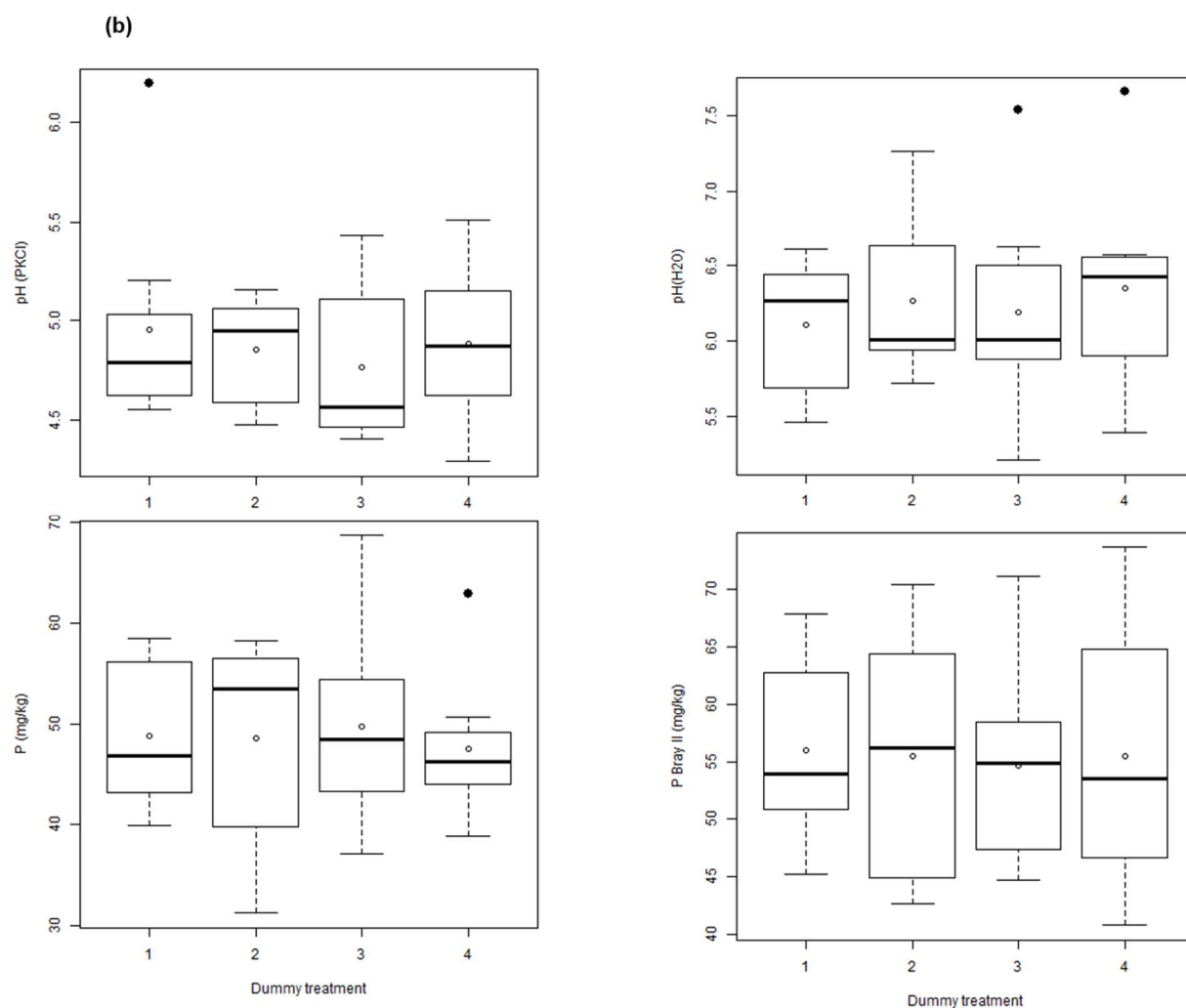


Figure S3.2B The box and whisker plots illustrating uniformity in soil chemical properties across dummy treatments of the randomized complete block designed field in a dryland system of South Africa. Each dummy treatment had 8 replications. Horizontal heavy bar = median, box=lower and upper quartiles, whiskers=minimum and maximum values, open dot=mean, filled dot=outliers.

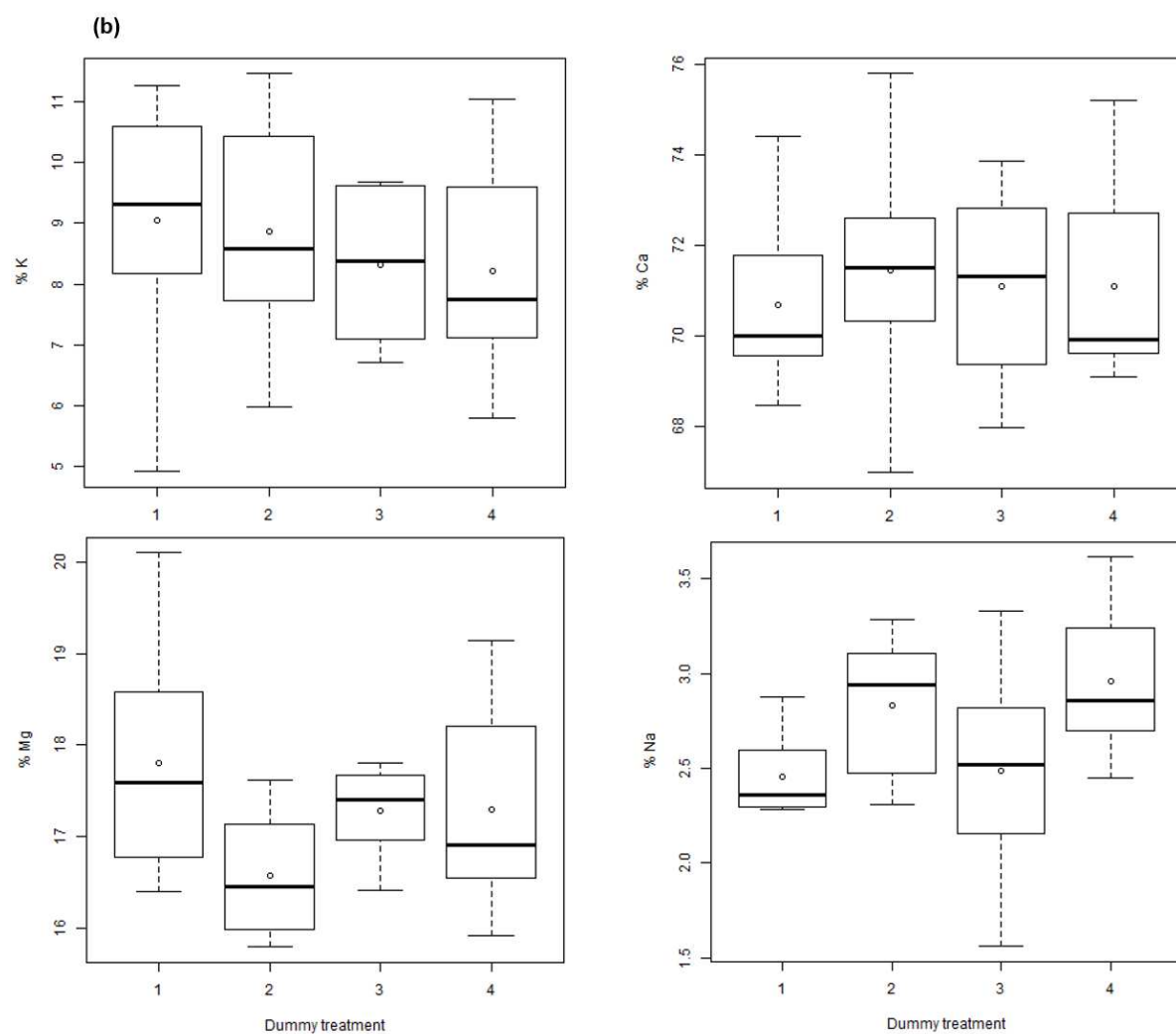


Figure S3.2B (continued) The box and whisker plots illustrating uniformity in soil chemical properties across dummy treatments of the randomized complete block designed field in a dryland system of South Africa. Each dummy treatment had 8 replications. Horizontal heavy bar = median, box=lower and upper quartiles, whiskers=minimum and maximum values, open dot=mean, filled dot=outliers.

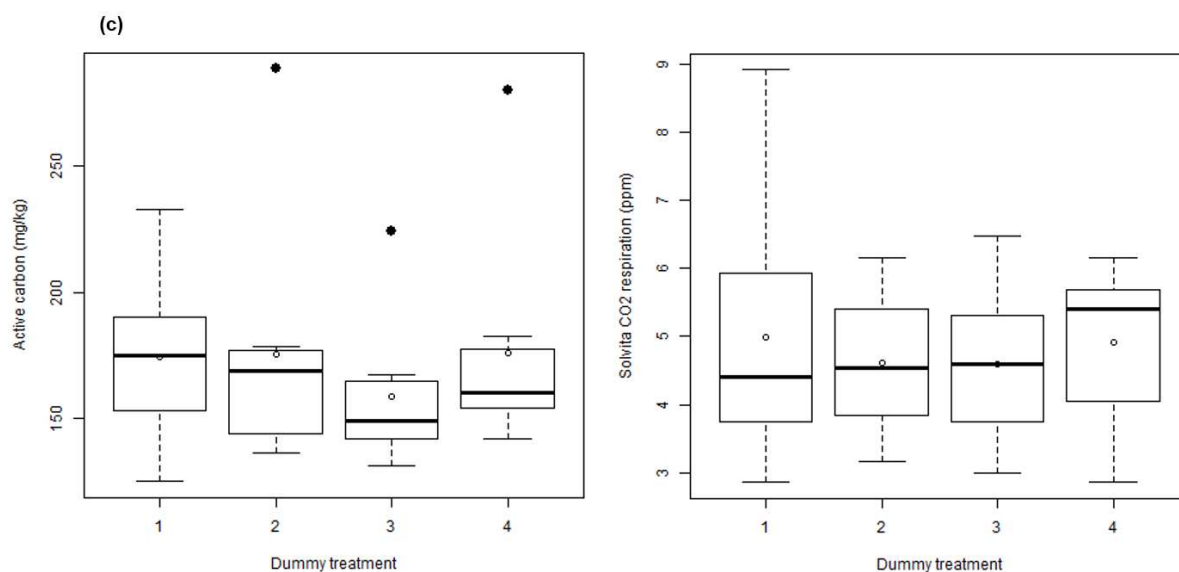


Figure S3.2C The box and whisker plots illustrating uniformity in soil biological properties across dummy treatments of the randomized complete block designed field in a dryland system of South Africa. Each dummy treatment had 8 replications. Horizontal heavy bar = median, box=lower and upper quartiles, whiskers=minimum and maximum values, open dot=mean, filled dot=outliers.

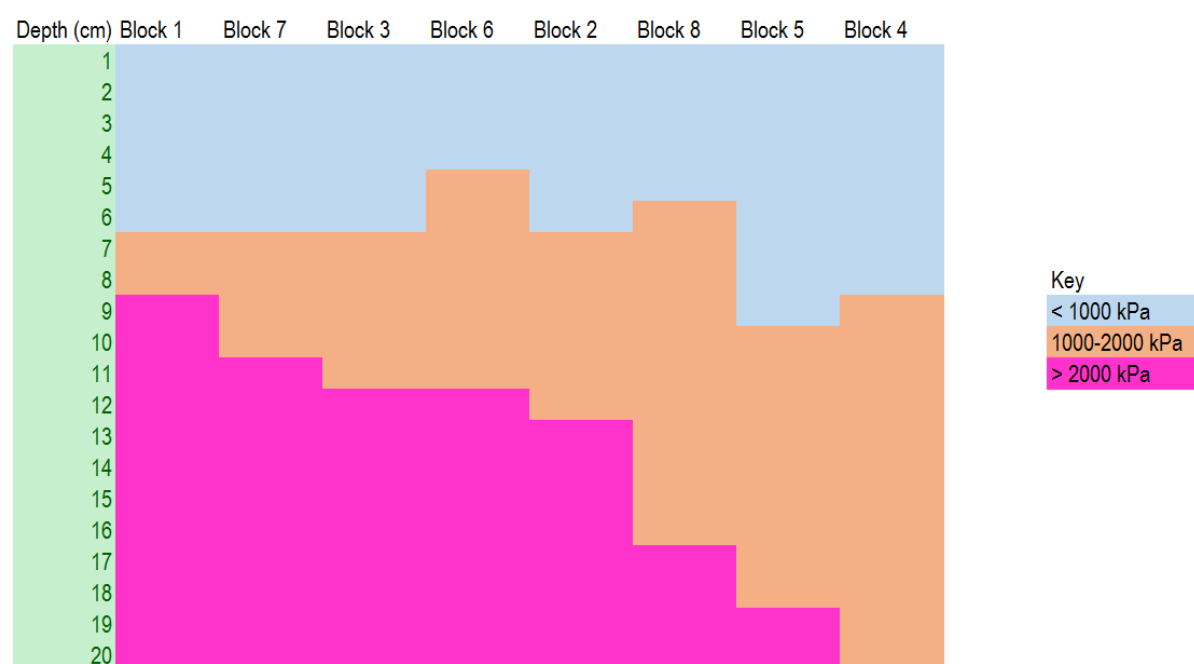


Figure S3.2D (i) Soil penetration resistance across blocks between rows.

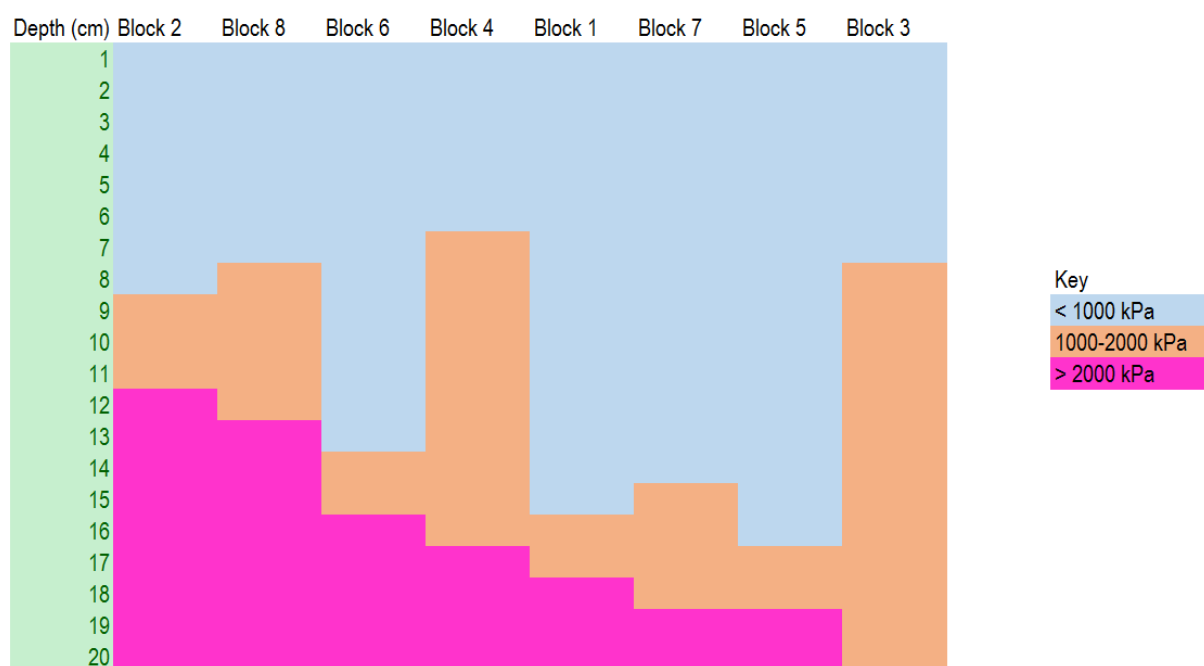


Figure S3.2D (ii) Soil penetration resistance across blocks in rows.

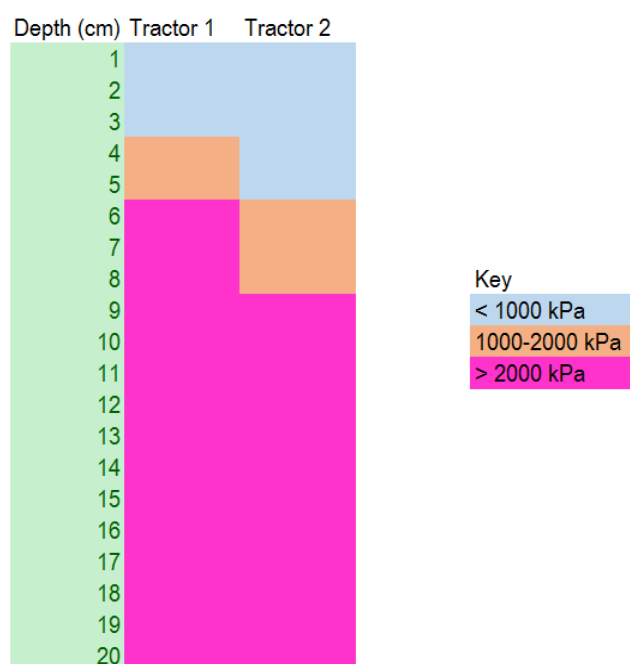


Figure S3.2D (iii) Soil penetration resistance across blocks between tractor rows.

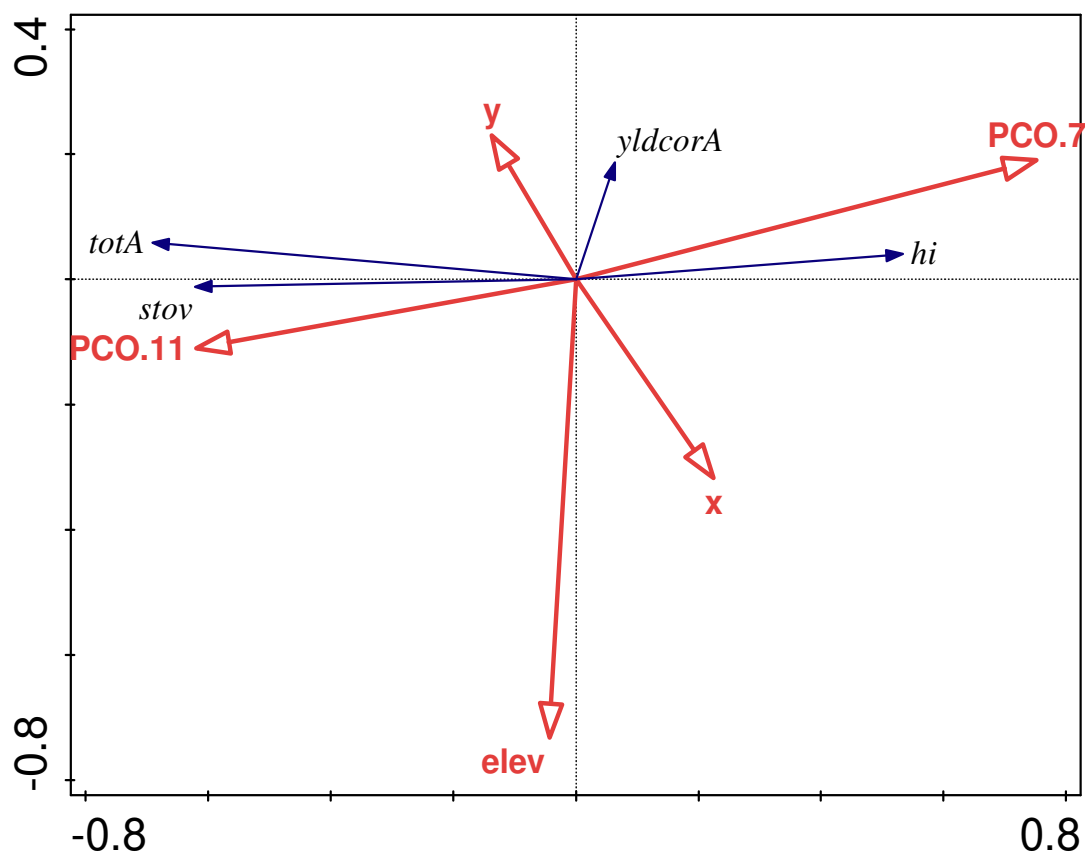


Figure S3.3 Biplot diagram of spatial variation of yield parameters. The harvest index (*hi*) and yield corrected for moisture (*yldcorA*) were both correlated with the PCO.7 spatial eigenfunction while the total plant biomass (*totA*) and stover (*stov*) were correlated with PCO.11. Arrows show variable direction of the change.

Supplementary Tables

Table S3.1A Analysis of variance table for harvest Index (%) using adjusted sum of squares (Sum Sq) for tests. d.f. degrees of freedom, mean squares (Mean Sq).

Source	d.f.	Sum Sq	Mean Sq	F-ratio	<i>p-value</i>
“Dummy” treatment	3	172.45	57.482	1.1996	0.328
Residuals	28	1341.72	47.918		

Table S3.1B Analysis of variance table for total plant biomass yield (kg/ha) using adjusted sum of squares (Sum Sq) for tests. d.f. degrees of freedom, mean squares (Mean Sq).

Source	d.f.	Sum Sq	Mean Sq	F-ratio	<i>p-value</i>
“Dummy” treatment	3	7723450	2574483	1.1138	0.3601
Residuals	28	64720806	2311457		

Table S3.1C Analysis of variance table for stover plant biomass yield (kg/ha) using adjusted sum of squares (Sum Sq) for tests. d.f. degrees of freedom, mean squares (Mean Sq).

Source	d.f.	Sum Sq	Mean Sq	F-ratio	<i>p-value</i>
“Dummy” treatment	3	10502410	3500803	1.1469	0.3474
Residuals	28	85463718	3052276		

Table S3.2A Perfect orthogonality in the randomized complete block design using dummy treatments and total plant biomass yield (kg/ha). Sequential sum of squares (Seq SS), adjusted sum of squares (Adj SS), d.f. degrees of freedom, mean squares (Mean Sq).

Response	d.f	Seq SS	Adj SS	MS	F-ratio	<i>p-value</i>
Block	7	9754028	9754028	1393433	0.5324	0.8002
Dummy treatment	3	7723450	7723450	2574483	0.9836	0.4194
Residuals	21	54966778	54966778	2617466		

Table S3.2B Perfect orthogonality in the randomized complete block design using dummy treatments and stover plant biomass yield (kg/ha). Sequential sum of squares (Seq SS), adjusted sum of squares (Adj SS), d.f. degrees of freedom, mean squares (Mean Sq).

Response	d.f	Seq SS	Adj SS	MS	F-ratio	<i>p-value</i>
Block	7	1622297 1	16222971	2317567	0.7029	0.6696
Dummy treatment	3	1050241 0	10502410	3500803	1.0618	0.3864
Residuals	21	6924074 7	69240747	3297178		

Table S3.2C Perfect orthogonality in the randomized complete block design using dummy treatments and harvest index (%). Sequential sum of squares (Seq SS), adjusted sum of squares (Adj SS), d.f. degrees of freedom, mean squares (Mean Sq).

Response	d.f	Seq SS	Adj SS	MS	F-ratio	<i>p-value</i>
Block	7	362.73	362.73	51.819	1.1116	0.3923
Dummy treatment	3	172.45	172.45	57.482	1.233	0.3226
Residuals	21	978.98	978.98	46.618		

Table S3.3 Soil properties per block (Mean±SE). N = 4 composite soil samples, SE represent standard error of the mean.

Soil property	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Block 7	Block 8
Physical								
Clay (%)	8± 0	8.5± 0.5	9.5± 0.5	8± 0	9.5± 0.5	9.5± 0.5	9± 0.58	9.5± 0.5
Silt (%)	10.8± 0.93	9.02± 0.49	9.21± 0.84	9.16± 0.71	10.3± 0.24	10.5± 0.07	10.6± 0.77	8.89± 0.36
Sand (%)	81.2± 0.93	82.5± 0.68	81.3± 0.59	82.8± 0.71	80.3± 0.54	80± 0.55	80.4± 0.53	81.6± 0.30
Density (g/cm³)	1.22 ±0.001	1.26 ±0.006	1.22 ±0.010	1.26 ±0.023	1.26 ±0.013	1.30 ±0.02	1.32 ±0.01	1.29 ±0.01
Biological								
Active carbon (mg/kg)	193± 33.3	142± 7.12	162± 6.07	157± 8.91	163± 12.8	215± 24.5	183± 17.6	153± 8.32
Solventa CO ₂ respiration (ppm)	4.87± 0.58	4.61± 0.40	4.96± 0.55	4.49± 0.35	3.58± 0.38	3.83± 0.78	5.80± 0.26	6.08± 0.99
Chemical								
pH(H ₂ O)	6.18± 0.26	6.2± 0.18	6.54± 0.29	6.05± 0.13	6.03± 0.12	6.03± 0.29	5.96± 0.27	6.86± 0.50
pH(KCl)	4.72± 0.13	5.15± 0.10	5.22± 0.16	5.24± 0.36	4.55± 0.05	4.63± 0.09	4.83± 0.07	4.57± 0.15
P Bray II (mg/kg)	48.5± 2.5	52.1± 2.48	50.3± 6.77	53.2± 1.31	58.2± 5.68	58.7± 4.25	53.9± 3.38	68.5± 2.31
P (mg/kg)	45.1± 2.24	48.3± 2.94	43.6± 4.94	46.7± 3.49	46.7± 5.34	52.5± 2.5	46.5± 3.28	60.6± 3.49
K (mg/kg)	108± 6.17	88.8± 6.41	82.3± 7.15	69.8± 9.18	102± 4.63	97.5± 5.63	86± 4.95	62.1± 3.29
Na (mg/kg)	16.2± 1.22	17.4± 1.04	14.6± 1.75	14.4± 2.04	16.4± 1.05	17.1± 0.73	17.3± 1.29	14.2± 1.85
Ca (mg/kg)	384± 14.60	412± 23.1	403± 18.9	389± 53.3	330± 12.7	376± 11.9	350± 7.98	314± 13.8
Mg (mg/kg)	60.5± 3.24	58.8± 4.31	58± 2.75	55.6± 8.7	49.2± 2.1	55± 4	54.3± 2.73	46.8± 3.47

Table S3.3 Soil properties per block (Mean±SE). N = 4 composite soil samples, SE represent standard error of the mean.

Soil property	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Block 7	Block 8
<i>Chemical</i>								
OUT H+Al cmol(+)/kg	0.0128 ±0.01	7.95E-08 ±4.0E-10	8.17E-08 ±6.7E-10	7.96E-08 ±1.5E-9	0.022 ±0.013	0.00663 ±0.0066	7.59E-08 ±5.3E-10	0.0283 ±0.02
%Ca	69.3± 0.49	72.4± 0.23	72.9± 1.19	73.5± 1.07	68.5± 0.61	70.7± 0.66	70.3± 0.31	71.3± 0.8
%Mg	17.8± 0.32	16.9± 0.26	17.2± 0.58	17.1± 0.45	16.7± 0.29	16.9± 0.58	17.8± 0.48	17.4± 0.93
%K	9.93± 0.21	7.98± 0.36	7.62± 0.65	7.07± 1.32	10.9± 0.40	9.35± 0.23	8.87± 0.67	7.19± 0.18
%Na	2.53± 0.14	2.67± 0.16	2.3± 0.26	2.37± 0.09	2.98± 0.24	2.79± 0.12	3.01± 0.16	2.8± 0.33
%Acid.V	0.431± 0.43	2.82E- 6± 1.54E-7	2.97E-6± 1.41E-7	3.13E-6± 3.05E-8	0.958± 0.57	0.26± 0.26	3.05E-6± 4.85E-8	1.36± 0.83
Ca:Mg (1.5-4.5)	3.89± 0.08	4.29± 0.07	4.26± 0.21	4.3± 0.10	4.1± 0.07	4.2± 0.17	3.95± 0.10	4.14± 0.22
(Ca+Mg)/K (10.0-20.0)	8.79± 0.18	11.3± 0.56	12.1± 1.18	14.1± 2.30	7.89± 0.34	9.38± 0.27	10.1± 0.79	12.3± 0.31
Mg:K (3.0-4.0)	1.8± 0.03	2.13± 0.12	2.29± 0.14	2.66± 0.46	1.55± 0.09	1.81± 0.05	2.05± 0.20	2.41± 0.09
Na:K	0.256± 0.02	0.339± 0.03	0.307± 0.04	0.363± 0.06	0.277± 0.03	0.3± 0.02	0.348± 0.04	0.39± 0.05
S-Value (cmol+)/kg	2.76± 0.12	2.85± 0.17	2.77± 0.12	2.64± 0.34	2.39± 0.08	2.65± 0.11	2.49± 0.06	2.18± 0.10

Table S3.3 Soil properties per block (Mean±SE). N = 4 composite soil samples, SE represent standard error of the mean.

Soil property	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Block 7	Block 8
<i>Chemical</i>								
CEC (cmol(+)/kg)	2.48± 0.12	2.18± 0.04	2.16± 0.04	2.41± 0.19	2.42± 0.07	2.35± 0.16	2.21± 0.20	1.94± 0.052
N - NH ₄ (mg/l)	0.414± 0.012	0.41± 0.003	0.42± 0.010	0.413± 0.005	0.431± 0.010	0.411± 0.006	0.412± 0.008	0.405± 0.012
N - NO ₃ (mg/l)	1.86± 0.08	1.63± 0.08	1.36± 0.18	1.47± 0.20	1.5± 0.11	1.39± 0.18	1.56± 0.20	1.53± 0.08
Al (mg/kg)	303± 21.8	282± 17.5	274± 10.3	268± 33.5	257± 16.4	283± 6.51	246± 12.7	281± 12.3
T (cmol(+)/kg)	2.78± 0.12	2.85± 0.17	2.77± 0.12	2.64± 0.34	2.41± 0.07	2.66± 0.10	2.49± 0.06	2.2± 0.09
Fe (mg/kg)	74.2± 6.33	65.9± 5.23	61.8± 3.65	76.9± 9.15	59.9± 6.15	66.9± 1.89	59.3± 5.54	63.4± 3.88
Mn (mg/kg)	26± 1.78	20.2± 1.44	18.8± 0.98	20.4± 2.54	17.9± 1.19	20± 1.4	16.2± 1.5	16.4± 1.17
Cu (mg/kg)	1.43± 0.08	1.36± 0.06	1.2± 0.04	1.18± 0.07	1.27± 0.03	1.43± 0.05	1.28± 0.03	1.18± 0.03
Zn (mg/kg)	2.46± 0.24	2.33± 0.09	2.66± 0.38	2.7± 0.23	2.29± 0.32	2.37± 0.24	2.41± 0.25	2.66± 0.06
S (mg/kg)	8.23± 0.79	8.3± 0.70	6.47± 0.92	11± 4.13	7.16± 0.35	8.28± 0.61	7.53± 0.19	6.78± 0.90
B (mg/kg)	0.449± 0.183	0.207± 0.011	0.211± 0.011	0.272± 0.091	0.175± 0.009	0.234± 0.027	0.179± 0.007	0.168± 0.002

Table S3.4A PCA of uniformity trial data Analysis 'Unconstrained' Method: PCA Total variation is 25.95349.

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.2701	0.2088	0.1611	0.0835
Explained variation (cumulative)	27.01	47.89	64	72.35

Table S3.4B Simple (marginal) effects of explanatory variables.

Name	Explains %	pseudo- <i>F</i>	<i>P</i>
%Acid.V	25.7	10.4	0.001
OUT H+Al (cmol+)/kg)	25.2	10.1	0.001
(Ca+Mg)/K (10.0-20.0)	20.5	7.7	0.001
%K	20.4	7.7	0.001
Mg:K (3.0-4.0)	19.9	7.5	0.001
K (mg/kg)	19.8	7.4	0.001
S-Value (cmol+)/kg)	18.8	7	0.001
pH(KCl)	18.7	6.9	0.001
Ca (mg/kg)	18.7	6.9	0.001
Mg (mg/kg)	18.1	6.6	0.001
T (cmol+)/kg)	18	6.6	0.001
%Ca	17.2	6.2	0.001
Na:K	16	5.7	0.001
S (mg/kg)	14.6	5.1	0.001
Mn (mg/kg)	13	4.5	0.001
Cu (mg/kg)	12.8	4.4	0.001
Na (mg/kg)	11.3	3.8	0.001
P Bray II (mg/kg)	10.6	3.6	0.001
%Na	10.3	3.4	0.001
B (mg/kg)	9.6	3.2	0.024
Fe (mg/kg)	9.5	3.2	0.004
P (mg/kg)	9.2	3	0.007
CEC (cmol+)/kg)	8.7	2.9	0.007
Silt (%)	8.7	2.9	0.007
Solvita CO ₂ respiration (ppm)	8.7	2.8	0.009
Sand (%)	8.3	2.7	0.009
Al (mg/kg)	8.1	2.6	0.011
Zn (mg/kg)	7.8	2.6	0.014
Active carbon (mg/kg)	7.3	2.4	0.033
%Mg	6.7	2.2	0.049
pH(H ₂ O)	6.7	2.2	0.046
Density (g/cm ³)	5.3	1.7	0.098
Ca:Mg (1.5-4.5)	5.2	1.6	0.118
N - NO ₃ (mg/l)	4.7	1.5	0.161
Clay (%)	4	1.3	0.26
N - NH ₄ (mg/l)	2.4	0.7	0.683

Table S3.4C Conditional effects of explanatory variables. %acid is the most significant factor for yield and its parameters, followed by %K and S.

Name	Explains %	pseudo-<i>F</i>	<i>P</i>
%Acid.V	25.71	10.4	0.001
%K	20.16	10.8	0.001
S (mg/kg)	14.19	10	0.001
Mn (mg/kg)	8.16	6.9	0.001
P (mg/kg)	7.35	7.8	0.001
Solvita CO ₂ respiration (ppm)	5.87	7.9	0.001
K (mg/kg)	3.92	6.4	0.001
Active carbon (mg/kg)	3.86	8.2	0.001
Na (mg/kg)	1.97	4.9	0.001
N - NO ₃ (mg/l)	1.67	4.9	0.001
Silt (%)	1.46	5.1	0.001
Zn (mg/kg)	1.2	5.1	0.001
Fe (mg/kg)	1.2	6.5	0.001
Mg:K (3.0-4.0)	0.99	7.3	0.001
Clay (%)	0.71	7.2	0.002
pH(H ₂ O)	0.51	7.1	0.001
B (mg/kg)	0.34	6.4	0.001
Al (mg/kg)	0.32	9.8	0.001
CEC (cmol+)/kg)	0.2	10.7	0.001
P Bray II (mg/kg)	0.17	37.6	0.001
pH(KCl)	0.02	9.2	0.083
Cu (mg/kg)	0.01	10.4	0.165
Ca:Mg (1.5-4.5)	<0.01	7.5	0.19

Table S3.5 Method: RDA Analysis Total variation is 128.0, explanatory variables account for 61.00% (adjusted explained variation is 0.00%).

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.485	0.1227	0.0023	0
Explained variation (cumulative)	48.5	60.77	61	61
Pseudo-canonical correlation	0.7812	0.7784	0.8994	0.7919
Explained fitted variation (cumulative)	79.51	99.62	100	100

Table S3.6 The significant axes of constrained and unconstrained ordination applied to the same response yield parameters data. The efficiency of the first constrained axis is 60.25% of the first unconstrained axis

Statistic	1	2	3	4
Explained by constrained axis [%]	48.5	12.27	0.23	0
Explained by unconstrained axis [%]*	80.5	19.21	0.28	0
Efficiency of constrained axis [%]	60.25	63.84	81.64	64.87

* Optional coloring of second row reflects comparison of unconstrained axes with broken stick model.

Table S3.7 Canonical variate analysis in discriminant analysis.

Statistic	Ax 1	Ax 2	Ax 3	Ax 4	Ax 5	Ax 6	Ax 7
Eigenvalue	276.12	47.42	32.53	9.01	4.86	1.77	1.01
Percent variance	74.1	86.8	95.5	98	99.3	99.7	100

Table S3.8 Canonical variate analysis in discriminant analysis to discriminant functions. N = 32 composite soil samples, SE represent standard error of the mean.

Predictors	Mean±SE	c1	c2	c3	c4	c5	c6	c7
Density (g/cm ³)	1.267± 0.01	27.2117	41.5166	116.159	41.1653	-17.703	-28.0822	-2.99145
Active carbon (mg/kg)	170.933± 6.76	0.069111	-0.0186	-0.03184	-0.01641	0.00897	5.73E-05	-0.02453
Solvita CO ₂ respiration (ppm)	4.77656± 0.23	0.089641	1.45745	1.05482	1.17205	-0.02967	-0.16748	0.355937
pH(H ₂ O)	6.23078± 0.10	1.10118	-2.09225	3.8533	0.630787	0.616574	-2.06511	-0.78826
pH(KCl)	4.86384± 0.07	-66.9	7.08418	-12.8049	-4.61676	13.0308	3.54507	2.98182
P Bray II (mg/kg)	55.415± 1.64	2.42549	1.78454	0.100944	0.692934	0.466281	-0.02339	0.196489
P (mg/kg)	48.74± 1.46	-3.93842	-2.00863	-0.02851	-0.98348	-0.17834	0.105635	-0.20135
K (mg/kg)	87.0714± 3.25	-0.08503	-0.40095	0.641531	0.196447	-0.24174	-0.13716	0.086886
Na (mg/kg)	15.9558± 0.50	-3.99569	-2.98591	4.0406	2.36835	-0.65696	-1.0506	0.348669
Ca (mg/kg)	369.834± 9.44	0.961061	-0.39682	0.68874	0.207083	-0.50171	-0.37759	0.071922
Mg (mg/kg)	54.7783± 1.57	-1.04113	-1.42436	1.85242	0.428021	-0.37005	-0.75374	-0.12705
S-Value (cmol(+)/kg)	2.59023± 0.06	0	0	0	0	0	0	0
T (cmol(+)/kg)	2.59895± 0.06	-80.0406	94.0234	-149.628	-43.5529	77.2084	72.2681	-13.3339
Fe (mg/kg)	66.0536± 2.06	0.795772	0.055993	0.961215	0.720685	-0.171	-0.1177	0.043645
Mn (mg/kg)	19.4946± 0.72	0.420422	-1.09885	-0.07991	0.808308	0.289648	-0.02349	0.09252
Cu (mg/kg)	1.28958± 0.02	139.645	66.7811	-68.5583	-58.4785	25.9083	10.2343	2.98625
Zn (mg/kg)	2.4873± 0.08	5.08945	-0.326	-0.72232	1.545	-0.4468	-0.76865	-0.35336
S (mg/kg)	7.96441± 0.55	3.3088	3.16143	-2.54209	-1.3185	0.027011	0.737955	0.158657
B (mg/kg)	0.23684± 0.03	-38.6819	-30.9598	0.974561	0.649614	0.603237	-3.11992	-6.58501
Al (mg/kg)	274.147± 6.36	-0.14564	0.233505	-0.14297	0.018147	0.026654	-0.00875	0.012494
CEC (cmol(+)/kg)	2.27002± 0.05	18.5814	2.33973	4.12729	2.92153	4.36486	-3.72842	0.617257
1:2 WATER EXTRACT N - NH ₄ (mg/l)	0.414656± 0.00297	-455.978	-340.734	-88.4928	-115.547	-79.3969	56.6034	-64.2034
1:2 WATER EXTRACT N - NO ₃ (mg/l)	1.53731± 0.05	-22.0797	-1.03327	-3.19831	-3.54934	5.9351	-1.14029	3.06641

Table S3.9 Class centroids

Class	Ax 1	Ax 2	Ax 3	Ax 4	Ax 5	Ax 6	Ax 7
Block 2	-8.35093	1.42899	-4.71954	-3.82185	-0.1393	-0.48889	2.07339
Block 3	-16.1529	-5.67043	-7.32973	-0.88525	-2.80984	-0.2403	-1.41354
Block 4	6.52259	9.05567	-3.24899	5.98301	-1.7042	0.072578	0.468455
Block 5	3.15967	-6.23264	3.93708	0.082684	-0.4531	3.11674	0.359698
Block 6	33.414	5.23959	1.33257	-3.73482	-0.5969	-0.33477	-0.80233
Block 7	-10.1355	-2.91674	11.9662	0.729185	-1.42678	-1.65141	0.160126
Block 8	-21.1218	9.24085	1.41362	-0.76435	3.51683	0.527864	-0.87117
Block 1	12.6649	-10.1453	-3.35118	2.41139	3.61329	-1.00182	0.025361

Table S3.10 The single summary approach ‘one plot-one-datapoint’ dataset for the legacy in for *F. graminearum* pathogen DNA quantities. As each plot is a separate datapoint, there is no need for the variable *Plot*. Na – not quantifiable.

Dummy treatment	Pre-plant soil DNA quantity ng/μl	Post-harvest soil DNA quantity ng/μl
D1	0.016	Na
D1	0.020	Na
D1	0.025	Na
D1	0.017	Na
D2	0.013	Na
D2	0.030	Na
D2	0.017	Na
D2	0.022	Na
D3	0.009	Na
D3	0.010	Na
D3	0.005	Na
D3	0.004	Na
D4	0.003	Na
D4	0.030	Na
D4	0.079	Na
D4	0.001	Na

Table S3.11A Analysis of variance table for soybean total plant biomass (g/plant) using adjusted sum of squares (Sum Sq) for tests. d.f. degrees of freedom, mean squares (Mean Sq).

Source	d.f.	Sum Sq	Mean Sq	F-ratio	<i>p-value</i>
Blocks	7	0.36517	0.052167	1.7814	0.1376
Residuals	24	0.70282	0.029284		

Table S3.11B Analysis of variance table for maize total plant biomass (g/plant) using adjusted sum of squares (Sum Sq) for tests. d.f. degrees of freedom, mean squares (Mean Sq).

Source	d.f.	Sum Sq	Mean Sq	F-ratio	<i>p-value</i>
Blocks	7	0.14265	0.020378	1.6577	0.1674
Residuals	24	0.29502	0.012293		

Table S3.11C Analysis of variance table for sugar bean total plant biomass (g/plant) using adjusted sum of squares (Sum Sq) for tests. d.f. degrees of freedom, mean squares (Mean Sq).

Source	d.f.	Sum Sq	Mean Sq	F-ratio	<i>p-value</i>
Blocks	7	0.20379	0.029112	0.7557	0.6288
Residuals	24	0.92460	0.038525		

Table S3.11D Analysis of variance table for sunflower total plant biomass (g/plant) using adjusted sum of squares (Sum Sq) for tests. d.f. degrees of freedom, mean squares (Mean Sq).

Source	d.f.	Sum Sq	Mean Sq	F-ratio	<i>p-value</i>
Blocks	7	0.050847	0.0072638	1.1235	0.3812
Residuals	24	0.155175	0.0064656		

Table S3.12 Significance tests for variation partitioning with spatial predictors using PCNM.

Tested fraction	F-statistics	<i>P-value</i>
$a + b + c$	5	0.002
$a + c$	5.3	0.007
$b + c$	5.5	0.003

Supplementary Material for Chapter 4

Supplementary figures

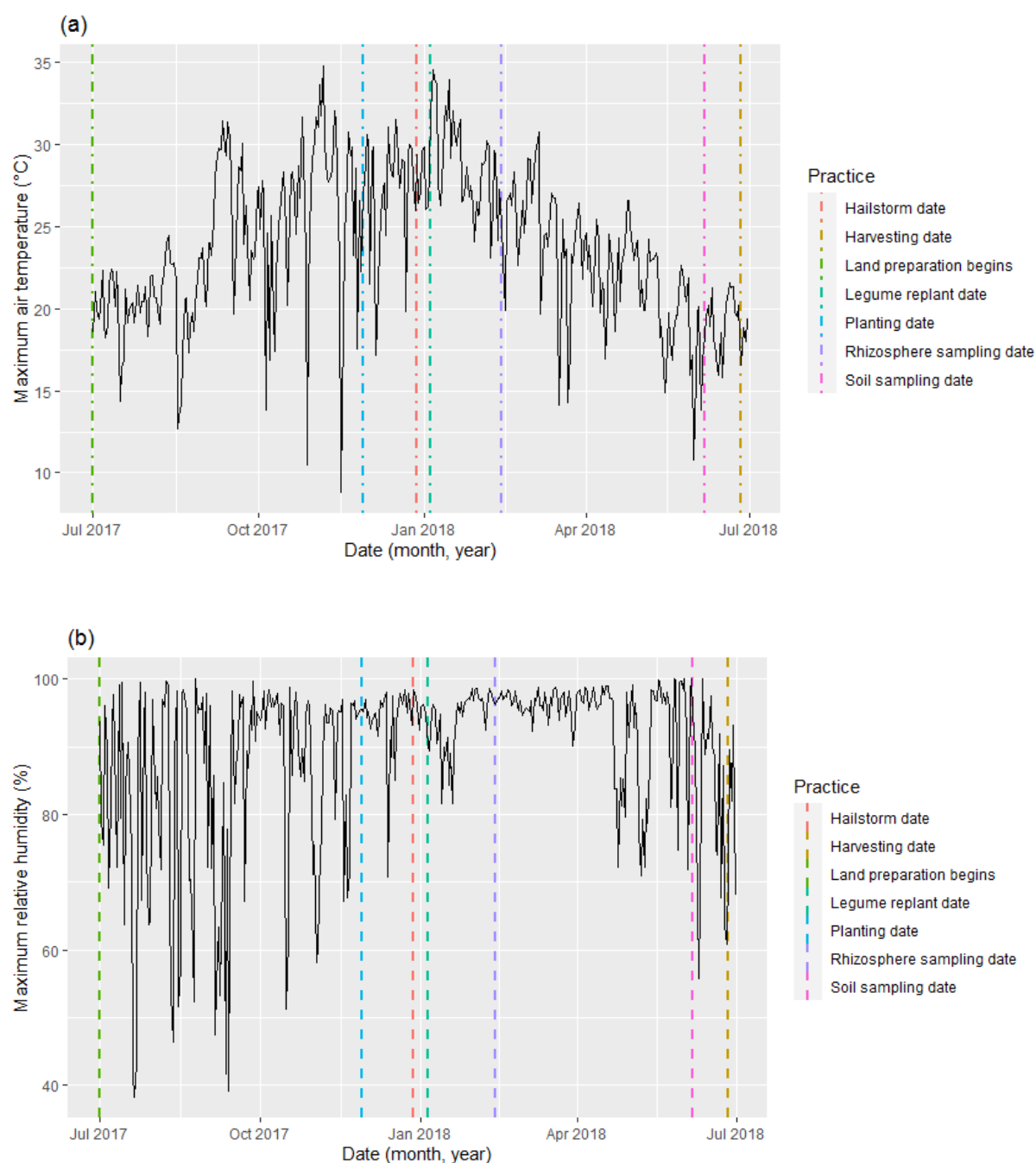


Figure S4.1A Climate conditions in the EFS field experiment. (a) Maximum air temperature (°C) and (b) maximum relative humidity (%) from planting to harvesting. Land preparation begins (1/7/2017), Planting date (28/11/2017), Hailstorm date (28/12/2017), Legume replant date (5/1/2018), Rhizosphere sampling date (13/02/2018), Soil sampling date (6/6/2018), and Harvesting date (26/06/2018).

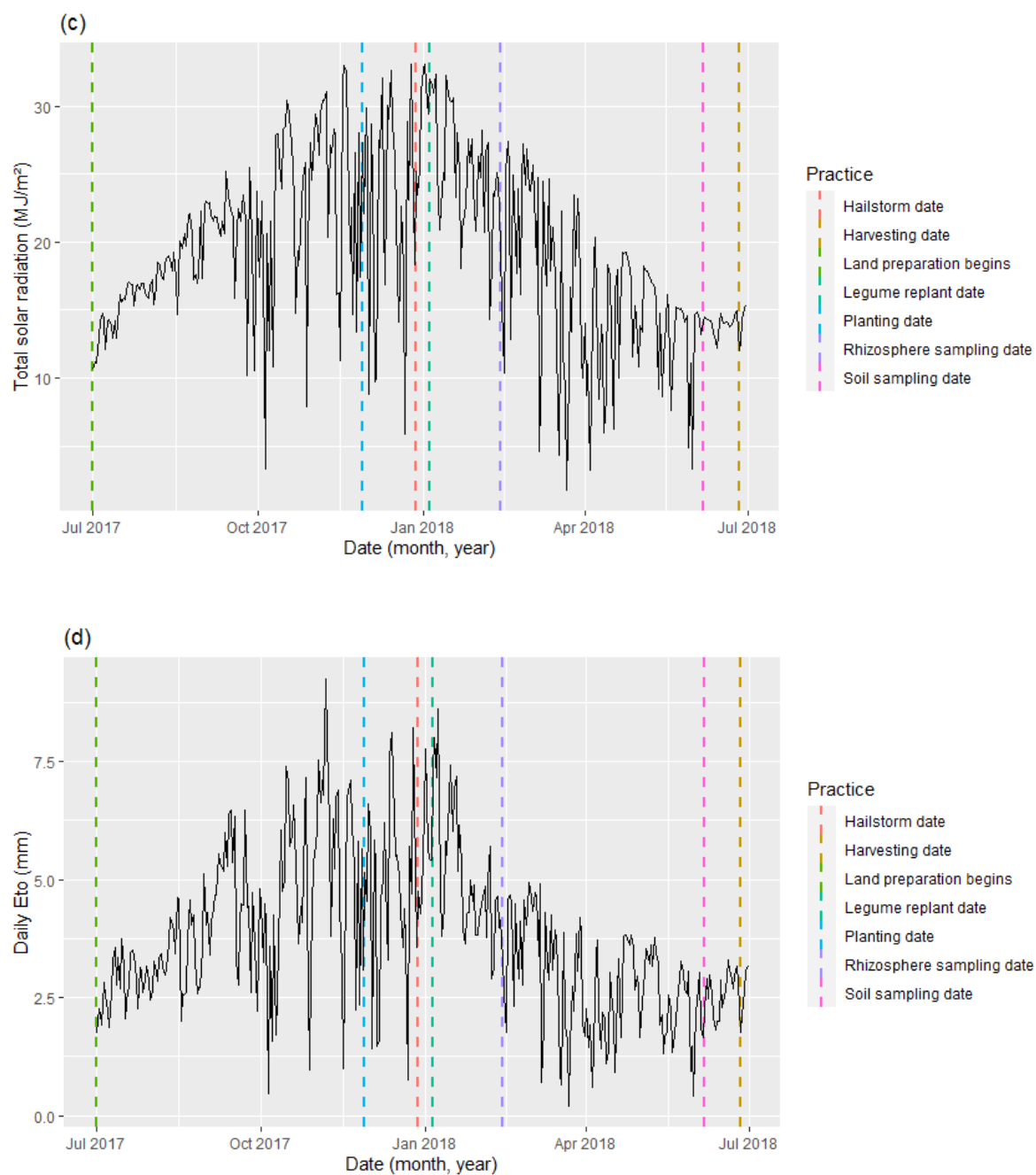


Figure S4.1B Climate conditions in the EFS field experiment. (c) Total solar radiation (MJ/m^2) and (d) evaporative demand (Eto) from planting to harvesting. Land preparation begins (1/7/2017), Planting date (28/11/2017), Hailstorm date (28/12/2017), Legume replant date (5/1/2018), Rhizosphere sampling date (13/02/2018), Soil sampling date (6/6/2018), and Harvesting date (26/06/2018).

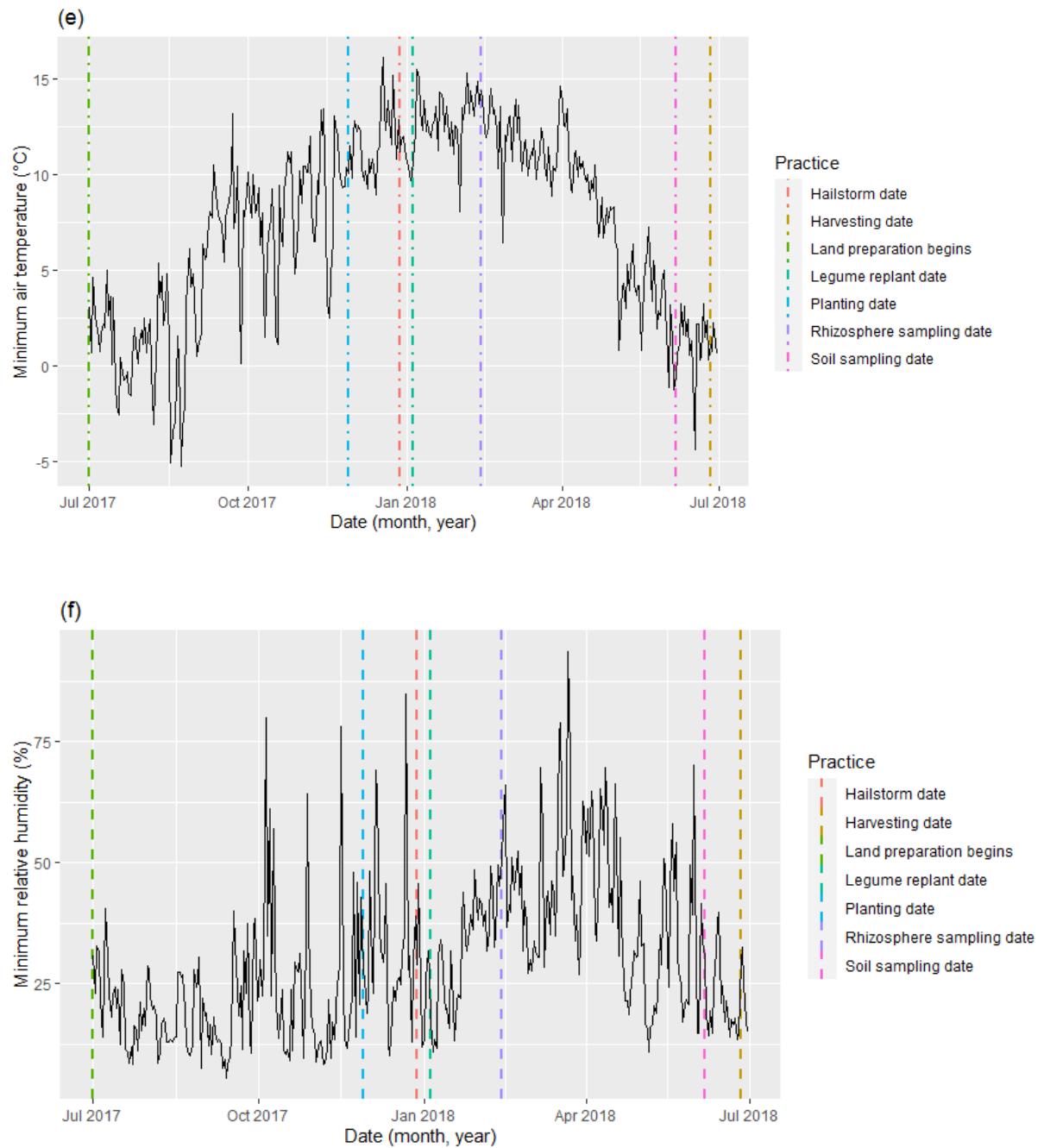


Figure S4.1C Climate conditions in the EFS field experiment. (e) Minimum air temperature (°C) and (f) minimum relative humidity (%) from planting to harvesting. Land preparation begins (1/7/2017), Planting date (28/11/2017), Hailstorm date (28/12/2017), Legume replant date (5/1/2018), Rhizosphere sampling date (13/02/2018), Soil sampling date (6/6/2018), and Harvesting date (26/06/2018).

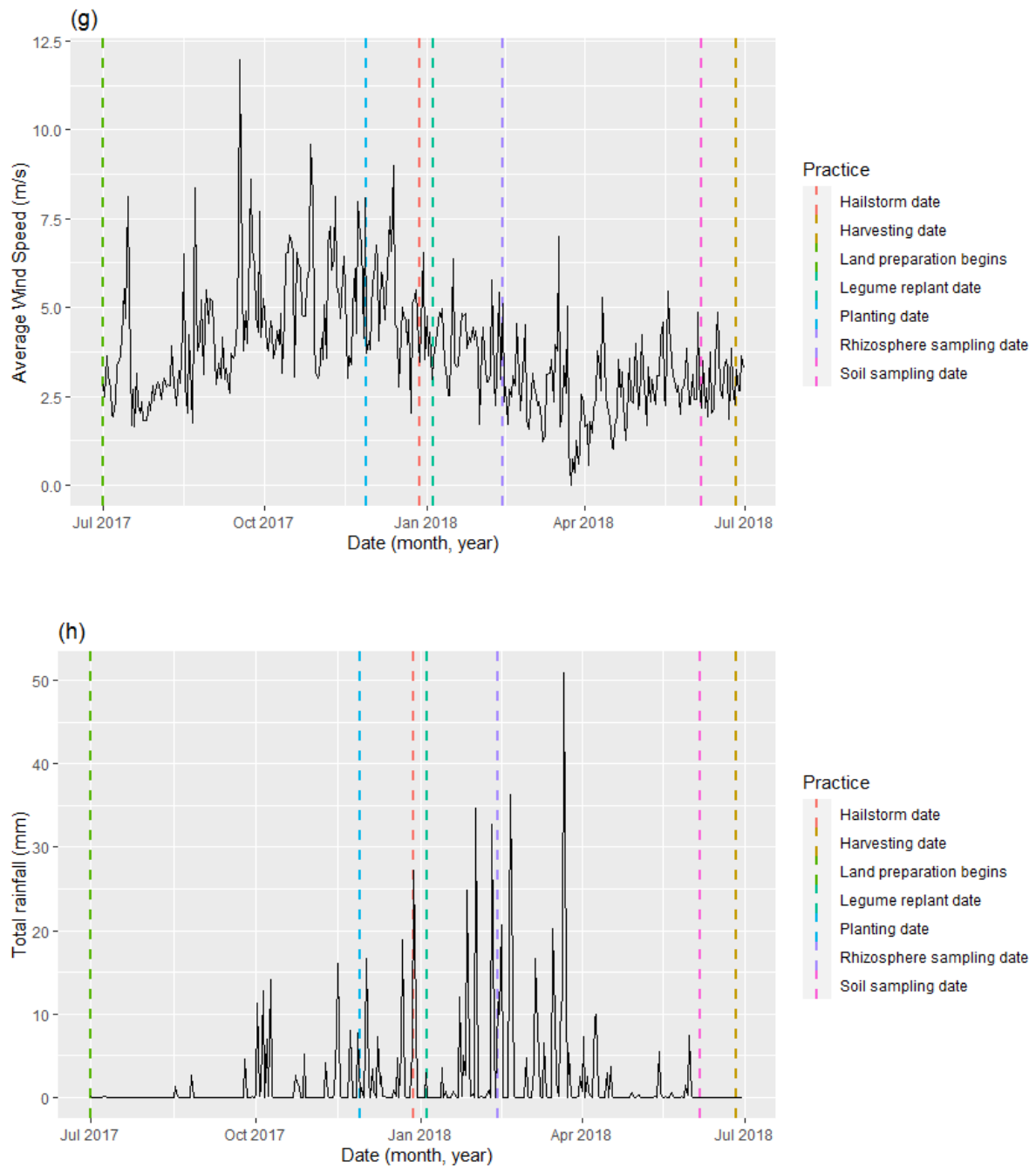


Figure S4.1D Climate conditions in the EFS field experiment. (g) Average wind speed (m/s) and (h) total rainfall (mm) from planting to harvesting. Land preparation begins (1/7/2017), Planting date (28/11/2017), Hailstorm date (28/12/2017), Legume replant date (5/1/2018), Rhizosphere sampling date (13/02/2018), Soil sampling date (6/6/2018), and Harvesting date (26/06/2018).

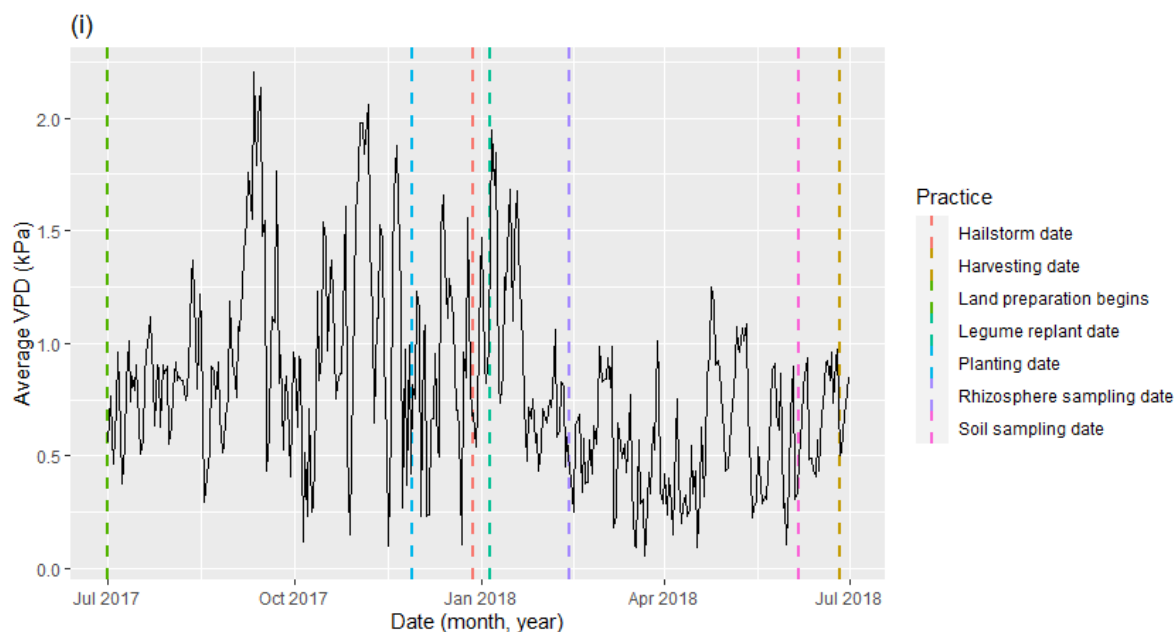


Figure S4.1E Climate conditions in the EFS field experiment. (i) Average vapour pressure deficit (kPa) from planting to harvesting. Land preparation begins (1/7/2017), Planting date (28/11/2017), Hailstorm date (28/12/2017), Legume replant date (5/1/2018), Rhizosphere sampling date (13/02/2018), Soil sampling date (6/6/2018), and Harvesting date (26/06/2018).

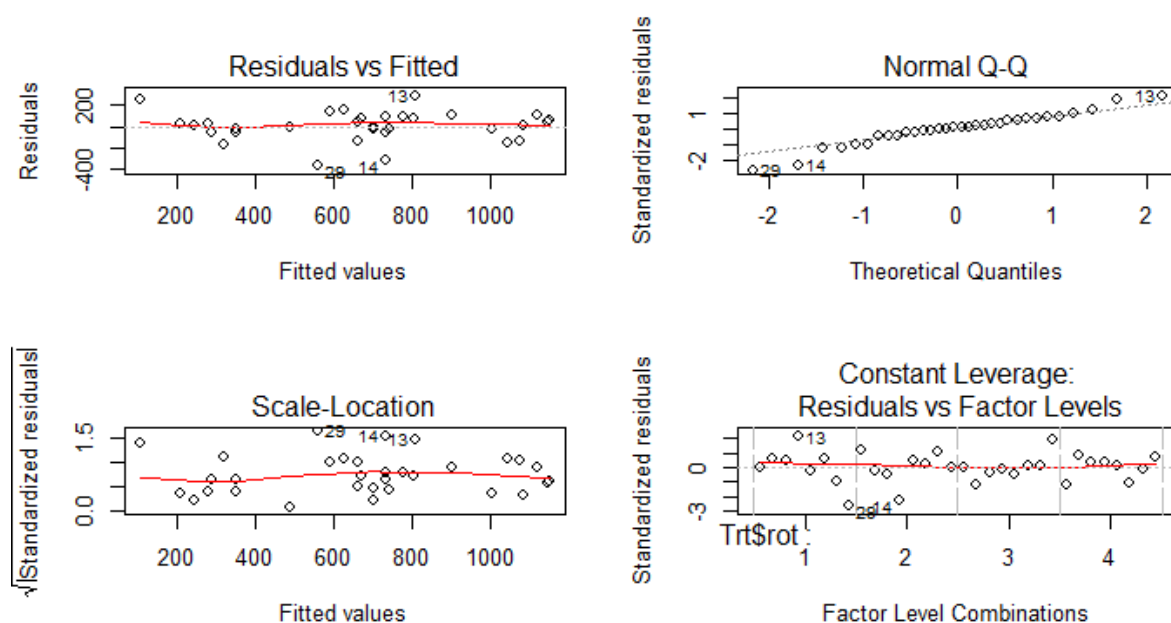


Figure S4.2A Diagnostic residual plots for the four dryland crop yield data following a normal distribution and homogeneity of variance.

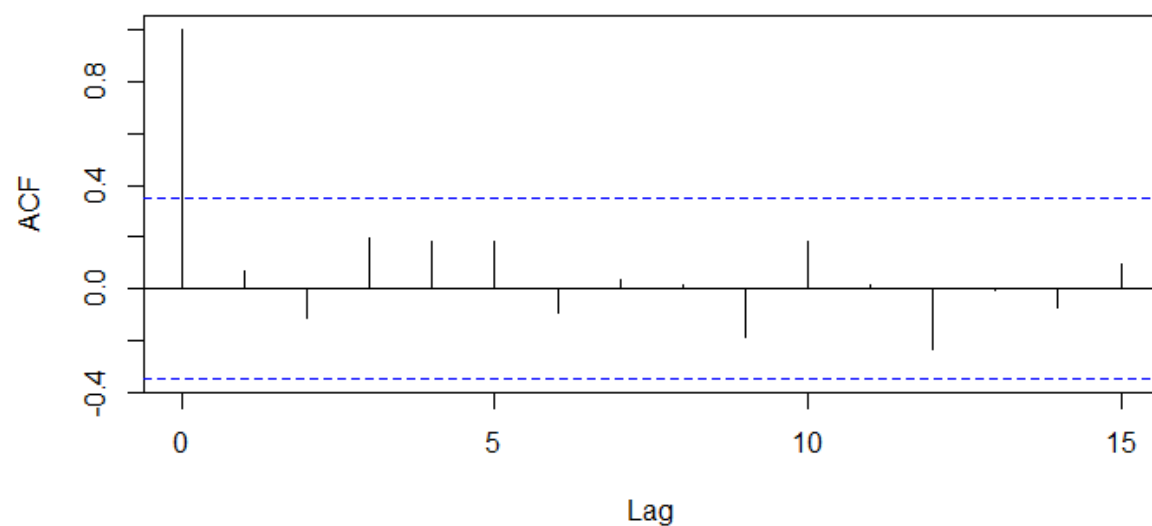


Figure S4.2B Diagnostic autocorrelation function (ACF) plots indicating independence of the crop yield data following a normal distribution and homogeneity of variance.

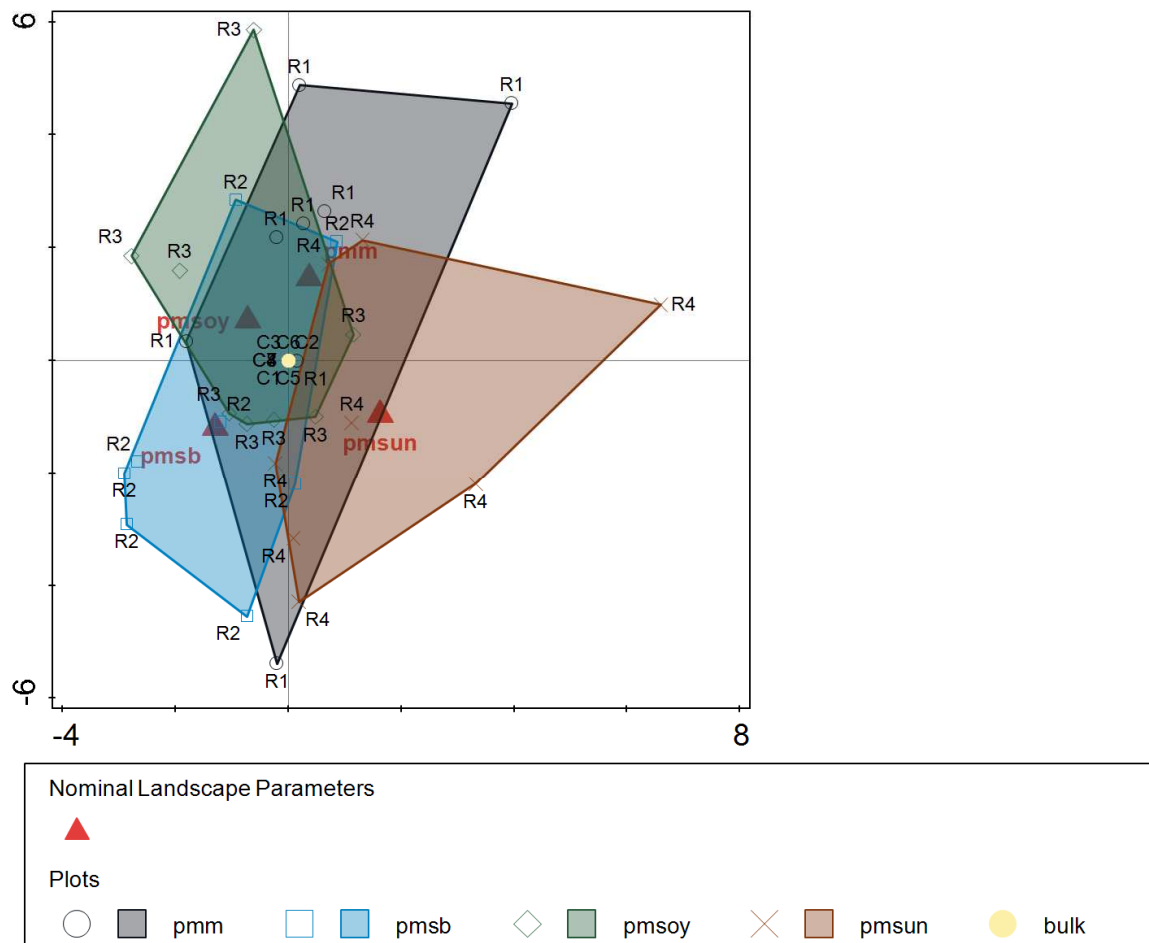


Figure S4.3 The RDA biplot of dissimilarity between the crop species of that plot and average plant health variable composition of the individual classes. Symbols of explanatory treatment and block parameter classes with plot symbols. Score scaling is focused on plant health variable scores (standardized) and not focused on the Euclidean distances between plots. R1R1 to R8R4 represents 32 ‘field plots’.

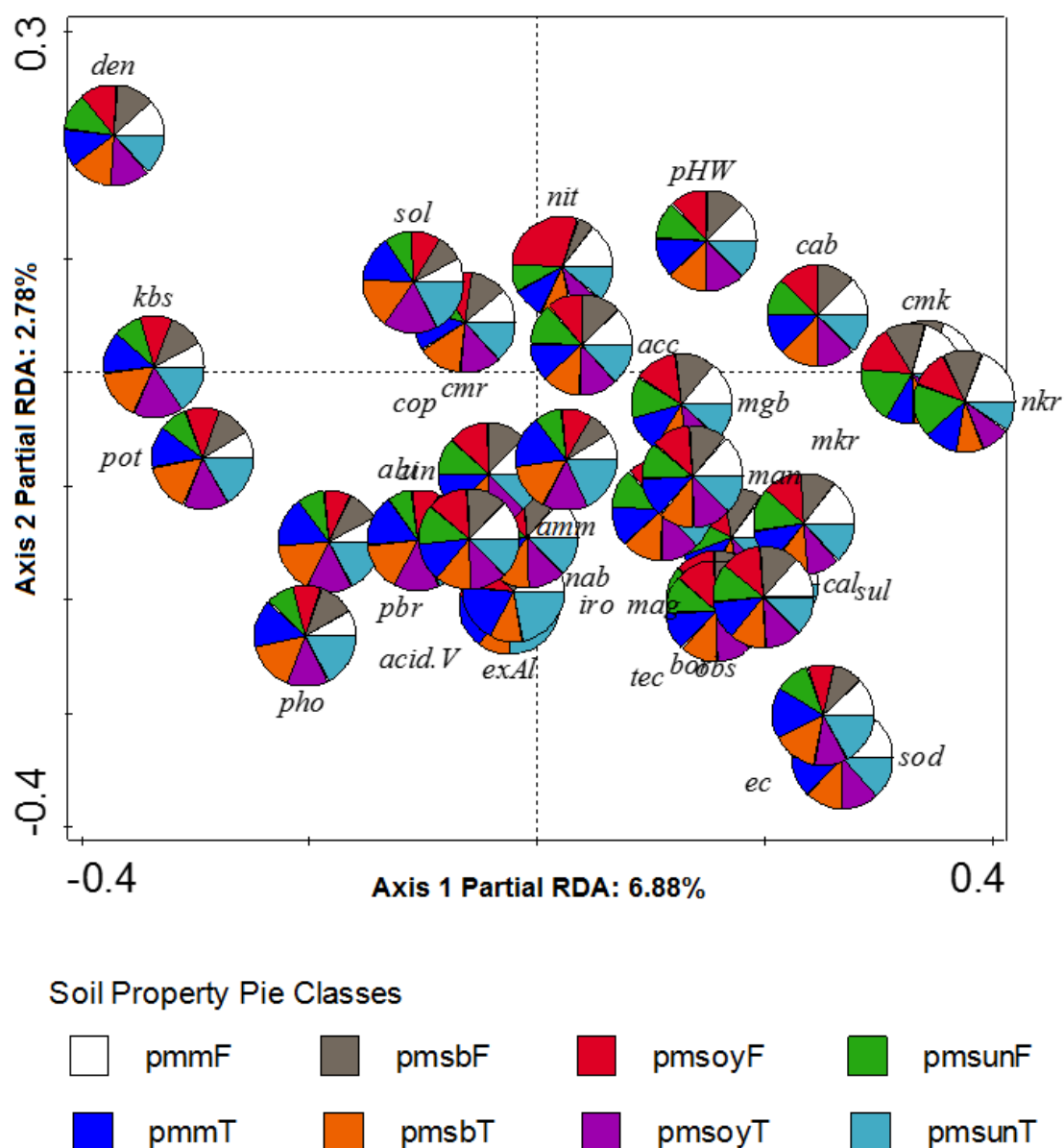


Figure S4.4 The partial redundancy analysis (RDA) biplot displaying the variation in legacies of soil health properties explained by soil depth (20cm-T and 40cm-F), after accounting for the effects of covariates (soil depth and blocks).

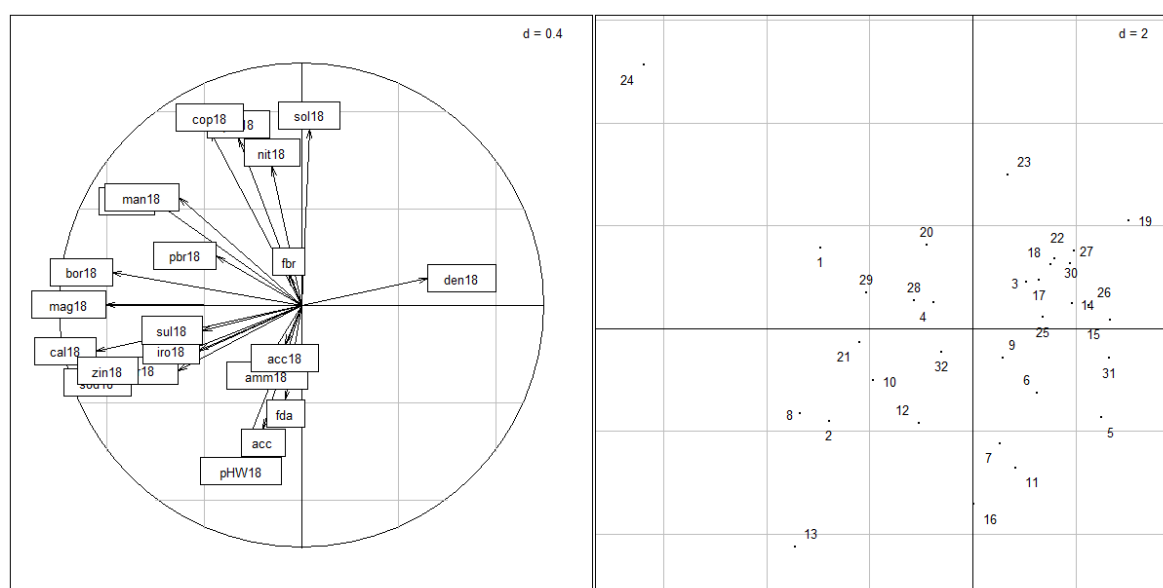


Figure S4.5 Normed Principal Component Analysis (PCA) of PLFA data for bacterial and fungal PLFA biomass and marker ratios (red arrows) associated with season 2018 soil health legacies (blue arrows) with Axis 1 PC: 22.146% and Axis 2 PC: 16.077% of explained variation. Four plant health properties include; Gram-positive bacteria (Gp) to Gram-negative bacteria (Gn) ratio (Gpnr), fungal: bacterial ratio (fbr) of PLFA markers, total fungal PLFA (tf), total bacterial PLFA (tb), acc - Active carbon (mg/kg), and fda - enzyme activity in FDA hydrolysed ($\mu\text{g}/\text{mg}$ soil). The whole numbers from 1 to 32 (left graph) represent the plot sites cultivated with *Z. mays* (1, 5, 9, 13, 17, 21, 25, 29), *P. vulgaris* (2, 6, 10, 14, 18, 22, 26, 30), *G. max* (3, 7, 11, 15, 19, 23, 27, 31) and *H. annuus* (4, 8, 12, 16, 20, 24, 28, 32) in season 2018. The blue arrows represent the soil health legacies for season 2018 where:

acc18 - Active carbon (mg/kg)	pHW18 - pH (H_2O)	man18 - Mn (mg/kg)
amm18 - 1:2 Water extract N - NH_4 (mg/l)	sod18 - Na (mg/kg)	bor18 - B (mg/kg)
sol18 - Solvita CO_2 respiration (ppm)	cop18 - Cu (mg/kg)	cal18 - Ca (mg/kg)
nit18 - 1:2 Water extract N - NO_3 (mg/l)	pot18 - K (mg/kg)	iro18 - Fe (mg/kg)
pbr18 - P Bray 2 (mg/kg)	zin18 - Zn (mg/kg)	mag18 - Mg (mg/kg)
den18 - Density (g/cm^3)	pho18 - P (mg/kg)	pHK18 - pH (KCl)

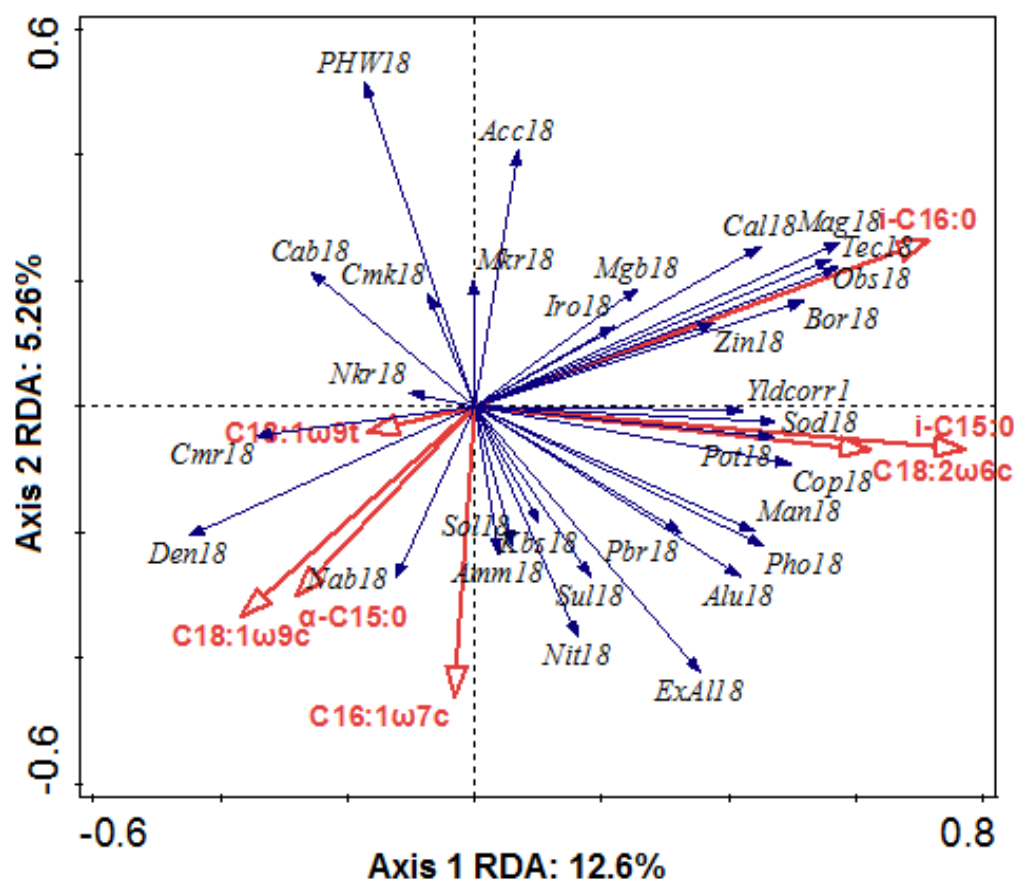


Figure S4.6 RDA of data for bacterial and fungal PLFA markers composition (red arrows) effects on soil health legacies (blue arrows). On First Axis; $pseudo-F=0.5$, $P=0.096$ while on All Axes; $pseudo-F=1.2$, $P=0.177$. The blue arrows represent the soil health legacies for season 2018 where:

Acc18 - Active carbon (mg/kg)	Cab18 - %Ca	Alu18 - Al (mg/kg)
Amm18 - 1:2 Water extract N - NH_4 (mg/l)	Cmr18 - Ca:Mg	Bor18 - B (mg/kg)
Cec18 - CEC (cmol(+)/kg)	Cop18 - Cu (mg/kg)	Cal18 - Ca (mg/kg)
Cmk18 - (Ca+Mg)/K	Hbs18 - %Acid.V	Iro18 - Fe (mg/kg)
Den18 - Density (g/cm ³)	Kbs18 - %K	Mag18 - Mg (mg/kg)
ExAl18 - salt-replaceable acidity (cmol(+)/kg)	Mgb18 - %Mg	Man18 - Mn (mg/kg)
Sol18 - Solvita CO ₂ respiration (ppm)	Nab18 - %Na	Zin18 - Zn (mg/kg)
Nit18 - 1:2 Water extract N - NO_3 (mg/l)	Nkr18 - Na:K	Pho18 - P (mg/kg)
Obs18 - S-Value (cmol(+)/kg)	Mkr18 - Mg:K	PHW18 - pH (H ₂ O)
Pbr18 - P Bray 2 (mg/kg)	Pot18 - K (mg/kg)	Sul18 - S (mg/kg)
Ec18 - electrical conductivity (ms/m)	Sod18 - Na (mg/kg)	

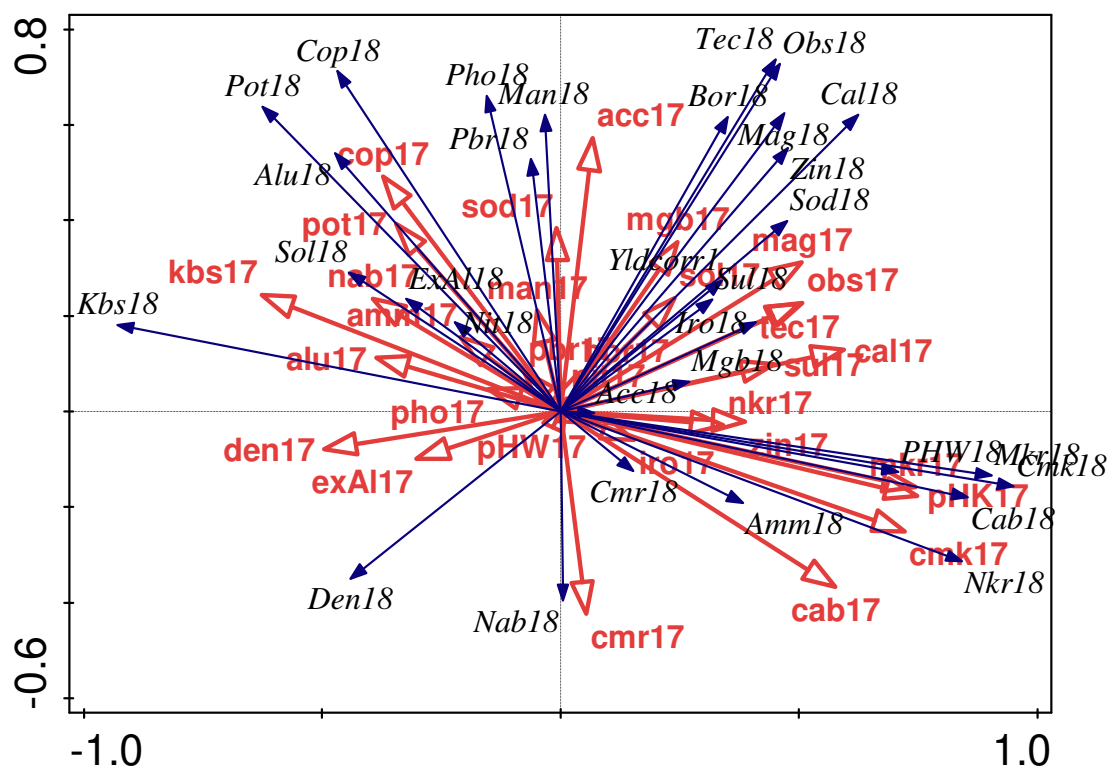


Figure S4.7 Soil-soil health properties composition feedbacks for seasons 2017 and 2018 using Redundancy Analysis (RDA) of the 31 measured soil health variables. Axis 1 RDA: 26.36% Axis 2 RDA: 19.03%, on First Axis; $pseudo-F < 0.1$, $P = 0.145$ while on All Axes; $pseudo-F = 1.4$, $P = 0.137$. The blue arrows represent the soil health legacies where:

acc - Active carbon (mg/kg)	cab - %Ca	alu - Al (mg/kg)
amm - 1:2 Water extract N - NH_4 (mg/l)	den - Density (g/cm^3)	bor - B (mg/kg)
cec - CEC (cmol(+)/kg)	cop - Cu (mg/kg)	cal - Ca (mg/kg)
cmk - (Ca+Mg)/K	hbs - % acid saturation	iro - Fe (mg/kg)
cmr - Ca:Mg	kbs - %K	mag - Mg (mg/kg)
exAl - salt-replaceable acidity (cmol(+)/kg)	mgb - %Mg	man - Mn (mg/kg)
sol - Solvita CO_2 respiration (ppm)	nab - %Na	zin - Zn (mg/kg)
nit - 1:2 Water extract N - NO_3 (mg/l)	nkr - Na:K	pho - P (mg/kg)
obs - S-Value (cmol(+)/kg)	mkr - Mg:K	pHW - pH (H_2O)
pbr - P Bray 2 (mg/kg)	sul - S (mg/kg)	pot - K (mg/kg)
sod - Na (mg/kg)		

Table S4.1A Tukey's HSD test of natural log-transformed total fungal PLFA markers averages for the four dryland crops. Different letters in subsets 1 and 2 indicate significant differences ($p < .05$).

Crop	N	Subset	
		1	2
Bulk soil	8	3.3081 (a)	
Maize	8	3.4276(ab)	3.4276 (ab)
Soybean	8	3.5731 (ab)	3.5731 (ab)
Sugar bean	8	3.6695 (ab)	3.6695 (ab)
Sunflower	8		3.8151 (b)
Sig.		0.142	0.100

Table S4.1B Tukey's HSD test of natural log-transformed fungal to bacterial PLFA ratio averages for the four dryland crops. Different letters in subsets 1 and 2 indicate significant differences ($p < .05$).

Crop	N	Subset	
		1	2
Bulk soil	8	-1.9487 (a)	
Maize	8	-1.8927 (ab)	-1.8927 (ab)
Soybean	8	-1.7766 (ab)	-1.7766 (ab)
Sugar bean	8	-1.6690 (ab)	-1.6690 (ab)
Sunflower	8		-1.4919 (b)
Sig.		0.410	0.107

Table S4.1C Tukey's HSD test of natural log-transformed total Gram positive PLFA markers averages for the four dryland crops. Different letters in subsets 1 and 2 indicate significant differences ($p < .05$).

Crop	N	Subset
		1
Bulk soil	8	2.1118(a)
Soybean	8	2.5622 (a)
Maize	8	2.7774 (a)
Sunflower	8	2.8195 (a)
Sugar bean	8	2.8854 (a)
Sig.		0.080

Table S4.1D Tukey's HSD test of natural log-transformed FDA enzyme activity averages for the four dryland crops. Different letters in subsets 1 and 2 indicate significant differences ($p < .05$).

Crop	N	Subset	
		1	2
Bulk soil	8	0.3176 (a)	
Maize	8	0.5289 (ab)	0.5289 (ab)
Sugar bean	8	0.5556 (ab)	0.5556 (ab)
Soybean	8	0.5686 (ab)	0.5686 (ab)
Sunflower	8		0.6929 (b)
Sig.		0.301	0.701

Table S4.2 Descriptive statistics for soil properties at 20cm (T) and 40cm (F) soil depths.

Descriptives	Treatment	pH(H ₂ O)	Bray II	P	K	Na	Ca	Mg	UIT H+Al
		-	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	cmol(+)/kg
		"pHW"	"pbr"	"pho"	"pot"	"sod"	"cal"	"mag"	"exAl"
Mean	pmmF	5.56	22	30.7	71.6	21.4	424	73.7	0.00411
	pmsbF	5.4	29.5	40.8	91.6	19.3	375	62.7	0.00535
	pmsoyF	5.5	25.5	34.6	91.9	18.5	393	68.2	0.00323
	pmsunF	5.47	24	32.3	72.8	19.5	382	65.4	1.81E-07
	pmmT	5.65	45.9	55.4	112	19.6	381	54.8	0.00471
	pmsbT	5.58	45.6	55.3	129	19.5	370	50.4	0.00274
	pmsoyT	5.63	42.3	50	121	18.1	346	49.8	4.25E-08
	pmsunT	5.54	49.5	62.3	137	21.1	392	58.2	0.0067
Std. error mean	pmmF	0.0933	1.86	1.82	6.36	1.29	20.8	4.41	0.00411
	pmsbF	0.0556	1.53	2.47	6.51	0.493	15.2	3.23	0.00535
	pmsoyF	0.074	1.53	1.86	6.46	0.488	8.6	3.44	0.00323
	pmsunF	0.0433	1.32	1.55	7.44	0.767	21.9	5.71	9.30E-08
	pmmT	0.108	2.23	3.04	7.52	0.477	24.1	3.77	0.00471
	pmsbT	0.0937	2.63	2.4	5.07	1.02	17.9	1.71	0.00274
	pmsoyT	0.0756	2.01	2.55	7.8	0.549	7.29	1.95	1.32E-08
	pmsunT	0.0898	3.14	3.69	11.4	0.697	13	2.65	0.00449
Shapiro-Wilk p	pmmF	0.838	0.291	0.767	0.463	0.272	0.762	0.769	< .001
	pmsbF	0.289	0.355	0.966	0.122	0.005	0.013	0.476	< .001
	pmsoyF	0.797	0.756	0.879	0.219	0.04	0.224	0.898	< .001
	pmsunF	0.305	0.49	0.008	0.708	0.718	0.085	0.095	< .001
	pmmT	0.905	0.857	0.835	0.69	0.853	0.021	0.096	< .001
	pmsbT	0.776	0.139	0.194	0.145	0.033	0.458	0.187	< .001
	pmsoyT	0.343	0.011	0.281	0.31	0.366	0.087	0.046	0.001
	pmsunT	0.152	0.452	0.55	0.725	0.262	0.585	0.525	< .001

Table S4.2 (continued) Descriptive statistics for soil content at 20cm (T) and 40cm (F) soil depths.

Descriptives	Treatment	%Ca	%Mg	%K	%Na	SUUR.V	Ca:Mg	(Ca+Mg)/K	Mg:K
		%	%	%	%	%	1.5-4.5	10.0-20.0	3.0-4.0
		"cab"	"mgb"	"kbs"	"nab"	"acid.V"	"cmr"	"cmk"	"mkr"
Mean	pmmF	70.5	20	6.17	3.1	0.166	3.53	16	3.52
	pmsbF	69.2	18.9	8.57	3.12	0.229	3.67	10.4	2.23
	pmsoyF	69.2	19.6	8.25	2.84	0.112	3.56	11.1	2.43
	pmsunF	70.4	19.6	6.86	3.15	7.44E-06	3.63	14.2	3.08
	pmmT	69.6	16.4	10.7	3.17	0.196	4.26	8.51	1.61
	pmsbT	68.9	15.4	12.4	3.18	0.117	4.47	6.88	1.26
	pmsoyT	68.5	16.1	12.3	3.11	1.71E-06	4.27	7.13	1.36
	pmsunT	68	16.5	12.1	3.19	0.229	4.13	7.31	1.43
Std. error mean	pmmF	0.595	0.345	0.588	0.139	0.166	0.0734	2.09	0.43
	pmsbF	0.271	0.389	0.308	0.131	0.229	0.0784	0.396	0.105
	pmsoyF	0.821	0.62	0.492	0.098	0.112	0.142	0.701	0.158
	pmsunF	0.81	0.71	0.648	0.0959	4.21E-06	0.178	1.68	0.35
	pmmT	0.934	0.275	0.826	0.155	0.196	0.088	0.909	0.157
	pmsbT	0.619	0.153	0.452	0.137	0.117	0.0813	0.322	0.0441
	pmsoyT	0.659	0.476	0.776	0.0605	5.36E-07	0.13	0.543	0.126
	pmsunT	0.806	0.386	0.872	0.109	0.157	0.107	0.644	0.121
Shapiro-Wilk p	pmmF	0.602	0.444	0.628	0.64	< .001	0.646	0.059	0.077
	pmsbF	0.251	0.357	0.31	0.073	< .001	0.367	0.347	0.501
	pmsoyF	0.1	0.413	0.248	0.342	< .001	0.367	0.24	0.032
	pmsunF	0.659	0.296	0.582	0.46	< .001	0.098	0.107	0.579
	pmmT	0.834	0.171	0.294	0.708	< .001	0.705	0.059	0.123
	pmsbT	0.233	0.327	0.285	0.264	< .001	0.606	0.053	0.38
	pmsoyT	0.612	0.048	0.183	0.789	0.003	0.918	0.194	0.052
	pmsunT	0.656	0.313	0.498	0.011	< .001	0.207	0.437	0.968

Table S4.2 (continued) Descriptive statistics for soil content at 20cm (T) and 40cm (F) soil depths.

Descriptives	Treatment	S-Waarde	Na:K	T	Density	Fe	Mn	Cu	Zn
		cmol(+)/kg	-	cmol(+)/kg	g/cm3	mg/kg	mg/kg	mg/kg	mg/kg
		"obs"	"nkr"	"tec"	"den"	"iro"	"man"	"cop"	"zin"
Mean	pmmF	3	0.561	3.01	1.14	101	40.3	1.66	1.39
	pmsbF	2.71	0.369	2.71	1.19	96.8	37.5	1.64	1.65
	pmsoyF	2.84	0.355	2.84	1.2	91.2	36.1	1.64	1.56
	pmsunF	2.72	0.497	2.72	1.14	88.6	35.9	1.59	1.32
	pmmT	2.73	0.309	2.73	1.22	91.2	37.3	1.54	2.84
	pmsbT	2.68	0.259	2.68	1.31	85.9	34.3	1.6	2.59
	pmsoyT	2.53	0.262	2.53	1.3	86.4	35.7	1.56	2.57
	pmsunT	2.88	0.277	2.88	1.25	91.4	35.7	1.63	2.99
Std. error mean	pmmF	0.142	0.0969	0.14	0.0151	18.4	2.92	0.0629	0.094
	pmsbF	0.116	0.024	0.114	0.00807	5.27	1.32	0.0556	0.105
	pmsoyF	0.0708	0.0281	0.071	0.0141	4.46	3.04	0.0472	0.0843
	pmsunF	0.165	0.0665	0.165	0.0104	4.06	1.97	0.0881	0.0395
	pmmT	0.153	0.0276	0.152	0.00766	5.96	1.77	0.0566	0.15
	pmsbT	0.112	0.015	0.111	0.0107	2.22	1.06	0.0261	0.218
	pmsoyT	0.0474	0.0198	0.0474	0.00854	4.51	1.69	0.0474	0.123
	pmsunT	0.0973	0.0282	0.0979	0.0131	2.73	1.51	0.0567	0.155
Shapiro-Wilk p	pmmF	0.776	0.004	0.712	0.653	< .001	0.243	0.528	0.777
	pmsbF	0.041	0.12	0.051	0.571	0.195	0.256	0.624	0.5
	pmsoyF	0.643	0.907	0.544	0.22	0.415	0.142	0.689	0.147
	pmsunF	0.09	0.024	0.09	0.442	0.495	0.35	0.611	0.915
	pmmT	0.052	0.156	0.071	0.555	< .001	0.555	0.797	0.103
	pmsbT	0.57	0.158	0.483	0.82	0.719	0.637	0.945	0.19
	pmsoyT	0.769	0.225	0.769	0.411	0.913	0.498	0.782	0.108
	pmsunT	0.206	0.117	0.245	0.66	0.268	0.248	0.475	0.856

Table S4.2 (continued) Descriptive statistics for soil properties at 20cm (T) and 40cm (F) soil depths.

Descriptives	Treatment	S	B	Al	EC	N - NH4	N - NO3	Active C	CO2
		mg/kg	mg/kg	mg/kg	ms/m	mg/kg	mg/kg	mg/kg	ppm
		"sul"	"bor"	"alu"	"ec"	"amm"	"nit"	"acc"	"sol"
Mean	pmmF	6.35	0.303	373	3.1	0.524	1.01	227	17.2
	pmsbF	5.06	0.286	385	2.42	0.46	0.399	222	17.7
	pmsoyF	5.37	0.285	382	2.31	0.619	2.02	230	21.8
	pmsunF	6.13	0.285	383	2.76	0.504	0.64	246	17.7
	pmmT	5.12	0.272	353	4.18	0.995	0.689	222	31.4
	pmsbT	5.3	0.265	346	3.73	0.895	0.767	230	35.5
	pmsoyT	4.5	0.259	341	2.84	0.853	0.693	235	37.9
	pmsunT	5.65	0.277	368	4.32	1.07	0.785	242	37
Std. error mean	pmmF	0.952	0.00923	16.9	0.449	0.0838	0.602	6.05	1.68
	pmsbF	0.287	0.0083	10.2	0.144	0.062	0.206	7.34	1.6
	pmsoyF	0.146	0.00763	11.3	0.12	0.16	1.12	6.47	2.39
	pmsunF	0.291	0.00681	13.3	0.14	0.0525	0.176	19.7	1.25
	pmmT	0.627	0.00709	17.9	0.643	0.184	0.149	6.13	5.01
	pmsbT	0.448	0.00501	8.71	0.518	0.154	0.156	17.2	4.13
	pmsoyT	0.163	0.00389	9.57	0.123	0.154	0.191	14.4	5.22
	pmsunT	0.306	0.00742	12.9	0.173	0.151	0.196	11	5.5
Shapiro-Wilk p	pmmF	< .001	0.618	0.007	0.006	0.238	< .001	0.489	0.017
	pmsbF	0.589	0.055	0.367	0.496	0.003	0.001	0.128	0.022
	pmsoyF	0.422	0.631	0.412	0.708	0.001	0.002	0.079	0.125
	pmsunF	0.681	0.332	0.492	0.512	0.695	0.294	0.003	0.157
	pmmT	0.014	0.601	0.048	0.009	0.497	0.451	0.351	0.855
	pmsbT	0.459	0.064	0.584	0.002	0.71	0.004	0.768	0.484
	pmsoyT	0.537	0.973	0.397	0.252	0.428	0.647	0.03	0.936
	pmsunT	0.793	0.002	0.369	0.976	0.718	0.923	0.012	0.55

Table S4.3 One-way ANOVA crop effects on legacies in soil health properties. Significant *p* values are <.05. *Statistical analysis done using Kruskal-Wallis ANOVA.

Effect	Soil property	df	<i>F</i>	<i>p</i>
Crop treatments	pHW	7	1.04	0.412
	pbr	7	28.8	< 0.001
	pho	7	23.4	< 0.001
	pot	7	11.1	< 0.001
	sod	7	2.17	0.051
	cal	7	1.72	0.124
	mag	7	5.78	< 0.001
	exAl*	7	30.6	< 0.001
	cab	7	1.53	0.177
	mgb	7	17.3	< 0.001
	kbs	7	15.2	< 0.001
	nab	7	0.885	0.524
	acid.V*	7	11.8	0.106
	cmr	7	10.7	< 0.001
	cmk	7	9.9	< 0.001
	mkr	7	14.4	< 0.001
	obs	7	1.47	0.197
	nkr	7	5.86	< 0.001
	den	7	32.8	< 0.001
	iro	7	0.462	0.858
	man	7	0.801	0.59
	cop	7	0.538	0.802
	zin	7	28.4	< 0.001
	sul	7	1.58	0.16
	bor	7	3.78	0.002
	alu	7	1.85	0.095
	ec	7	4.9	< 0.001
	amm	7	3.25	0.006
	nit*	7	3.69	0.815
	acc	7	2.12	0.953
	sol	7	6.18	< 0.001

Supplementary Material for Chapter 5

Supplementary Tables

Table S5.1 Independent *t*-test analysis of the effect of soil sterilization on plant performance (total biomass, root-to-shoot ratio and root mass ratio) of *G. max*, *H. annuus*, *P. vulgaris* and *Z. mays* in the conditioning phase. Significant *p* values are < 0.05.

Plant species	Root to shoot ratio		Root mass ratio	
	<i>t</i> ₆₂	p	<i>t</i> ₆₂	p
<i>G. max</i>	1.544	0.128	1.499	0.139
<i>H. annuus</i>	0.488	0.627	0.425	0.672
<i>P. vulgaris</i>	-1.498	0.139	-1.562	0.123
<i>Z. mays</i>	3.892	<0.001	4.207	<0.001

Table S5.2 ANOVA test results of plant-soil feedbacks among three soil types (field, n = 8; sterilized, n= 8; inoculated, n = 8) within groups of phytometer species using original total biomass data. Significant *p* values are < 0.05.

Experimental group		Total biomass	
Phytometer species	Conditioning species	<i>F</i>	p
<i>Zea mays</i>	<i>Zea mays</i>	7.24	0.004
<i>Zea mays</i>	<i>Phaseolus vulgaris</i>	0.202	0.819
<i>Zea mays</i>	<i>Helianthus annuus</i>	22.4	<0.001
<i>Zea mays</i>	<i>Glycine max</i>	0.047	0.954
<i>Phaseolus vulgaris</i>	<i>Zea mays</i>	0.927	0.411
<i>Phaseolus vulgaris</i>	<i>Helianthus annuus</i>	0.057	0.945
<i>Phaseolus vulgaris</i>	<i>Glycine max</i>	3.07	0.068
<i>Phaseolus vulgaris</i>	<i>Phaseolus vulgaris</i>	0.194	0.825
<i>Helianthus annuus</i>	<i>Zea mays</i>	1.58	0.229
<i>Helianthus annuus</i>	<i>Helianthus annuus</i>	9.79	0.001
<i>Helianthus annuus</i>	<i>Glycine max</i>	0.480	0.626
<i>Helianthus annuus</i>	<i>Phaseolus vulgaris</i>	0.848	0.442
<i>Glycine max</i>	<i>Zea mays</i>	29.9	<0.001
<i>Glycine max</i>	<i>Helianthus annuus</i>	3.31	0.056
<i>Glycine max</i>	<i>Glycine max</i>	12.5	<0.001
<i>Glycine max</i>	<i>Phaseolus vulgaris</i>	2.99	0.072

Table S5.3 *Post hoc* comparison test for soil FDA hydrolyzed ($\mu\text{g}/\text{mg}$ soil) legacies after conditioning phase across five categories of soil origin at $p = 0.05$. $n_1 = n_2 = n_3 = n_4 = n_5 = 3$, $N = 15$. Significant p values are < 0.05 .

Soil origin	Mean difference	Confidence interval	P value
He – Ctrl	0.530	[0.033, 1.028]	0.035
He – Gl	0.437	[-0.061, 0.934]	0.093
He – Ph	0.020	[-0.477, 0.517]	1.000
He – Ze	0.318	[-0.180, 0.815]	0.290
Ph – Ctrl	0.510	[0.013, 1.007]	0.044
Ph – Gl	0.416	[-0.081, 0.913]	0.114
Ph – Ze	0.297	[-0.200, 0.794]	0.345
Ze – Ctrl	0.213	[-0.284, 0.710]	0.636
Ze – Gl	0.119	[-0.378, 0.616]	0.928
Gl – Ctrl	0.094	[-0.403, 0.591]	0.968

Table S5.4 Tukey's honestly significant difference (Tukey HSD) *post hoc* comparison test for soil FDA hydrolyzed ($\mu\text{g}/\text{mg}$ soil) legacies after feedback phase across five categories of soil origin at $p = 0.05$. $n_1 = n_2 = n_3 = n_4 = n_5 = 3$, $N = 15$. Significant p values are < 0.05 .

Soil origin	Mean difference	Confidence interval	p-value
He/Ph – Ctrl	1.298	[0.327, 2.269]	0.009
He/Ph – He/Gl	0.944	[-0.027, 1.916]	0.058
He/Ph – He/Ze	0.521	[-0.450, 1.492]	0.441
He/Ph – He/He	0.718	[-0.253, 1.689]	0.183
He/Ze – Ctrl	0.777	[0.194, 1.748]	0.137
He/Ze – He/Gl	0.424	[-0.548, 1.395]	0.621
He/Ze – He/He	0.197	[-0.774, 1.168]	0.959
He/He – Ctrl	0.580	[-0.391, 1.551]	0.346
He/He – He/Gl	0.226	[-0.745, 1.198]	0.934
He/Gl – Ctrl	0.354	[-0.618, 1.325]	0.753

Table S5.5 Kruskal-Wallis test for soil nitrogen legacies across nine categories of soil origin at $p=0.05$. $n_1 = n_2 = n_3 = n_4 = n_5 = 3$, $n_6 = n_7 = n_8 = n_9 = 9$, $N = 51$. Significant p values are < 0.05 .

Effect	d.f.	Soil nitrogen							
		NO ₃ – N		NH ₄ – N		NO ₃ : NH ₄ ratio		Total nitrogen	
		H	p	H	p	H	p	H	p
Soil origin	8	25.4	0.001	18.9	0.015	25.4	0.001	24.03	0.002

Table S5.6 Kruskal-Wallis pairwise comparisons for soil nitrogen legacies across plant-soil feedback procedures at $p = 0.05$. ‡ Heterospecifics, £ Conspecifics, Ctrl Procedure Control. Coefficient of variation (CV) in soil nitrogen were; 2.3 %, 0.4 %, 1.4 % and 1.5 % for $\text{NO}_3 - \text{N}$, $\text{NH}_4 - \text{N}$, Total nitrogen and $\text{NO}_3 : \text{NH}_4$ ratio, respectively. Significant p values are < 0.05 .

Feedback effect	Soil nitrogen							
	$\text{NO}_3 - \text{N}$		$\text{NH}_4 - \text{N}$		$\text{NO}_3 : \text{NH}_4$ ratio		Total nitrogen	
	H	p	H	P	H	p	H	P
‡He/£Gl	6.33	1.000	1.22	1.000	7.11	1.000	6.50	1.000
‡He/£He	11.2	1.000	19.1	1.000	22.5	0.835	12.2	1.000
‡He/£Ze	-14.1	1.000	-4.22	1.000	-13.1	1.000	-8.94	1.000
‡He/£Gl	14.6	1.000	1.78	1.000	12.2	1.000	7.50	1.000
‡He/£Ph	19.7	1.000	16.1	1.000	15.2	1.000	19.7	1.000
‡He/£Ph	-20.4	0.123	-7.28	1.000	-19.5	0.194	-13.6	1.000
‡He/£Ze	32.7	0.033	3.22	1.000	34.8	0.016	25.8	0.328
‡He/Ctrl	38.1	0.004	27.6	0.191	37.2	0.006	33.5	0.026
‡Gl/£He	4.89	1.000	20.3	1.000	15.4	1.000	18.7	1.000
‡Gl/£Ze	-7.78	1.000	-3.00	1.000	-5.94	1.000	-2.44	1.000
‡Gl/£Gl	8.22	1.000	0.556	1.000	5.06	1.000	1.00	1.000
‡Gl/£Ph	13.4	1.000	14.9	1.000	8.06	1.000	13.2	1.000
‡Gl/£Ph	-14.1	1.000	-6.06	1.000	-12.4	1.000	-7.11	1.000
‡Gl/£Ze	26.4	0.271	4.44	1.000	27.7	0.186	19.3	1.000
‡Gl/Ctrl	31.7	0.047	26.4	0.278	30.1	0.087	27.0	0.231
£He/£Ze	-2.89	1.000	-23.3	0.676	-9.44	1.000	-21.1	1.000
£He/£Gl	3.33	1.000	20.8	1.000	10.3	1.000	19.7	1.000
£He/£Ph	-8.50	1.000	-35.2	0.135	-7.33	1.000	-31.8	0.313
£He/£Ph	-9.22	1.000	-26.3	0.283	-3.00	1.000	-25.8	0.334
£He/£Ze	-21.5	1.000	-15.8	1.000	-12.3	1.000	-38.0	0.063
£He/Ctrl	26.8	0.955	46.7	0.004	14.7	1.000	45.7	0.006
‡Ze/£Gl	0.444	1.000	2.44	1.000	0.889	1.000	1.44	1.000
‡Ze/£Ph	5.61	1.000	11.9	1.000	2.11	1.000	10.7	1.000
‡Ze/£Ph	6.33	1.000	3.06	1.000	6.44	1.000	4.67	1.000
‡Ze/£Ze	18.6	1.000	7.44	1.000	21.8	1.000	16.9	1.000
‡Ze/Ctrl	23.9	0.552	23.4	0.656	24.1	0.539	24.6	0.475
£Gl/£Ph	-5.17	1.000	-14.3	1.000	-3.00	1.000	-12.2	1.000
£Gl/£Ph	-5.89	1.000	-5.50	1.000	-7.33	1.000	-6.11	1.000
£Gl/£Ze	-18.2	1.000	-5.00	1.000	-22.7	1.000	-18.3	1.000
£Gl/Ctrl	23.5	1.000	25.8	1.000	25.0	1.000	26.0	1.000
£Ph/£Ph	-0.722	1.000	8.83	1.000	-4.33	1.000	-6.06	1.000
£Ph/£Ze	-13.0	1.000	-19.3	1.000	-19.7	1.000	-6.17	1.000
£Ph/Ctrl	18.3	1.000	11.5	1.000	22.0	1.000	13.8	1.000
‡Ph/£Ze	12.3	1.000	10.5	1.000	15.3	1.000	12.2	1.000
‡Ph/Ctrl	17.6	1.000	20.3	1.000	17.7	1.000	19.9	1.000
£Ze/Ctrl	5.33	1.000	30.8	0.398	2.33	1.000	7.67	1.000

Table S5.7 Total abundance (nmol/g) and ratios of viable microbes present in soil of different origin at the end of phase 1 monocultures and phase 2 rotations using fungal and bacterial PLFA signatures. Ctrl – procedural bare control soil, Gl – *G. max*, He – *H. annuus*, Ph – *P. vulgaris*, and Ze – *Z. mays*, He/Ze heterospecific *Z. mays* feedback, He/He conspecific *H. annuus* feedback, He/Ph heterospecific *P. vulgaris* feedback and He/Gl heterospecific *G. max* feedback, GN – Gram-negative bacteria and GP – Gram-positive bacteria.

Soil origin	Total GP bacteria	Total GN bacteria	GP to GN ratio	Total bacterial PLFAs	Total fungal PLFAs	Fungal to bacterial ratio
Phase 1						
Ze	35.30	411.51	0.086	534.25	664.44	1.24
Ph	20.73	384.64	0.054	490.01	606.19	1.24
He	11.97	382.30	0.031	462.65	607.22	1.31
Gl	23.12	382.27	0.060	475.26	606.19	1.28
Ctrl	34.46	830.98	0.041	939.52	551.10	0.59
Phase 2						
He/Ze	25.68	132.17	0.19	192.86	110.95	0.58
He/He	0	135.11	0.00	160.13	191.97	1.20
He/Gl	0	128.98	0.00	162.62	123.49	0.76
He/Ph	0	129.90	0.00	152.87	93.90	0.61
Ctrl	0	131.49	0.00	162.76	107.31	0.66

Table S5.8 PLFA biomarkers in phases 1 (P1) and 2 (P2).

Soil origin	C18:2 ω6c	C18:1ω 9t	C18:1ω 9c	C18:2ω 6t	C18:3ω 3c	C14:0	i-C15:0	a-C15:0	C15:0	i-C16:0	C16:19	C17:0	i-C17:0	C18:17
P1	Fungal fatty acids biomarkers					Bacterial fatty acids biomarkers								
Ze	122.61	10.71	372.12	145.28	13.72	62.93	25.29	0	12.33	10.01	411.51	12.18	0	0
Ph	110.75	9.56	331.36	138.74	15.88	62.88	8.71	0	11.15	12.02	384.64	10.61	0	0
He	98.03	5.59	360.30	135.47	7.83	55.32	6.76	0	9.02	5.21	382.30	4.04	0	0
Gl	109.24	10.15	340.33	137.72	8.76	56.01	10.47	0	9.70	12.65	382.27	4.17	0	0
Ctrl	95.90	9.77	301.26	137.38	6.78	59.10	22.46	0	14.98	12.00	830.98	0	0	0
P2	Fungal fatty acids biomarkers					Bacterial fatty acids biomarkers								
He/Ze	8.92	0	29.07	72.97	0	2.53	12.71	12.97	0	0	132.17	0	0	32.47
He/He	78.90	0	27.33	73.86	11.89	0	0	0	0	0	135.11	0	0	25.02
He/Gl	6.53	0	31.14	85.82	0	9.64	0	0	0	0	128.98	0	0	24.00
He/Ph	7.28	0	17.01	69.61	0	0	0	0	0	0	129.90	0	0	22.96
Ctrl	6.74	0	17.35	83.22	0	12.24	0	0	0	0	131.49	0	0	19.02

Supplementary Figures

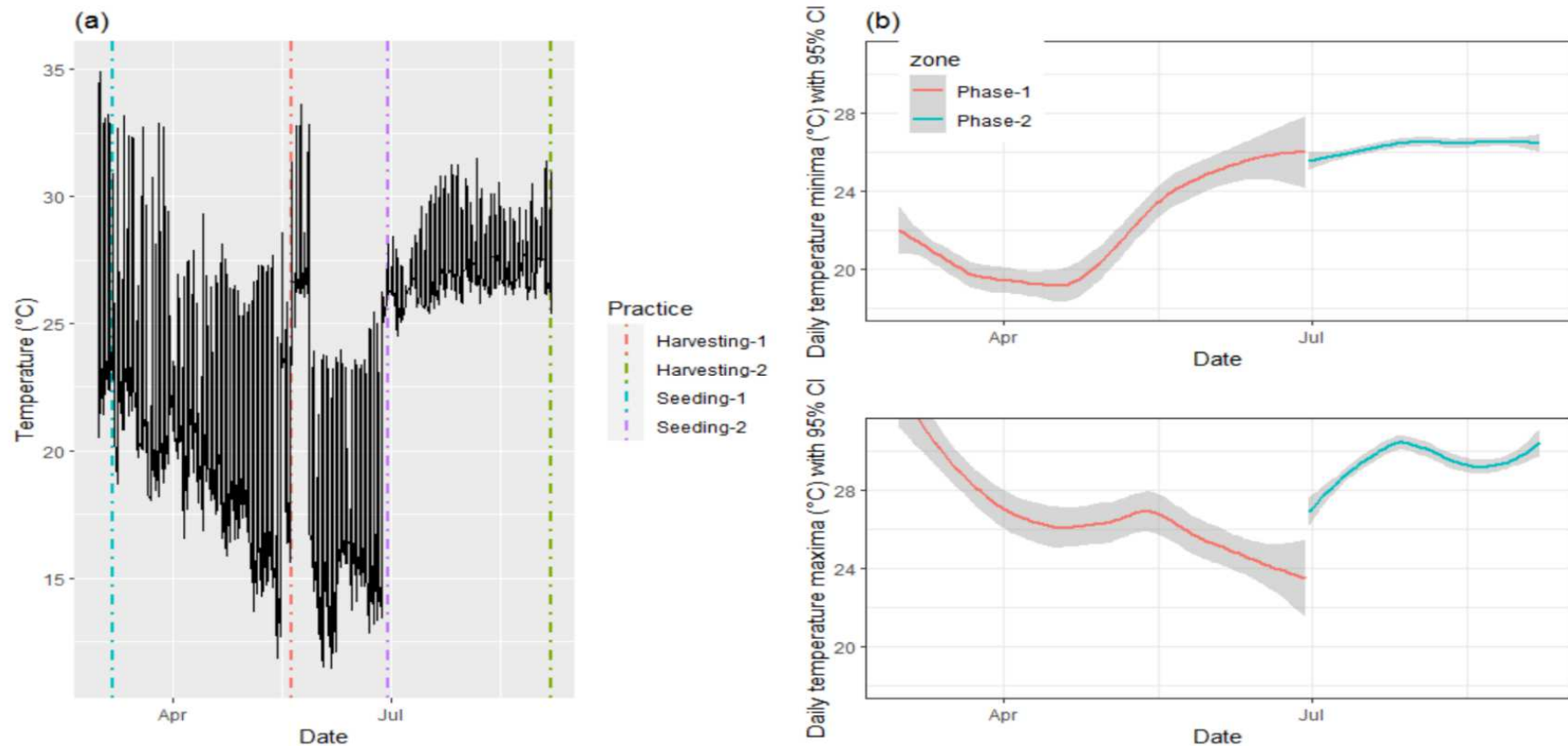


Figure S5.1 Temperature data of conditioning phase (phase-1) and feedback phase (phase-2) from seeding (planting) to harvesting. (a) Temperature differences are higher in phase-1 (early March to early June) than phase-2 (late June to early September). (b) The daily temperature maxima in phase-2 and phase-1 are slightly different but their minima are different by a wide margin. Seeding-1 07/03/2018, Harvesting-1 21/05/2018, Seeding-2 30/06/2018, Harvesting-2 06/09/2018.

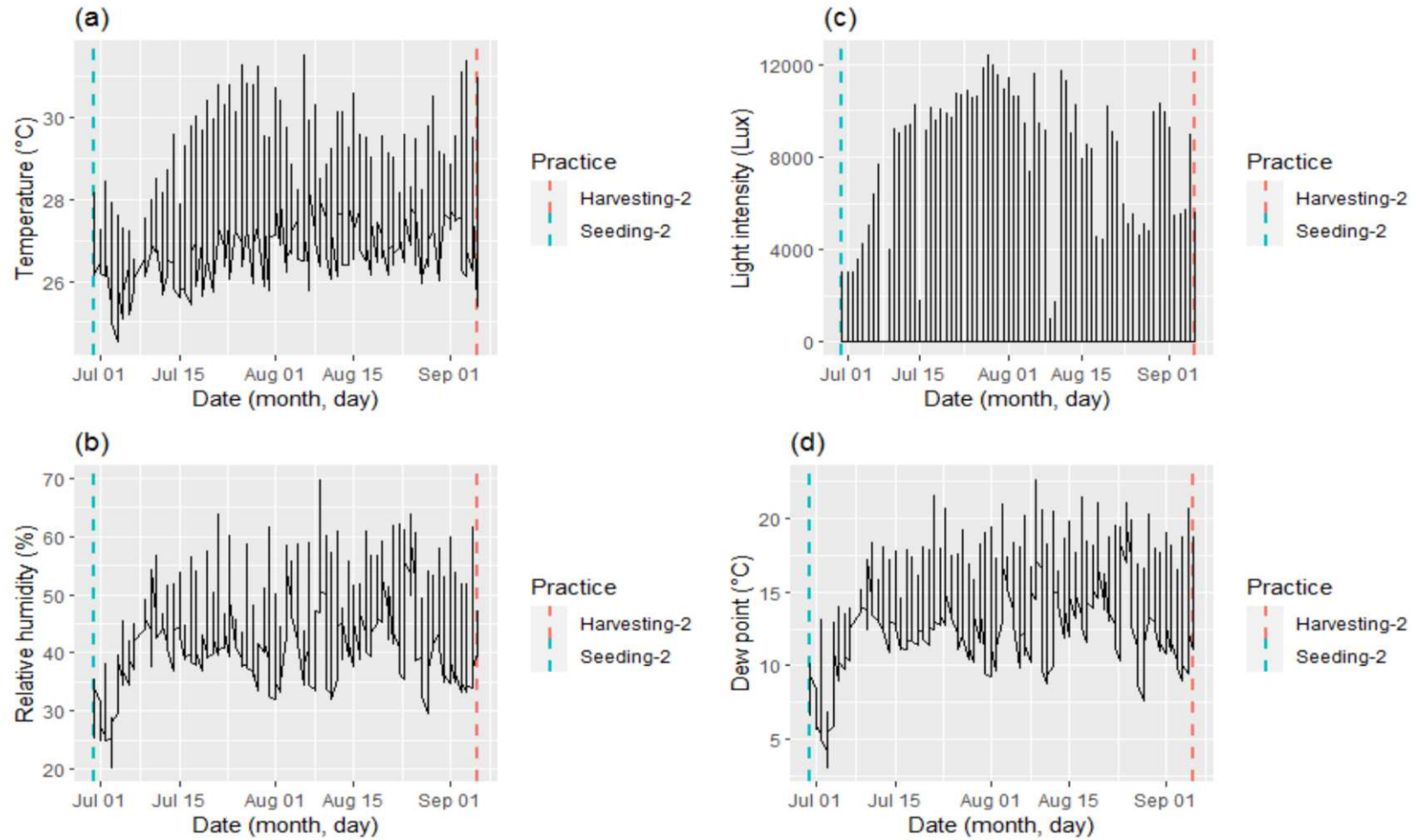


Figure S5.2 (a) Temperature (°C) and (b) relative humidity (%) data of feedback phase (phase-2) from seeding (planting) to harvesting. (c) Light intensity (Lux) and (d) dew point (°C) data of feedback phase (phase-2) from seeding (planting) to harvesting. Seeding-2 30/06/2018, Harvesting-2 06/09/2018.

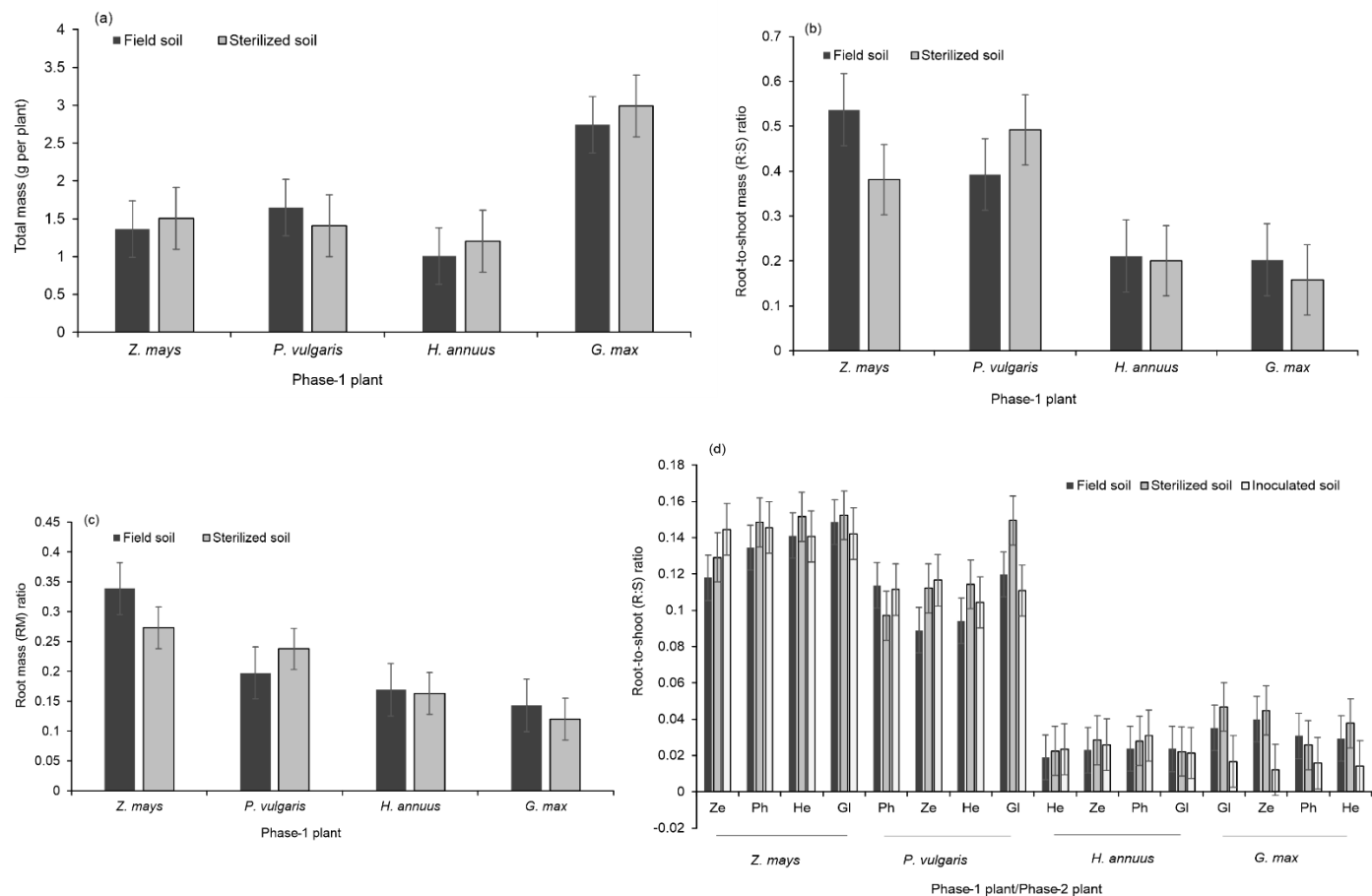


Figure S5.3 Effect of soil treatments on (a) total biomass, (b) root-to-shoot ratio and (c) root mass ratio of phase-1 plants after the conditioning phase. *Zea mays* and *P. vulgaris* were superior than *H. annuus* and *G. max* in the (d) root-to-shoot ratio at the end of the feedback phase. Bars represent the mean values, error bars show standard error (n = 24 for (a) to (c), n = 8 for (d)). Black indicates natural field soil, grey indicates sterilized soil and white indicates inoculated soil.

Supplementary Material for Chapter 6

Supplementary Figures

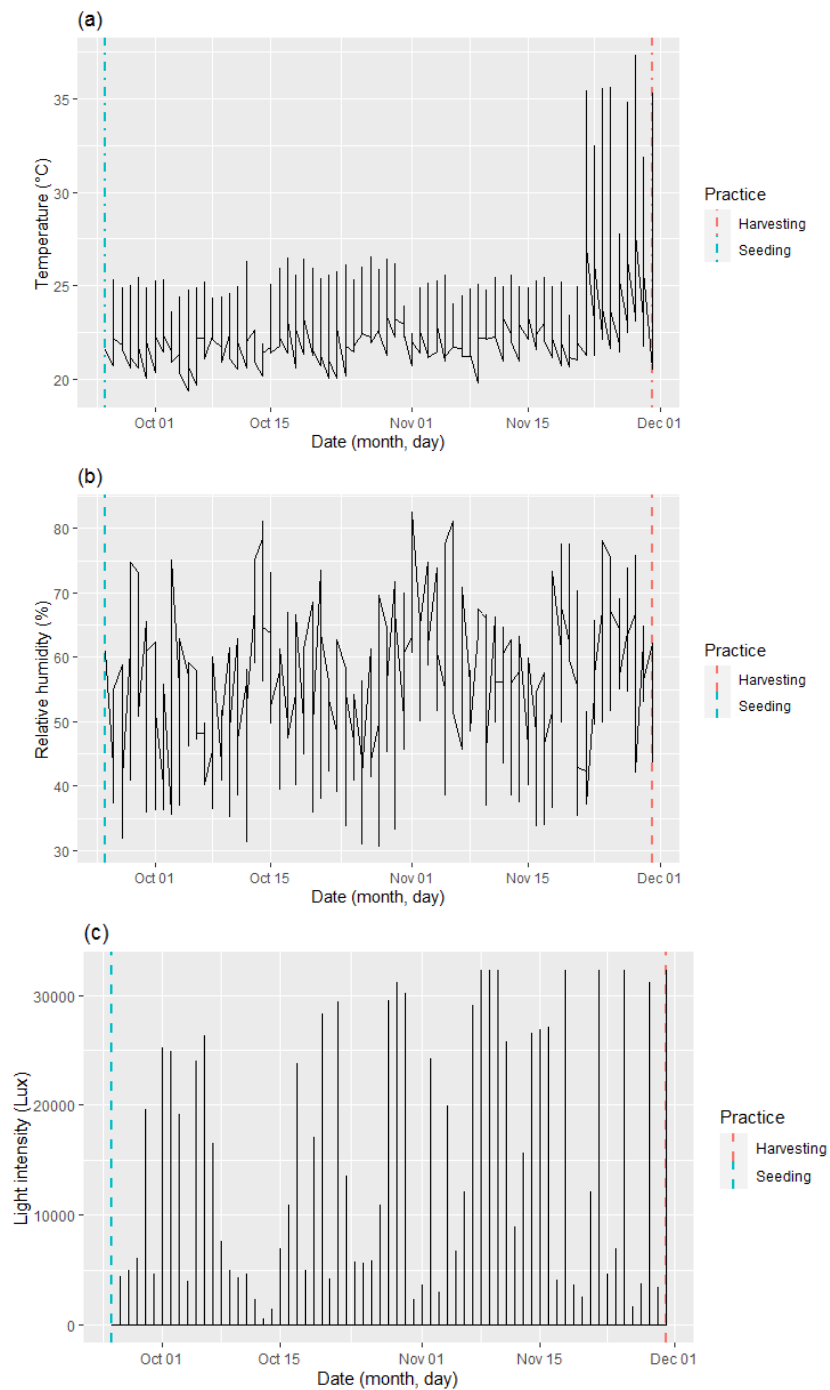


Figure S6.1 (a) Temperature, (b) Relative humidity and (c) Light intensity variables of the greenhouse experiments conducted at the University of Pretoria between September and December 2018.

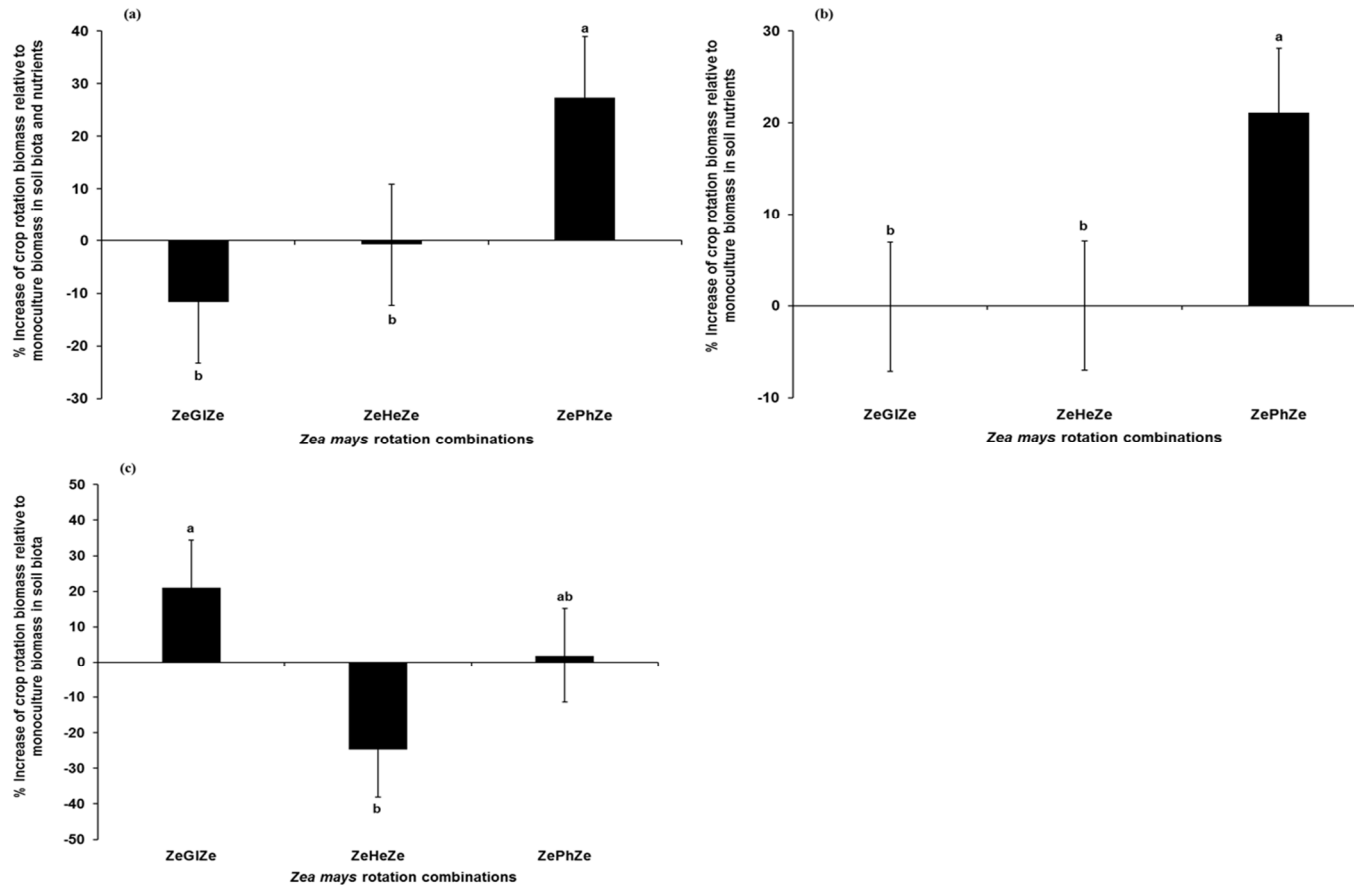


Figure S6.2 The resistance responses of *Z. mays* crop rotation to monoculture in (a) soil biota and nutrients and (b) soil biota only relative to that grown in sterilized soil controls. Different letters represent significant differences ($p < .05$) among crop rotation combinations or a strong resistance value, while shared letters represent non-significant differences ($p > .05$) among crop rotation combinations or a weak resistance value based on the one-way ANOVA *post hoc* Tukey HSD test. All bars represent the standard error of the mean ($n = 24$). *Zea mays* (Ze), *Phaseolus vulgaris* (Ph), *Glycine max* (Gl) and *Helianthus annuus* (He).

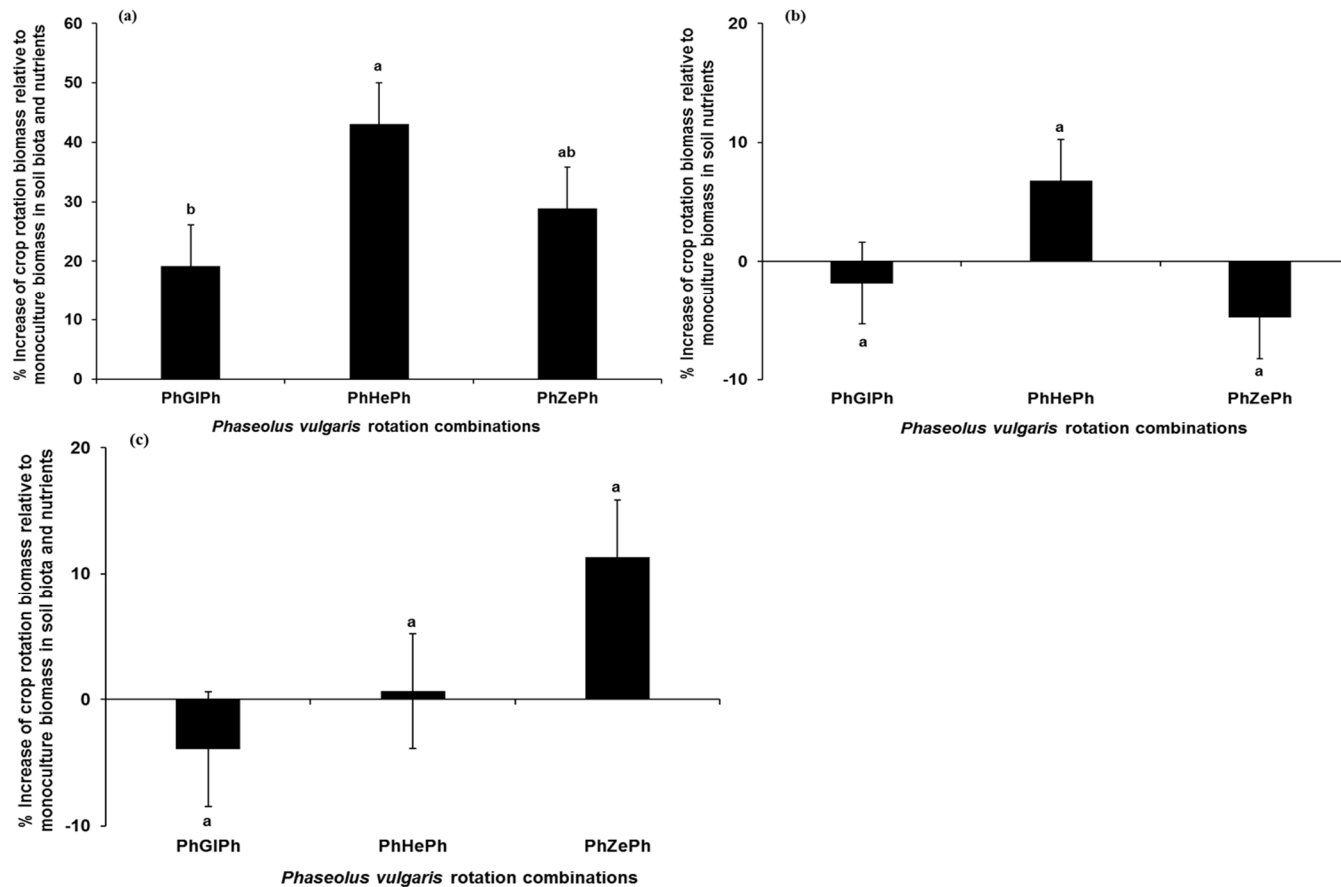


Figure S6.3 The resistance responses of *P. vulgaris* crop rotation to monoculture in (a) soil biota and nutrients and (b) soil biota only relative to that grown in sterilized soil controls. Different letters represent significant differences ($p < .05$) among crop rotation combinations or a strong resistance value, while shared letters represent non-significant differences ($p > .05$) among crop rotation combinations or a weak resistance value based on the one-way ANOVA *post hoc* Tukey HSD test. All bars represent the standard error of the mean ($n = 24$). *Zea mays* (Ze), *Phaseolus vulgaris* (Ph), *Glycine max* (Gl) and *Helianthus annuus* (He).

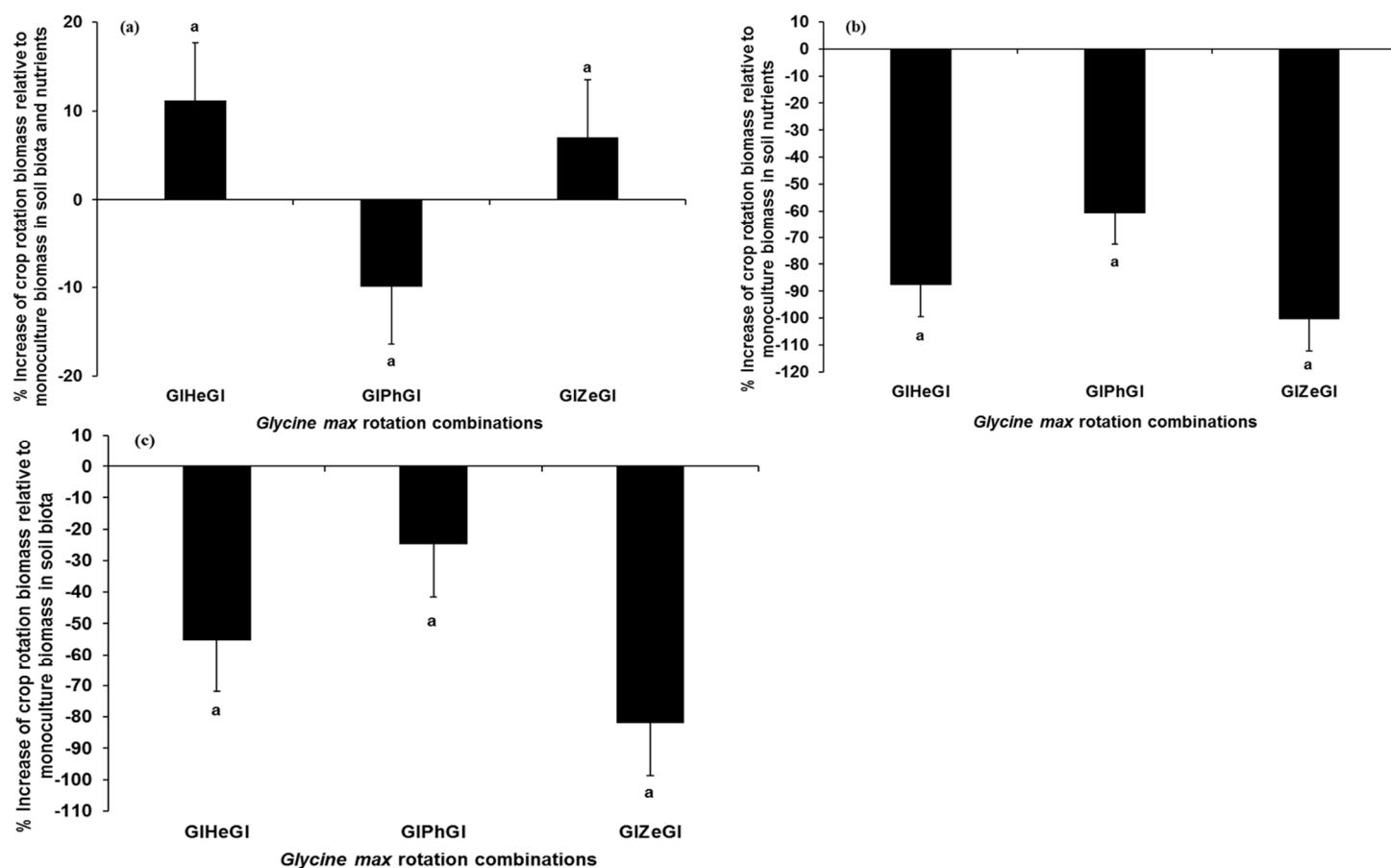


Figure S6.4 The resistance responses of *G. max* crop rotation to monoculture in (a) soil biota and nutrients and (b) soil biota only relative to that grown in sterilized soil controls. Different letters represent significant differences ($p < .05$) among crop rotation combinations or a strong resistance value, while shared letters represent non-significant differences ($p > .05$) among crop rotation combinations or a weak resistance value based on the one-way ANOVA *post hoc* Tukey HSD test. All bars represent the standard error of the mean ($n = 24$). *Zea mays* (Ze), *Phaseolus vulgaris* (Ph), *Glycine max* (Gl) and *Helianthus annuus* (He).

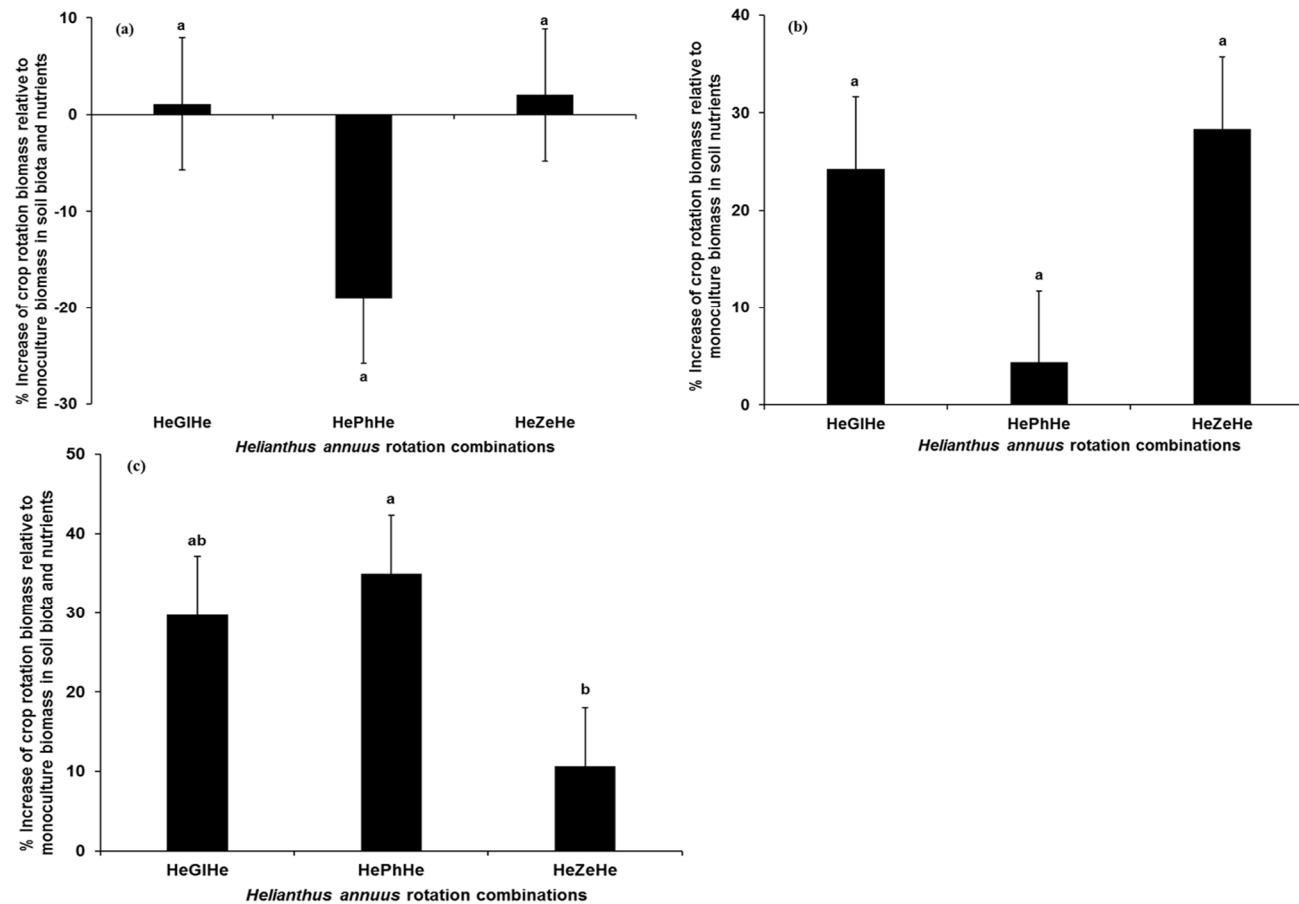


Figure S6.5 The resistance responses of *H. annuus* crop rotation to monoculture in (a) soil biota and nutrients and (b) soil biota only relative to that grown in sterilized soil controls. Different letters represent significant differences ($p < .05$) among crop rotation combinations or a strong resistance value, while shared letters represent non-significant differences ($p > .05$) among crop rotation combinations or a weak resistance value based on the one-way ANOVA *post hoc* Tukey HSD test. All bars represent the standard error of the mean ($n = 24$). *Zea mays* (Ze), *Phaseolus vulgaris* (Ph), *Glycine max* (Gl) and *Helianthus annuus* (He).

Supplementary Tables

Table S6.1 Soil N content (Mean $\pm$ SE) legacies after the training-phase 1 (¥change) and training-phase 2 (\$change) from a plant-soil feedback experiment (Mushonga et al., 2020). *Zea mays* (Ze), *Phaseolus vulgaris* (Ph), *Glycine max* (Gl) and *Helianthus annuus* (He). N = 3 composite samples. SE = standard error.

Soil origin	Ammonium-Nitrogen (mg/l)	Nitrate-Nitrogen (mg/l)
ZeZe\$	0.29 $\pm$ 0.071	0.767 $\pm$ 0.079
ZePh\$	0.177 $\pm$ 0.019	0.143 $\pm$ 0.052
ZeHe\$	0.347 $\pm$ 0.075	0.103 $\pm$ 0.052
ZeGl\$	0.195 $\pm$ 0.068	0.255 $\pm$ 0.135
PhZe\$	0.36 $\pm$ 0.064	0.093 $\pm$ 0.038
PhPh\$	0.483 $\pm$ 0.088	0.323 $\pm$ 0.198
PhHe\$	0.297 $\pm$ 0.018	0.017 $\pm$ 0.003
PhGl\$	0.417 $\pm$ 0.038	0.043 $\pm$ 0.029
HeZe\$	0.337 $\pm$ 0.111	0.107 $\pm$ 0.078
HePh\$	0.47 $\pm$ 0.05	0.537 $\pm$ 0.277
HeHe\$	0.135 $\pm$ 0.01	0.104 $\pm$ 0.008
HeGl\$	0.36 $\pm$ 0.055	0.027 $\pm$ 0.012
GlZe\$	0.36 $\pm$ 0.021	0.43 $\pm$ 0.158
GlPh\$	0.497 $\pm$ 0.038	0.53 $\pm$ 0.212
GlHe\$	0.313 $\pm$ 0.037	0.01 $\pm$ 0.00
GlGl\$	0.325 $\pm$ 0.037	0.112 $\pm$ 0.042
Ctrl¥	0.743 $\pm$ 0.041	3.87 $\pm$ 1.14
Ze¥	0.188 $\pm$ 0.029	0.405 $\pm$ 0.133
Ph¥	0.407 $\pm$ 0.024	0.843 $\pm$ 0.284
He¥	0.37 $\pm$ 0.03	0.053 $\pm$ 0.026
Gl¥	0.45 $\pm$ 0.055	0.407 $\pm$ 0.236

Table S6.2 Kruskal-Wallis test for significant differences in macronutrient composition of plant tissue after cultivation of phytometers in; field soil (‡), sterilized soil (£) and (ii) inoculated soil (§) under greenhouse conditions. $n_1 = 6$, $N_1 = 18$, $n_2 = n_3 = 5$, $N_2 = N_3 = 15$. Asterisk (*) test statistic (H) at $(n - 1)$ degrees of freedom (d.f.) represent significant p values that are $< .05$. Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S).

	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Effect	H, d.f.	H, d.f.	H, d.f.	H, d.f.	H, d.f.	H, d.f.
Plant origin (‡)	15.348*, 5	15.913*, 5	14.793*, 5	16.175*, 5	12.700*, 5	11.0130, 5
Plant origin (£)	10.367*, 4	12.517*, 4	11.233*, 4	12.923*, 4	11.654*, 4	11.229*, 4
Plant origin (§)	12.433*, 4	12.928*, 4	11.379*, 4	12.900*, 4	12.969*, 4	11.214*, 4

Table S6.3 Kruskal-Wallis test for significant differences in micronutrient composition of plant tissue after cultivation of phytometers in; field soil (‡), sterilized soil (£) and inoculated soil (§) under greenhouse conditions. $n_1 = 6$, $N_1 = 18$, $n_2 = n_3 = 5$, $N_2 = N_3 = 15$. Asterisk (*) test statistic (H) at $(n - 1)$ degrees of freedom (d.f.) represents significant p values that are $< .05$. Sodium (Na), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), molybdenum (Mo) and boron (B).

	Na mg/kg	Fe mg/kg	Mn mg/kg	Cu mg/kg	Zn mg/kg	Mo mg/kg	B mg/kg
Effect	H, d.f.	H, d.f.	H, d.f.	H, d.f.	H, d.f.	H, d.f.	H, d.f.
Plant origin (‡)	10.568, 5	14.694*, 5	15.737*, 5	16.801*, 5	14.638*, 5	16.158*, 5	14.752, 5
Plant origin (£)	11.713*, 4	10.577*, 4	12.633*, 4	12.299*, 4	11.972*, 4	12.848*, 4	12.380*, 4
Plant origin (§)	6.895, 4	9.233, 4	12.367*, 4	12.998*, 4	12.299*, 4	13.500*, 4	12.324*, 4

Table S6.4 Kruskal-Wallis test for significant differences in macronutrient composition of plant tissue after cultivation of *Zea mays* phytometer in; field soil (‡), sterilized soil (£) and inoculated soil (§) under greenhouse conditions. $n = 6$, $N = 18$. Asterisk (*) test statistic (H) at $(n - 1)$ degrees of freedom (d.f.) represent significant p values that are $< .05$. Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S).

	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Effect	H, d.f.	H, d.f.	H, d.f.	H, d.f.	H, d.f.	H, d.f.
Plant origin (‡,£,§)	9.615, 5	10.994, 5	7.837, 5	10.063, 5	13.609*, 5	1.438, 5

Table S6.5 Kruskal-Wallis test for significant differences in micronutrient composition of plant tissue after cultivation of *Zea mays* phytometer in; field soil (‡), sterilized soil (£) and inoculated soil (§) under greenhouse conditions n = 6, N = 18. Asterisk (*) test statistic (*H*) at (n – 1) degrees of freedom (d.f.) represent significant *p* values that are < .05. Sodium (Na), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), molybdenum (Mo) and boron (B).

	Na mg/kg	Fe mg/kg	Mn mg/kg	Cu mg/kg	Zn mg/kg	Mo mg/kg	B mg/kg
Effect	<i>H</i> , d.f.	<i>H</i> , d.f.	<i>H</i> , d.f.	<i>H</i> , d.f.	<i>H</i> , d.f.	<i>H</i> , d.f.	<i>H</i> , d.f.
Plant origin (‡,£,§)	9.615, 5	8.602, 5	6.369, 5	4.250, 5	9.833, 5	7.773, 5	4.567, 5

Table S6.6 Kruskal-Wallis test for significant differences in macronutrient composition of plant tissue after cultivation of *Phaseolus vulgaris* phytometer in; field soil (‡), sterilized soil (£) and inoculated soil (§) under greenhouse conditions. n = 3, N = 9. Asterisk (*) test statistic (*H*) at (n – 1) degrees of freedom (d.f.) represent significant *p* values that are < .05. Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S).

	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Effect	<i>H</i> , d.f.	<i>H</i> , d.f.	<i>H</i> , d.f.	<i>H</i> , d.f.	<i>H</i> , d.f.	<i>H</i> , d.f.
Plant origin (‡,£,§)	5.422, 2	3.832, 2	3.467, 2	1.422, 2	1.390, 2	2.979, 2

Table S6.7 Kruskal-Wallis test for significant differences in micronutrient composition of plant tissue after cultivation of *Phaseolus vulgaris* phytometer in; field soil (‡), sterilized soil (£) and inoculated soil (§) under greenhouse conditions n = 3, N = 9. Asterisk (*) test statistic (*H*) at (n – 1) degrees of freedom (d.f.) represent significant *p* values that are < .05. Sodium (Na), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), molybdenum (Mo) and boron (B).

	Na mg/kg	Fe mg/kg	Mn mg/kg	Cu mg/kg	Zn mg/kg	Mo mg/kg	B mg/kg
Effect	<i>H</i> , d.f.	<i>H</i> , d.f.	<i>H</i> , d.f.	<i>H</i> , d.f.	<i>H</i> , d.f.	<i>H</i> , d.f.	<i>H</i> , d.f.
Plant origin (‡,£,§)	0.157, 2	5.067, 2	4.356, 2	< 0.001, 2	0.874, 2	1.867, 2	1.175, 2

Table S6.8 Kruskal-Wallis test for significant differences in macronutrient composition of plant tissue after cultivation of *Glycine max* phytometer in; field soil (‡), sterilized soil (£) and inoculated soil (§) under greenhouse conditions. n = 4, N = 12. Asterisk (*) test statistic (*H*) at (n – 1) degrees of freedom (d.f.) represent significant *p* values that are < .05. Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S).

	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Effect	<i>H</i> , d.f.	<i>H</i> , d.f.	<i>H</i> , d.f.	<i>H</i> , d.f.	<i>H</i> , d.f.	<i>H</i> , d.f.
Plant origin (‡,£,§)	7.513, 3	8.135*, 3	9.464*, 3	8.774*, 3	9.497*, 3	1.963, 3

Table S6.9 Kruskal-Wallis test for significant differences in micronutrient composition of plant tissue after cultivation of *Glycine max* phytometer in; field soil (‡), sterilized soil (£) and inoculated soil (§) under greenhouse conditions n = 4, N = 12. Asterisk (*) test statistic (*H*) at (n – 1) degrees of freedom (d.f.) represent significant *p* values that are < .05. Sodium (Na), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), molybdenum (Mo) and boron (B).

	Na mg/kg	Fe mg/kg	Mn mg/kg	Cu mg/kg	Zn mg/kg	Mo mg/kg	B mg/kg
Effect	<i>H</i>, d.f.	<i>H</i>, d.f.	<i>H</i>, d.f.	<i>H</i>, d.f.	<i>H</i>, d.f.	<i>H</i>, d.f.	<i>H</i>, d.f.
Plant origin (‡,£,§)	2.664, 3	6.662, 3	7.513, 3	3.000, 3	2.449, 3	7.732, 3	6.029, 3

Table S6.10 Kruskal-Wallis test for significant differences in macronutrient composition of plant tissue after cultivation of *Helianthus annuus* phytometer in; field soil (‡), sterilized soil (£) and inoculated soil (§) under greenhouse conditions. n = 3, N = 9. Asterisk (*) test statistic (*H*) at (n – 1) degrees of freedom (d.f.) represent significant *p* values that are < .05. Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S).

	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Effect	<i>H</i>, d.f.	<i>H</i>, d.f.	<i>H</i>, d.f.	<i>H</i>, d.f.	<i>H</i>, d.f.	<i>H</i>, d.f.
Plant origin (‡,£,§)	5.647, 2	4.475, 2	2.756, 2	0.089, 2	5.535, 2	5.429, 2

Table S6.11 Kruskal-Wallis test for significant differences in micronutrient composition of plant tissue after cultivation of *Helianthus annuus* phytometer in; field soil (‡), sterilized soil (£) and inoculated soil (§) under greenhouse conditions n = 3, N = 9. Asterisk (*) test statistic (*H*) at (n – 1) degrees of freedom (d.f.) represent significant *p* values that are < .05. Sodium (Na), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), molybdenum (Mo) and boron (B).

	Na mg/kg	Fe mg/kg	Mn mg/kg	Cu mg/kg	Zn mg/kg	Mo mg/kg	B mg/kg
Effect	<i>H</i>, d.f.	<i>H</i>, d.f.	<i>H</i>, d.f.	<i>H</i>, d.f.	<i>H</i>, d.f.	<i>H</i>, d.f.	<i>H</i>, d.f.
Plant origin (‡,£,§)	3.854, 2	5.956, 2	3.467, 2	2.740, 2	4.356, 2	1.867, 2	5.600, 2

Supplementary Material for Chapter 7

Supplementary Figures

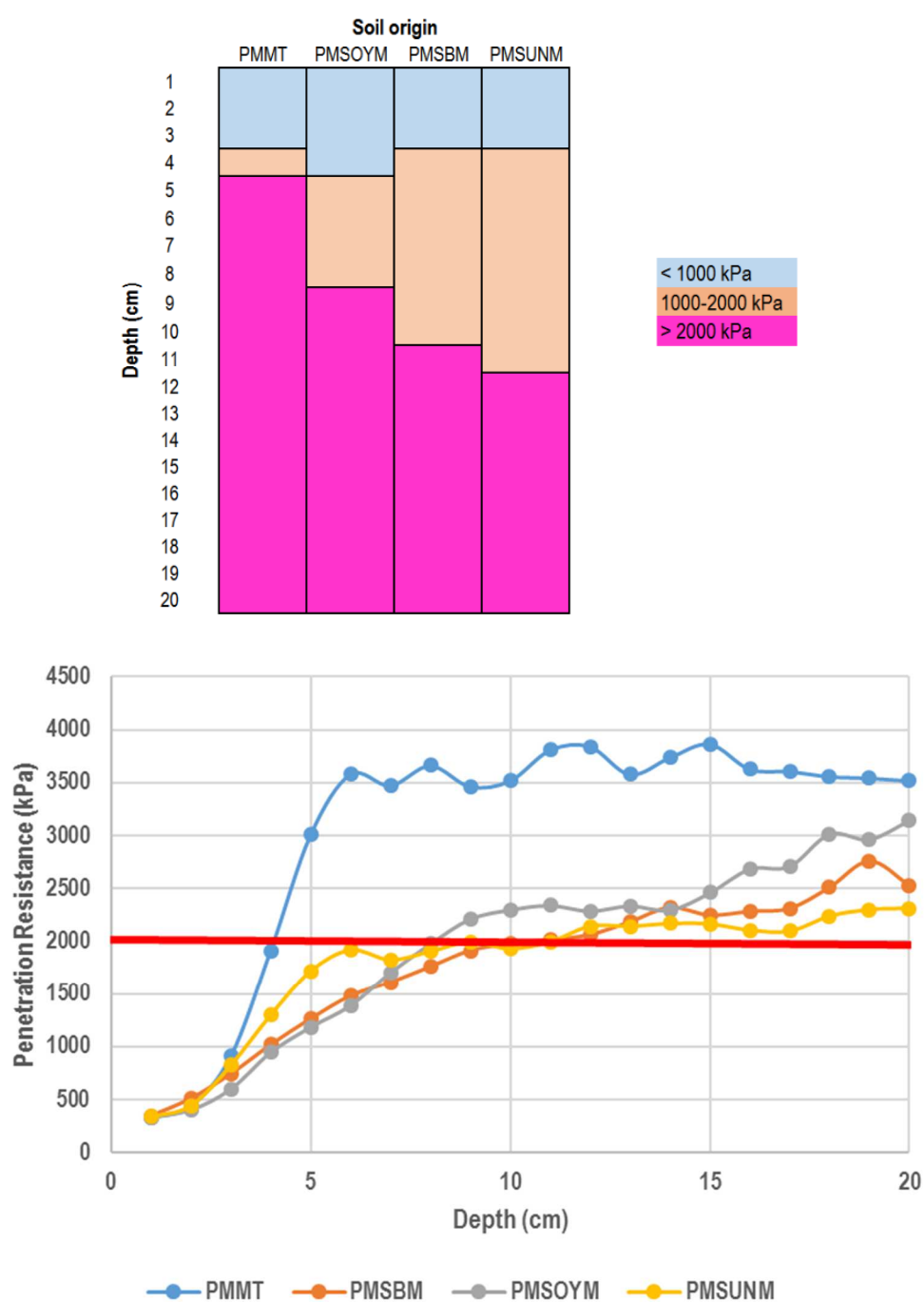


Figure S7.1 Crop sequence effects on soil strength (kPa, penetrometer resistance). The different colours represent different soil strength ranges, as indicated by the legend. Values represent averages across replications per treatment. PMMT – Potatoes-Maize-Maize-Teff, PMSBM – Potatoes-Maize-Sugar beans-Maize, PMSOYM – Potatoes-Maize-Soybeans-Maize, and PMSUNM – Potatoes-Maize-Sunflower-Maize rotations.

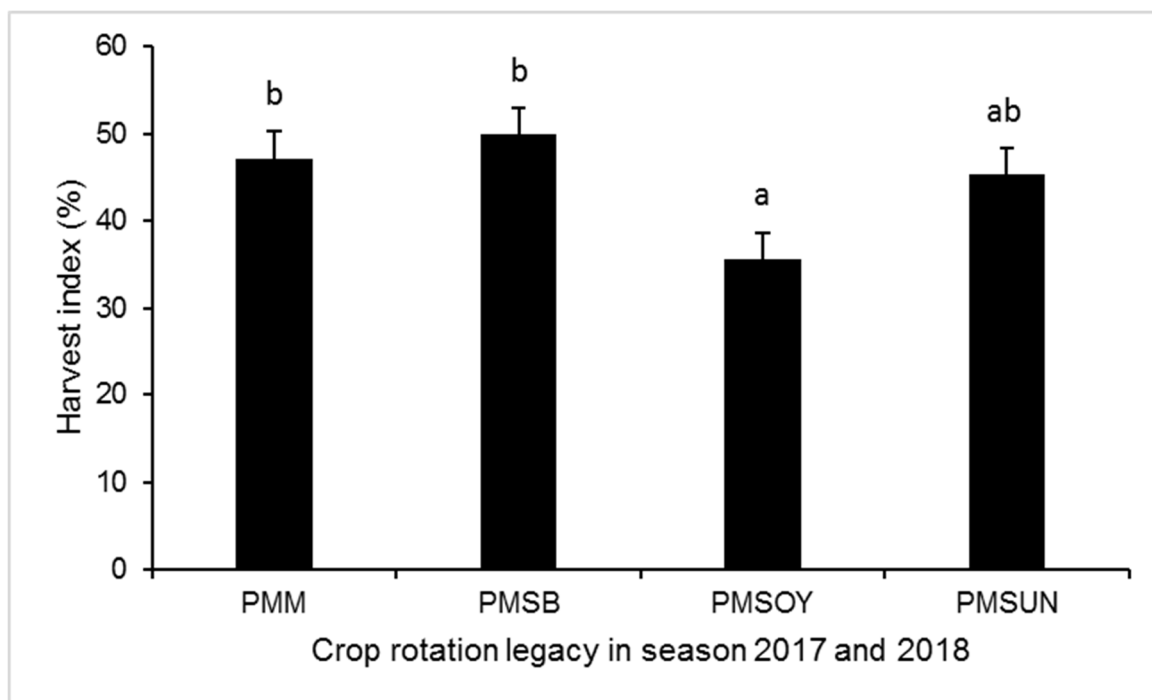


Figure S7.2 Relationships between harvest index (%) of combined season 2017 and season 2018 biomass legacies of dryland crop rotations. PMM: Potatoes-Maize-Maize, PMSB: Potatoes-Maize-Sugar bean, PMSOY: Potatoes-Maize-Soybean and PMSUN: Potatoes-Maize-Sunflower. Different letters represent significant differences ($p < .05$) among crop rotation combinations while shared letters represents non-significant differences ($p > .05$) among crop rotation combinations based on one-way ANOVA using the Tukey HSD test *post hoc* multiple comparisons. All bars represent the standard error of the mean ($n = 12$).

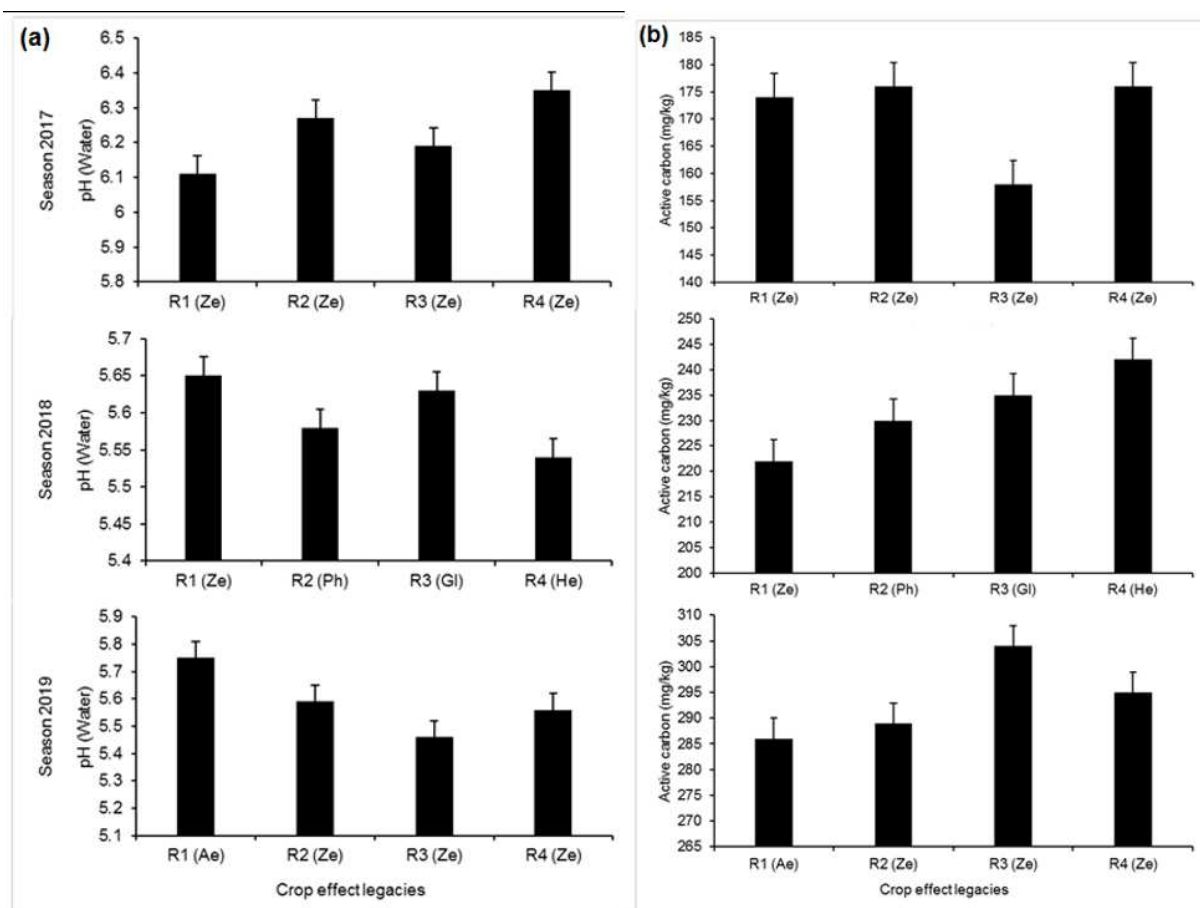


Figure S7.3A The impact of crop species legacies on soil (a) pH and (b) active C from seasons 2017 to 2019. *Zea mays* (Ze), *P. vulgaris* (Ph), *H. annuus* (He), *G. max* (Gl) and *Eragrostis tef* (Ae). All bars represent the standard error of the mean (n =8).

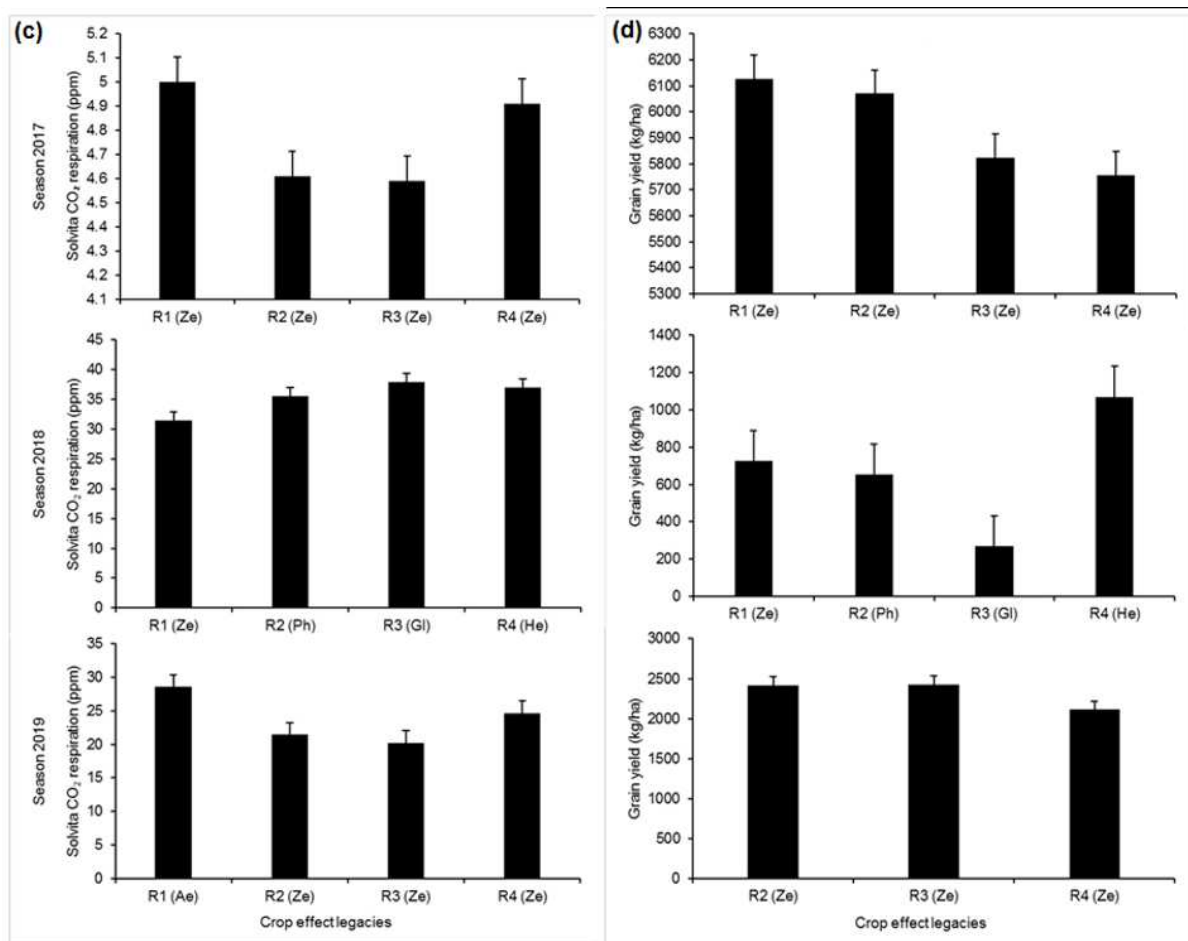


Figure S7.3B The impact of crop species legacies on soil (c) Solvita microbial respiration and (d) maize test crop grain yields from seasons 2017 to 2019. *Zea mays* (Ze), *P. vulgaris* (Ph), *H. annuus* (He), *G. max* (Gl) and *Eragrostis tef* (Ae). All bars represent the standard error of the mean (n=8).

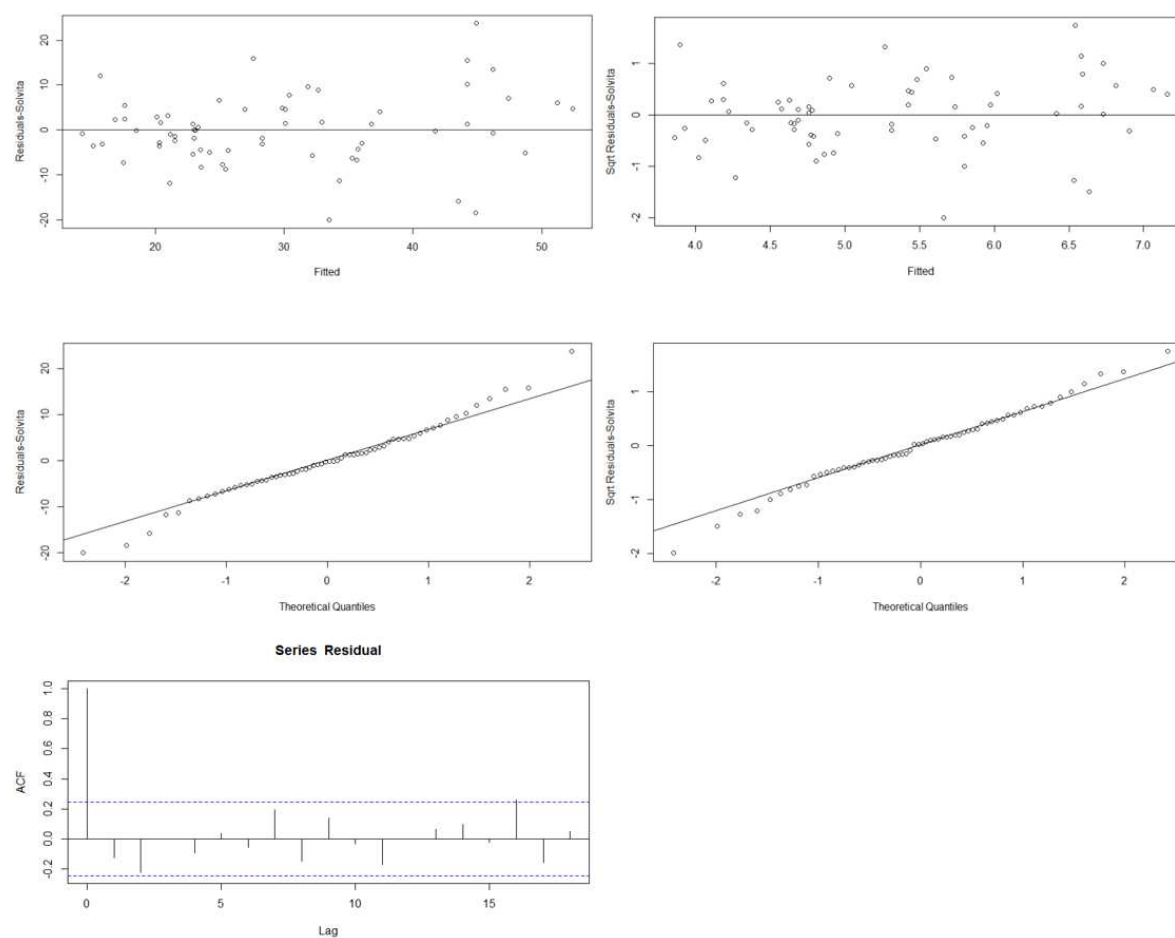


Figure S7.4A Residual, Q-Q and ACF plots of the unstandardized and standardized fitted Solvita soil respiration values.

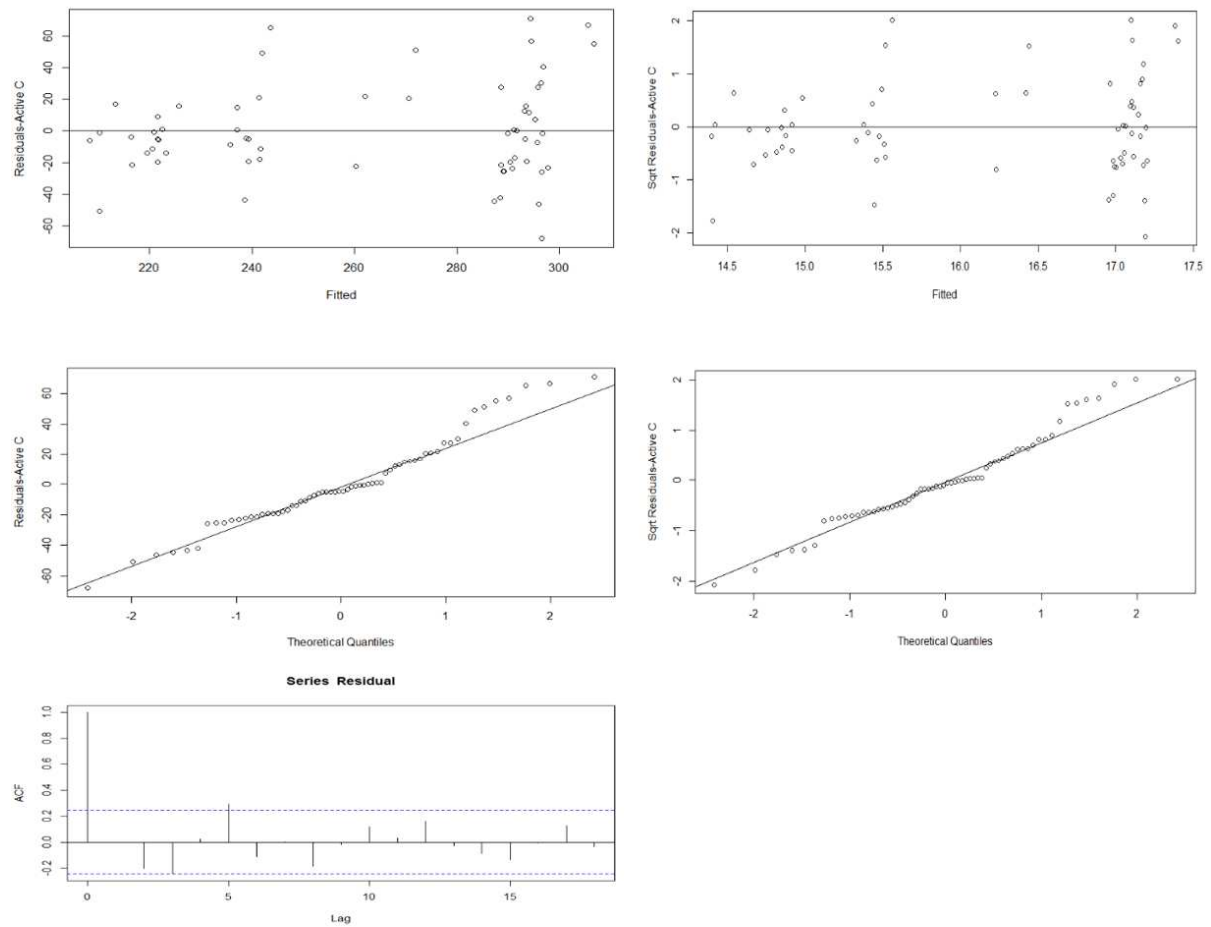


Figure S7.4B Residual, Q-Q and ACF plots of the unstandardized and standardized fitted soil active C values.

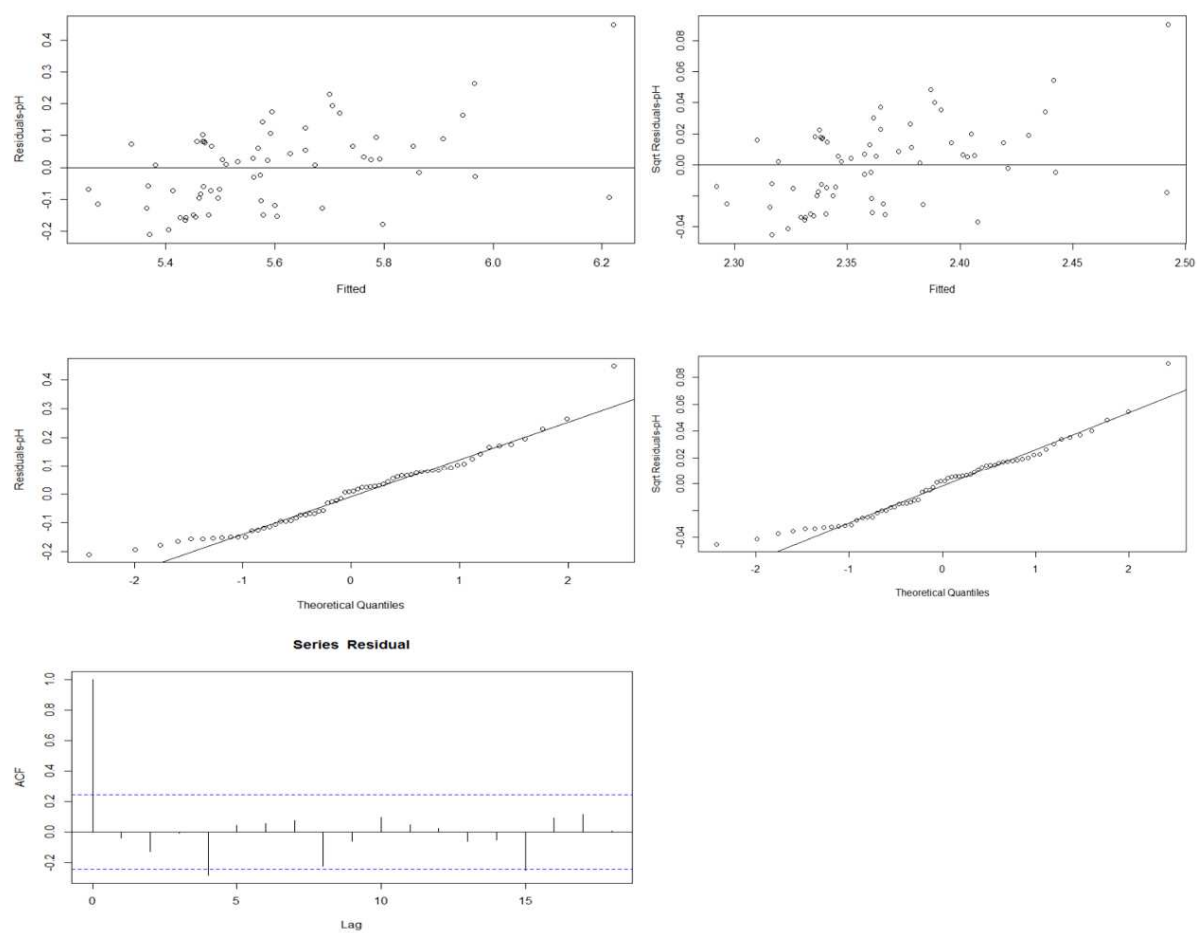


Figure S7.4C Residual, Q-Q and ACF plots of the unstandardized and standardized fitted soil pH values.

Supplementary Tables

Table S7.1 Fertilization regime (N – nitrogen, P – phosphorus, K – potassium) for the crop rotation field experiment from season 2017 to season 2019.

Crop	N	P	K	Row spacing	Population	Variable cost	Over-heads [¥]	Returns
	Kg/ha			m	Plants/ha	Rands/ha		Rands/tonne
Maize 2017	90	22.5	6	0.91	28000	6408	3434	1977
Maize 2018	100	24	6	0.91	28000	5755	6317	1808
Sugar bean 2018	45	27	27	0.91	120000	10693	1808	13000
Soybean 2018	45	27	27	0.91	240000	4002	4438	4800
Sunflower 2018	100	24	6	0.91	45000	4002	4438	4494
Maize 2019	120	20	6	0.91	32000	6317	5755	2300
Teff 2019	83	8	0	Broad- cast	7 kg/ha	2800	1500	R50/25kg bale
[¥] hire, administration, labour, salary								

Table S7.2 The seasonal climate conditions in the Eastern Free State field trial from 2017 to 2019.

Parameter	Mean 2017/2018/2019	Maximum 2017/2018/2019	Minimum 2017/2018/2019
Maximum temperature (°C)	23.9/23.9/25.7	33.8/34.8/35.7	6.89/8.83/8.49
Total rainfall(mm)	772*/706*/539*	62.8/50.9/41.5	0.00/0.00/0.00
Maximum relative humidity (%)	90/89.1/88.1	100/100/99.8	45.4/38.3/26.0
Total solar radiation (MJ/m ²)	19.2/19.3/21.5	32.5/33.1/33.3	3.65/1.77/2.89
Daily evaporative demand (mm)	3.74/3.80/4.49	7.65/9.25/9.86	0.228/0.202/0.404
Wind speed (m/s)	3.43/3.76/4.03	11/12/10.5	0.724/0.00/0.753
Vapour pressure deficit (kPa)	0.78/0.812/0.98	1.91/2.20/2.51	0.009/0.058/0.068

*represents total and not the average value

Table S7.3 Descriptive statistics of physical indicators of soil quality measured at 0–20 cm depth in four rotation sequence treatments measured at harvest in season 3 (year 2018/19). Bulk density is generally moderate across rotation legacies. PMMT – Potatoes-Maize-Maize-Teff, PMSBM – Potatoes-Maize-Sugar beans-Maize, PMSOYM – Potatoes-Maize-Soybeans-Maize, and PMSUNM – Potatoes-Maize-Sunflower-Maize rotations. Each rotation sequence treatment had 8 replications. Each block had 4 treatments. SEM = standard error of mean, CV = coefficient of variation (%).

Properties	Rotation	Mean	Median	Minimum	Maximum	SEM	CV
Density at 20 cm soil depth (g/cm ³)	PMMT	1.55	1.56	1.48	1.62	0.04	5
	PMSBM	1.32	1.32	1.29	1.36	0.02	3
	PMSOYM	1.40	1.38	1.40	1.47	0.04	4
	PMSUNM	1.45	1.44	1.35	1.50	0.03	3
Density at 40 cm soil depth (g/cm ³)	PMMT	1.54	1.53	1.50	1.57	0.02	2
	PMSBM	1.58	1.61	1.47	1.67	0.06	6
	PMSOYM	1.55	1.61	1.42	1.61	0.06	7
	PMSUNM	1.47	1.44	1.40	1.56	0.05	6
Soil strength (kPa, penetrometer resistance)	PMMT	3520	3600	2362	4837	438	28
	PMSBM	2528	2050	1125	5275	338	55
	PMSOYM	3144	3437	1512	4812	277	34
	PMSUNM	2312	2337	1012	4025	178	34

Table S7.4 Biological and physicochemical properties of sandy (%Clay 8.94 ± 0.18 , %Silt 9.80 ± 0.24 , and % Sand 81.26 ± 0.26) topsoil collected at 0–20 cm depth from a crop rotation field trial. Phosphorus Bray 2 – PBR2, Mehlich III –M3, Solvita CO₂ respiration – Solvita, Ammonium nitrogen – Ammonium-N, Nitrate nitrogen – Nitrate-N, PMMT – Potatoes-Maize-Maize-Teff, PMSBM – Potatoes-Maize-Sugar beans-Maize, PMSOYM – Potatoes-Maize-Soybeans-Maize, and PMSUNM – Potatoes-Maize-Sunflower-Maize rotations. The field is located in a grassland biome of the Eastern Free State province of South Africa. N = 8 composite samples. SE = standard error.

Soil factor	Soil origin (Mean $\pm$ SE)				
	Bulk soil	PMMT	PMSBM	PMSOYM	PMSUNM
Density (g/cm ³)	1.43 $\pm$ 0.014	1.35 $\pm$ 0.036	1.38 $\pm$ 0.009	1.39 $\pm$ 0.006	1.39 $\pm$ 0.011
pH (H ₂ O)	5.55 $\pm$ 0.035	5.75 $\pm$ 0.170	5.59 $\pm$ 0.123	5.46 $\pm$ 0.078	5.56 $\pm$ 0.026
Phosphorus Mehlich 3 (mg/kg)	45.60 $\pm$ 1.85	36.40 $\pm$ 1.74	40.10 $\pm$ 2.28	43.60 $\pm$ 2.15	46.10 $\pm$ 2.00
Phosphorus Bray 2 (mg/kg)	46.50 $\pm$ 2.81	38.60 $\pm$ 3.29	44.30 $\pm$ 2.23	46.50 $\pm$ 1.93	49.10 $\pm$ 2.23
Potassium (mg/kg)	140.00 $\pm$ 7.30	129.00 $\pm$ 11.2	96.90 $\pm$ 7.83	85.80 $\pm$ 6.73	127.00 $\pm$ 7.68
Ammonium-Nitrogen (mg/l)	0.556 $\pm$ 0.02	0.365 $\pm$ 0.029	0.380 $\pm$ 0.022	0.371 $\pm$ 0.031	0.588 $\pm$ 0.156
Nitrate-Nitrogen (mg/l)	0.909 $\pm$ 0.148	1.64 $\pm$ 0.167	1.29 $\pm$ 0.168	2.29 $\pm$ 0.466	2.780 $\pm$ 1.60
Active-carbon (mg/kg)	212.00 $\pm$ 20.3	286.00 $\pm$ 8.96	289.00 $\pm$ 13.9	304.00 $\pm$ 15.1	295.00 $\pm$ 14.2
Solvita (ppm)	24.50 $\pm$ 3.87	28.50 $\pm$ 6.83	21.40 $\pm$ 3.06	20.20 $\pm$ 1.74	24.60 $\pm$ 2.86
Soil organic matter (%)	0.263 $\pm$ 0.018	0.345 $\pm$ 0.017	0.346 $\pm$ 0.015	0.294 $\pm$ 0.020	0.320 $\pm$ 0.019

Table S7.5 Negative Binomial regression model (a) Goodness-of-Fit test indicating the reduction of overdispersion using the Deviance ratio and (b) Parameter Estimates used in the building of Equations 7.4 and 7.5 using the generalized model of Equation 7.2. df represents degrees of freedom, SE is the standard error, k is an estimate of the dispersion coefficient.

(a) Goodness-of-fit						
Description				Value	Value/df	
Deviance				104.096	1.170	
Pearson Chi-Square				64.971	0.730	
Log-likelihood				-292.539		
Akaike’s Information Criterion (AIC)				599.079		
Bayesian Information Criterion (BIC)				617.029		
(b) Parameter Estimates						
Parameter	Estimate	SE	Wald	df	P value	Incident rate
Intercept	2.274	0.3716	37.459	1	< 0.0001	9.721
R1	0.681	0.6249	1.187	1	0.276	1.975
R2	0.189	0.4680	0.164	1	0.686	1.209
R3	0.057	0.4674	0.015	1	0.904	1.058
R4	0 ^a	-	-	-	-	1
W1	-1.170	0.5631	4.318	1	0.038	0.310
W2	0.035	0.4036	0.008	1	0.930	1.036
W3	0 ^a	-	-	-	-	1
K	2.452	0.4321				

^a Superscript – parameter set to zero because it is redundant.

Table S7.6 Analysing the soil health factors for crop rotation sequences. All F-statistics are based on 3 and 28 degrees of freedom for crop rotation treatment and error, respectively. Aims 1 to 3 we used one-way ANOVA for statistical analysis while for Aim 4, the Pillai's criterion

in estimating the MANOVA was used. Asterisk (*) *p*-values indicate a significant difference ($p < 0.05$).

Soil factor[§]	F	<i>p</i>
Solvita CO ₂ respiration (ppm)		
Aim 1	0.5259	0.6681
Aim 2	0.4325	0.7314
Aim 3	1.1010	0.3652
Aim 4	0.4133	0.9247
Active carbon (mg/kg)		
Aim 1	0.3298	0.8038
Aim 2	0.4716	0.7045
Aim 3	0.1774	0.9108
Aim 4	0.3951	0.9343
pH(H ₂ O)		
Aim 1	1.0890	0.3699
Aim 2	0.4641	0.7097
Aim 3	1.518	0.2314
Aim 4	0.6975	0.7094
1:2 Water extract N - NO ₃ (mg/l)		
Aim 1	0.9056	0.4508
Aim 2	0.1004	0.9591
Aim 3	0.3264	0.8062
Aim 4	0.3493	0.9553
1:2 Water extract N - NH ₄ (mg/l)		
Aim 1	2.136	0.1181
Aim 2	0.2919	0.8309
Aim 3	0.117	0.9494
Aim 4	1.6335	0.1189
K (mg/kg)		
Aim 1	6.382	0.0020*
Aim 2	4.903	0.0073*
Aim 3	7.562	0.0074*
Aim 4	3.442	0.0012*
P (mg/kg)		
Aim 1	4.316	0.0127*
Aim 2	1.986	0.139
Aim 3	2.748	0.0615
Aim 4	2.122	0.0363*
P Bray 2 (mg/kg)		
Aim 1	3.688	0.0235*
Aim 2	0.5337	0.6629
Aim 3	2.258	0.1036
Aim 4	1.4207	0.1924
Na (mg/kg)		
Aim 1	0.6979	0.5612
Aim 2	0.1595	0.9227
Aim 3	2.169	0.1139
Aim 4	1.7365	0.0933
Table S7.6 continued		
Mg (mg/kg)		
Aim 1	2.426	0.0865
Aim 2	1.829	0.1648
Aim 3	1.515	0.2324

Aim 4	1.5624	0.1400
Ca (mg/kg)		
Aim 1	0.6117	0.6131
Aim 2	1.223	0.3197
Aim 3	0.9759	0.4181
Aim 4	0.7577	0.6553
Density (g/cm ³)		
Aim 1	1.18	0.3351
Aim 2	3.516	0.0279*
Aim 3	2.224	0.1075
Aim 4	2.9052	0.0048*
B (mg/kg)		
Aim 1	0.5323	0.6639
Aim 2	0.9204	0.4437
Aim 3	0.2518	0.8593
Aim 4	1.3268	0.2356
Cu (mg/kg)		
Aim 1	0.2261	0.8774
Aim 2	0.2465	0.8631
Aim 3	0.6119	0.6129
Aim 4	0.3782	0.9425
Fe (mg/kg)		
Aim 1	0.4664	0.708
Aim 2	0.6725	0.5761
Aim 3	0.1292	0.9419
Aim 4	0.4590	0.8979
Mn (mg/kg)		
Aim 1	0.4406	0.7258
Aim 2	0.5858	0.6293
Aim 3	0.1126	0.952
Aim 4	0.4908	0.877
Zn (mg/kg)		
Aim 1	8.907	< 0.001*
Aim 2	2.583	0.0733
Aim 3	5.835	0.0032*
Aim 4	3.000	0.0038*

[§]Aim 1- to determine the impact of crop rotation sequence on legacy of soil health factor(s) after three seasons i.e. at the end of season 2019. Aim 2- to determine the fastest rate of change in soil health factor(s) over the first two seasons (difference between seasons 2017 and 2018). Aim 3- to determine the impact of grasses on rate of change in soil health factor(s) over the last two seasons (difference between seasons 2018 and 2019). Aim 4- to test whether there were differences or no differences in soil health factor(s) across the three seasons among the four crop rotations.

Table S7.7 The results of the LMM results for the Solvita soil respiration legacies analysis. npar- nonparametric, AIC- Akaike information criterion, BIC- Bayesian information criterion,

LogLik- Maximum log-likelihood, Deviance - Minus twice maximum log-likelihood, Df - degrees of freedom.

Models	npar	AIC	BIC	LogLik	Deviance	(Calculation)	Df	P value
Model 7.1b	8	495.19	512.46	-239.6	479.19			
Model 7.1a	9	496.80	516.23	-239.4	478.80	0.3958 (479.19 – 478.08)	1	0.5292

Table S7.8 The results of the LMM results for the soil active C legacies analysis. npar- nonparametric, AIC- Akaike information criterion, BIC- Bayesian information criterion, LogLik- Maximum log-likelihood, Deviance - Minus twice maximum log-likelihood, Df - degrees of freedom.

Models	npar	AIC	BIC	LogLik	Deviance	(Calculation)	Df	P value
Model 7.2b	8	649.94	667.21	-316.97	633.94			
Model 7.2a	9	651.93	671.36	-316.97	633.93	0.0059 (633.94 – 633.93)	1	0.9386

Table S7.9 The results of the LMM results for the soil pH (water) legacies analysis. npar- nonparametric, AIC- Akaike information criterion, BIC- Bayesian information criterion, LogLik- Maximum log-likelihood, Deviance - Minus twice maximum log-likelihood, Df - degrees of freedom.

Models	npar	AIC	BIC	LogLik	Deviance	(Calculation)	Df	P value
Model 7.3b	8	22.167	39.438	-3.0836	6.1671			
Model 7.3a	9	23.700	43.130	-2.8500	5.7000	0.4671 (6.1671– 5.7000)	1	0.4943

Supplementary Material for Chapter 8

Supplementary Figures

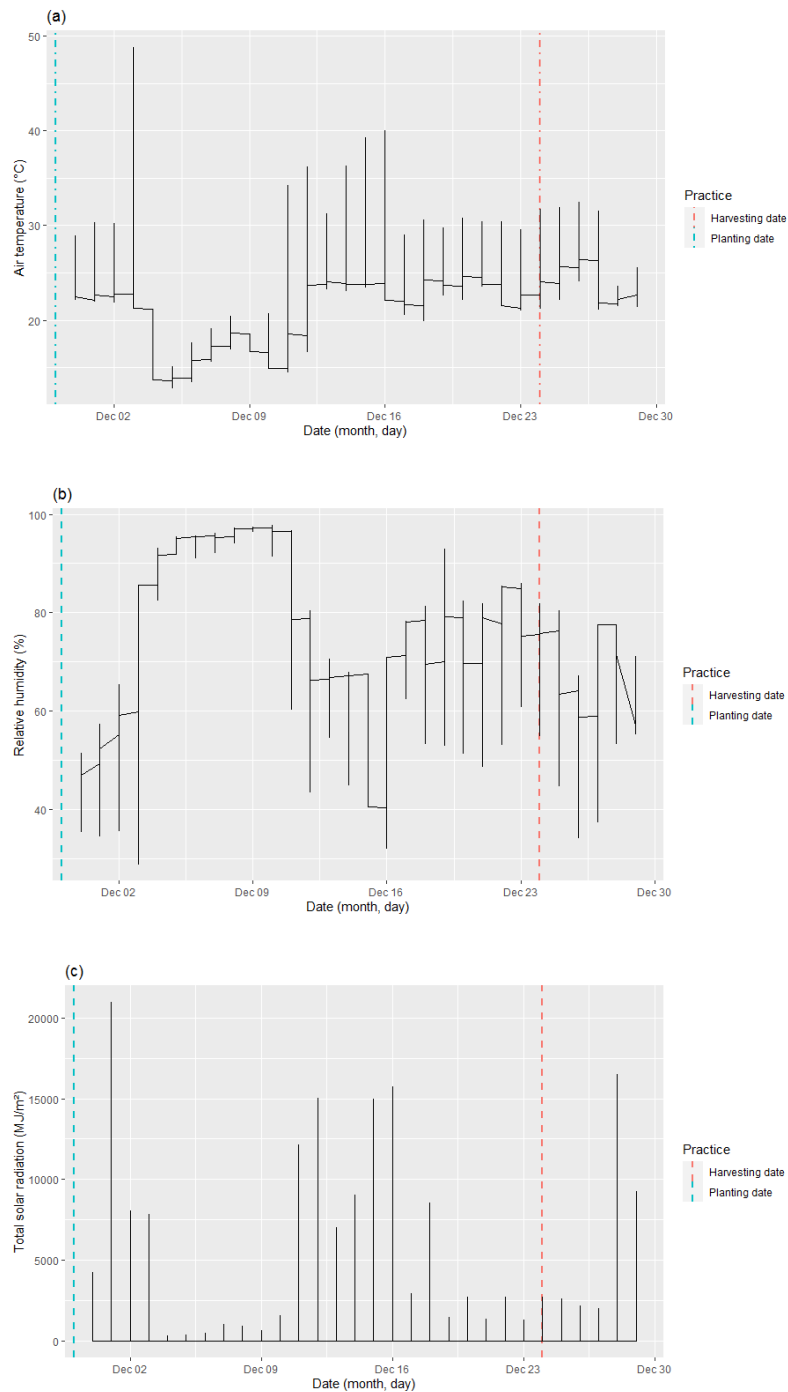


Figure S8.1 (a) Temperature, (b) Relative humidity and (c) Light intensity variables during the greenhouse experiments conducted at the Hillcrest Campus of the University of Pretoria between November and December 2019.

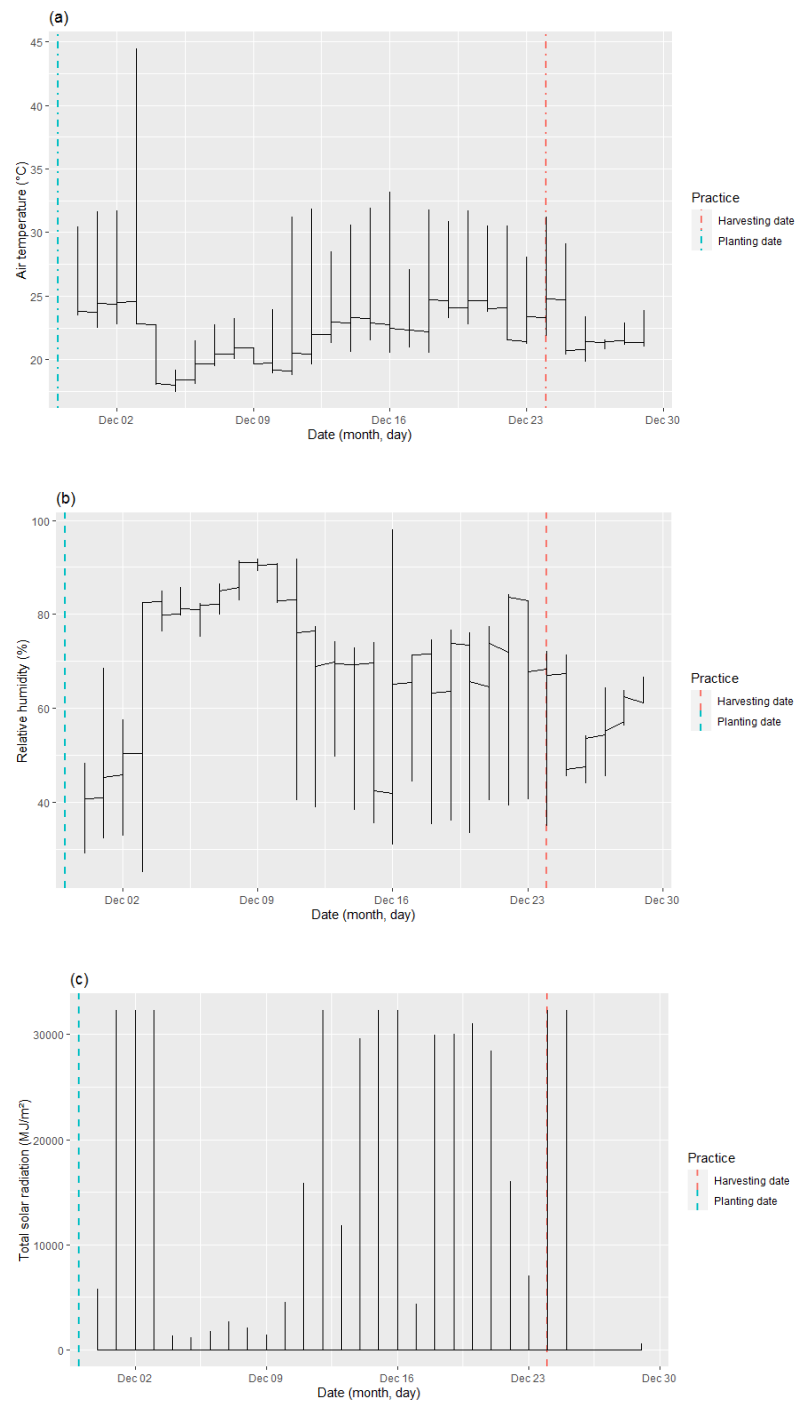


Figure S8.2 (a) Temperature, (b) Relative humidity and (c) Light intensity variables during the greenhouse experiments conducted at the Hatfield Campus of the University of Pretoria between November and December 2019.

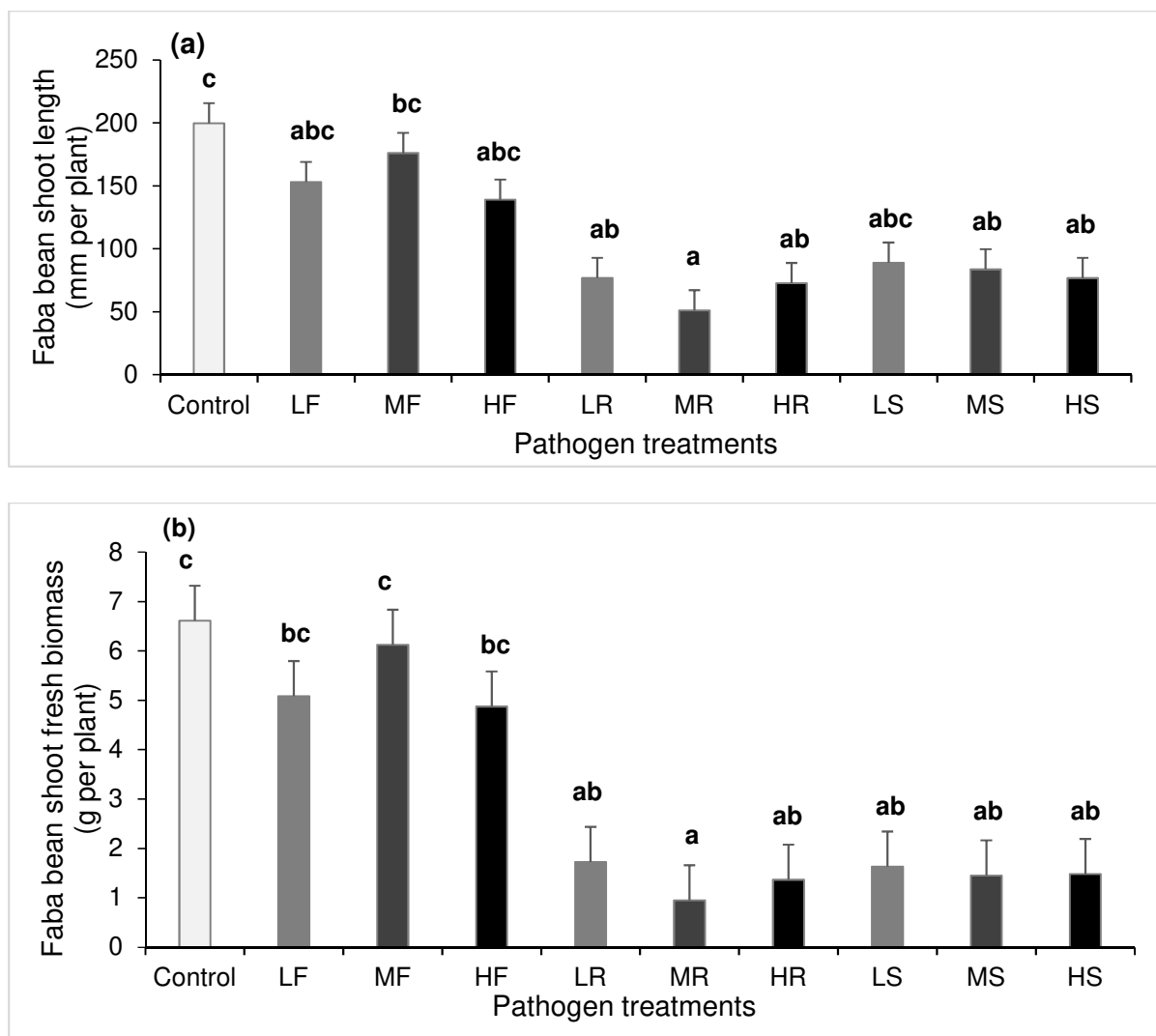


Figure S8.3A Mean differences in (a) shoot length and (b) shoot biomass response of faba bean plants grown in steam-sterilized sand soil with added artificial inoculum of *R. solani* AG 2-2 IIIB and *F. graminearum* under greenhouse conditions. LF – low inoculum soil for *Fusarium* disease, MF – medium inoculum soil for *Fusarium* disease, HF – high inoculum soil for *Fusarium* disease, LR – low inoculum soil for *Rhizoctonia* disease, MR – medium inoculum soil for *Rhizoctonia* disease, HR – high inoculum soil for *Rhizoctonia* disease, LS – low inoculum soil for *Fusarium* + *Rhizoctonia* coinfection disease, MS – medium inoculum soil for *Fusarium* + *Rhizoctonia* coinfection disease, HS – high inoculum soil for *Fusarium* + *Rhizoctonia* coinfection disease. Control represents pasteurized sand soil without artificial inoculum addition of pathogens. N = 64 per pathogen treatment, error bars represent standard error of the mean. Values with the same letter are not significantly different ($p > .05$) from one another according to the Tukey HSD test.

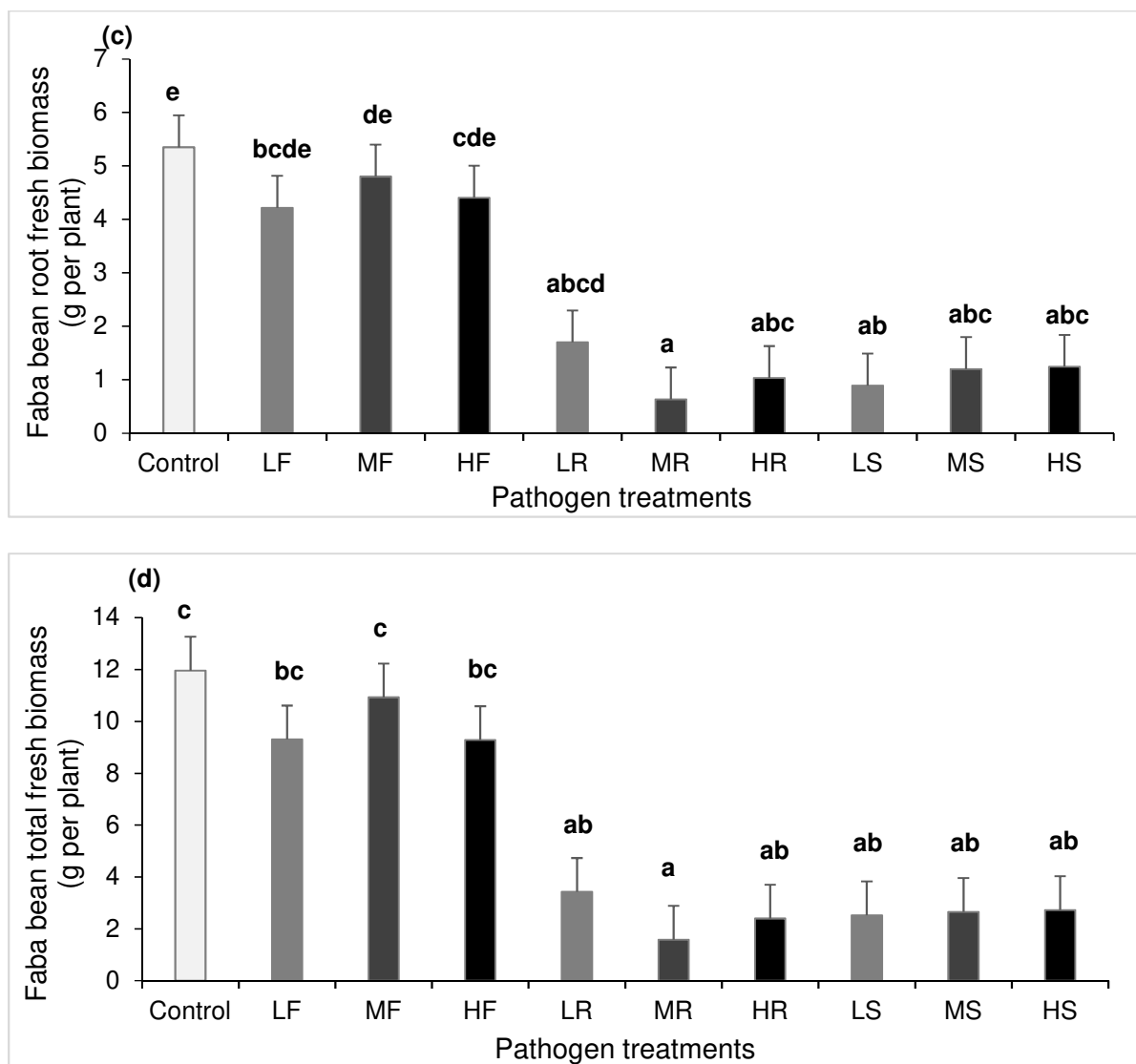


Figure S8.3B Mean differences in (c) root biomass and (d) total biomass response of faba bean plants grown in steam-sterilized sand soil with added artificial inoculum of *R. solani* AG 2-2 IIIB and *F. graminearum* under greenhouse conditions. LF – low inoculum soil for *Fusarium* disease, MF – medium inoculum soil for *Fusarium* disease, HF – high inoculum soil for *Fusarium* disease, LR – low inoculum soil for *Rhizoctonia* disease, MR – medium inoculum soil for *Rhizoctonia* disease, HR – high inoculum soil for *Rhizoctonia* disease, LS – low inoculum soil for *Fusarium* + *Rhizoctonia* coinfection disease, MS – medium inoculum soil for *Fusarium* + *Rhizoctonia* coinfection disease, HS – high inoculum soil for *Fusarium* + *Rhizoctonia* coinfection disease. Control represents pasteurized sand soil without artificial inoculum addition of pathogens. N = 64 per pathogen treatment, error bars represent standard error of the mean. Values with the same letter are not significantly different ($p > .05$) from one another according to the Tukey HSD test.

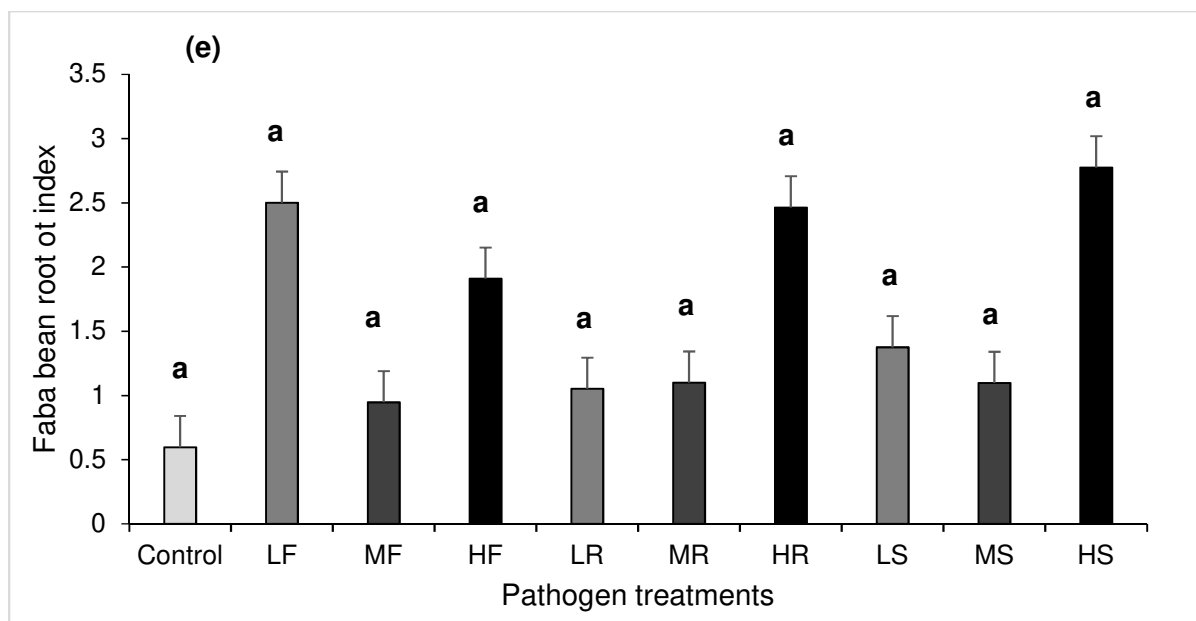


Figure S8.3C Mean differences in (e) root rot index response of faba bean plants grown in steam-sterilized sand soil with added artificial inoculum of *R. solani* AG 2-2 IIIB and *F. graminearum* under greenhouse conditions. LF – low inoculum soil for *Fusarium* disease, MF – medium inoculum soil for *Fusarium* disease, HF – high inoculum soil for *Fusarium* disease, LR – low inoculum soil for *Rhizoctonia* disease, MR – medium inoculum soil for *Rhizoctonia* disease, HR – high inoculum soil for *Rhizoctonia* disease, LS – low inoculum soil for *Fusarium* + *Rhizoctonia* coinfection disease, MS – medium inoculum soil for *Fusarium* + *Rhizoctonia* coinfection disease, HS – high inoculum soil for *Fusarium* + *Rhizoctonia* coinfection disease. Control represents pasteurized sand soil without artificial inoculum addition of pathogens. N = 64 per pathogen treatment, error bars represent standard error of the mean. Values with the same letter are not significantly different ($p > .05$) from one another according to the Tukey HSD test.

Supplementary Tables

Table S8.1 The interpretation of soil properties according to FERTASA (2016) guidelines for grains. The values outside the normal values indicate imbalances that can be expected in farming practice

Soil property	Low levels	Normal levels	High levels
pH (H ₂ O)	< 4	5.5 – 7.5	> 7.5
Phosphorus (mg/kg)	< 8	8 – 30	> 35
Potassium (mg/kg)	< 40	80 – 160	> 250
Calcium (mg/kg)	< 200	300 – 2000	> 3000
Magnesium (mg/kg)	< 50	80 – 300	> 300
Zinc (mg/kg)	< 2	2 – 5	> 4
Acid saturation (%)	Imbalances	5 – 30	Imbalances
Aluminium saturation (%)	Imbalances	< 1.5	Imbalances
Ca:Mg ratio	Imbalances	1.5 – 4.5	Imbalances
Mg:K ratio	Imbalances	3 – 4	Imbalances
(Ca+Mg)/K ratio	Imbalances	10 – 20	Imbalances
Base saturation (% of CEC)	Imbalances	70 – 95	Imbalances

Table S8.2 Spearman's rho correlation coefficients (r_s) for faba bean plant health data between the Hatfield experiment and the Hillcrest experiment. N = 96. The $p \leq .05$ values represent significant differences between experiments while $p > .05$ represents non-significant differences. Na – omitted from the analysis.

Variables	Statistic	Hatfield_shoot length	Hatfield_shoot biomass	Hatfield_root biomass	Hatfield_disease rating
Hillcrest_shoot length	r_s	-0.069	Na	Na	Na
	p	0.502			
Hillcrest_shoot biomass	r_s	Na	0.044	Na	Na
	p		0.671		
Hillcrest_root biomass	r_s	Na	Na	0.063	Na
	p			0.542	
Hillcrest_disease rating	r_s	Na	Na	Na	0.080
	p				0.441

Table S8.3 Impact of suppressiveness of crop rotations (PMMT, PMSBM, PMSOYM and PMSUNM) on *R. solani* AG 2-2 IIIB (*RS*) and *F. graminearum* (*FG*) effects and plant shoot length. *P* values < .05 represent significant differences. The *RS* post hoc of the Kruskal-Wallis test statistic = 9.849, degrees of freedom = 3, N = 50, *P* = .020. The *FG* post hoc of the Kruskal-Wallis test statistic *H* = 8.732, degrees of freedom = 3, N = 52, *P* = .033.

Sample 1 – Sample 2	<i>H</i>		<i>P</i> values	
	<i>RS</i>	<i>FG</i>	<i>RS</i>	<i>FG</i>
PMSOYM-PMSBM	3.875	12.375	1	.259
PMSOYM-PMMT	12.321	2.594	.173	1
PMSOYM-PMSUNM	-16.125	-8.417	.035	1
PMSBM-PMMT	8.446	14.969	.805	.054
PMSBM-PMSUNM	-12.250	-3.958	.218	1
PMMT-PMSUNM	-3.804	-11.010	1	.327

Table S8.4 Impact of suppressiveness of crop rotations (PMMT, PMSBM, PMSOYM and PMSUNM) on *R. solani* AG 2-2 IIIB (*RS*) and *F. graminearum* (*FG*) effects and plant shoot biomass. Significant *p* values are < .05. The *RS* post hoc of Kruskal-Wallis test statistic = 10.592, degrees of freedom = 3, N = 50, *P* = .014. The *FG* post hoc of the Kruskal-Wallis test statistic *H* = 11.094, degrees of freedom = 3, N = 52, *P* = .011.

Sample 1 – Sample 2	<i>H</i>		<i>P</i> values	
	<i>RS</i>	<i>FG</i>	<i>RS</i>	<i>FG</i>
PMSOYM-PMSBM	6.500	15.667	1	.062
PMSOYM-PMMT	14.262	0.146	.065	1
PMSOYM-PMSUNM	-16.792	-11.250	.023	.394
PMSBM-PMMT	7.762	15.812	.994	.034
PMSBM-PMSUNM	-10.292	-4.417	.459	1
PMMT-PMSUNM	-2.530	-11.396	1	.278

Table S8.5 Impact of suppressiveness of crop rotations (PMMT, PMSBM, PMSOYM and PMSUNM) on *R. solani* AG 2-2 IIIB (*RS*) and *F. graminearum* (*FG*) effects and plant root biomass. Significant *p* values are < .05. The *RS* post hoc of the Kruskal-Wallis test statistic = 9.822, degrees of freedom = 3, N = 50, *P* = .020. The *FG* post hoc of the Kruskal-Wallis test statistic *H* = 8.214, degrees of freedom = 3, N = 52, *P* = .042.

Sample 1 – Sample 2	<i>H</i>		<i>P values</i>	
	<i>RS</i>	<i>FG</i>	<i>RS</i>	<i>FG</i>
PMSOYM-PMSBM	3.792	12.917	1	.201
PMSOYM-PMMT	13.750	1.854	.079	1
PMSOYM-PMSUNM	-14.542	-7.250	.069	1
PMSBM-PMMT	9.958	14.771	.436	.056
PMSBM-PMSUNM	-10.750	-5.667	.371	1
PMMT-PMSUNM	-0.792	-9.104	1	.656

Table S8.6 Impact of suppressiveness of crop rotations (PMMT, PMSBM, PMSOYM and PMSUNM) on *R. solani* AG 2-2 IIIB (*RS*) and *F. graminearum* (*FG*) effects and whole plant biomass. Significant *p* values are < .05. The *RS* post hoc of the Kruskal-Wallis test statistic = 10.944, degrees of freedom = 3, N = 50, *P* = .012. The *FG* post hoc of the Kruskal-Wallis test statistic *H* = 10.980, degrees of freedom = 3, N = 52, *P* = .012.

Sample 1 – Sample 2	<i>H</i>		<i>P values</i>	
	<i>RS</i>	<i>FG</i>	<i>RS</i>	<i>FG</i>
PMSOYM-PMSBM	5.667	16.000	1	.053
PMSOYM-PMMT	14.595	0.271	.055	1
PMSOYM-PMSUNM	-16.542	-11.083	.027	.419
PMSBM-PMMT	8.929	15.729	.665	.036
PMSBM-PMSUNM	-10.875	-4.458	.368	1
PMMT-PMSUNM	-1.946	-10.812	1	.352

Table S8.7 Multicollinearity data for the soil health properties.

Soil factors	VIF	Tolerance
pH	1.97	0.5081
P Bray 2	2.15	0.4644
Density	2.06	0.4866
K	1.99	0.5021
P	2.17	0.4618
NO ₃ -N	2.22	0.4498
NH ₄ -N	2.53	0.3947
Z	2.67	0.3748
Active-C	1.19	0.8386
Solvita microbial respiration	1.45	0.691
SOM	20	0.05
Ash-C	19.96	0.0501

Supplementary Material for Chapter 9

Supplementary Figures

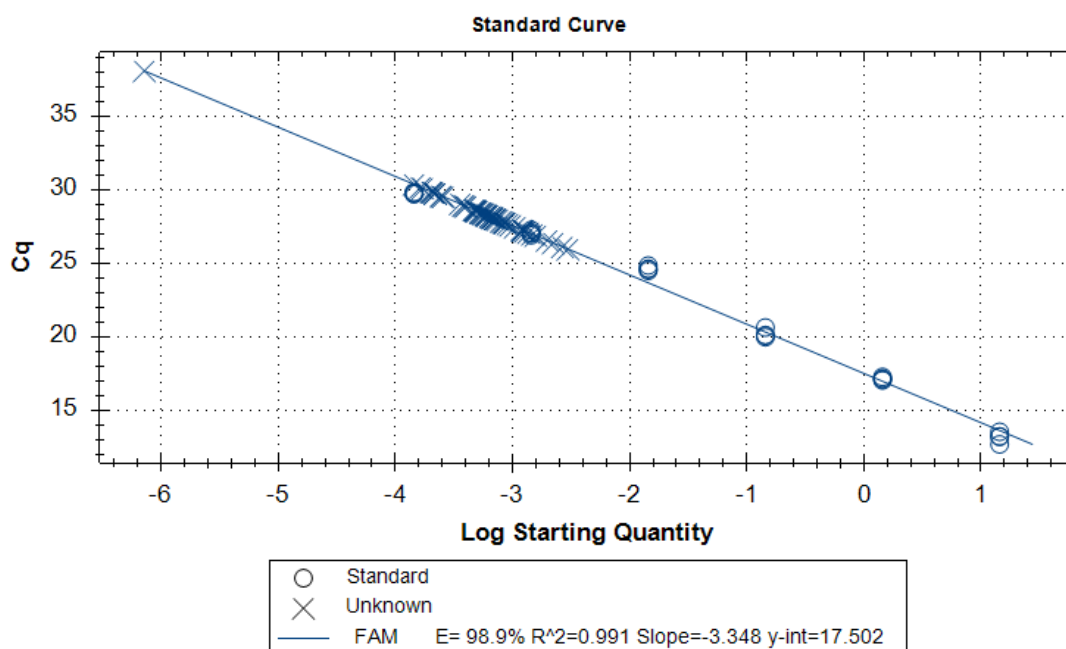


Figure S9.1 Standard curve for sensitivity and linearity of the real-time PCR quantification. Standard curve of quantification cycle number (C_q) plotted versus the log of the initial template concentration ($\log Q$) in ng/ μ l. The six points are ten-fold serial dilutions that are linear over five logs. Data represent four replicates of each *R. solani* AG 3-PT DNA concentrations (14.4–0.000014 ng). Efficiency (E) = $10^{-1/\text{slope}}$. The plotted symbol (X) represents season 2015/2016 samples and the open circles represent ten-fold serial dilution points.

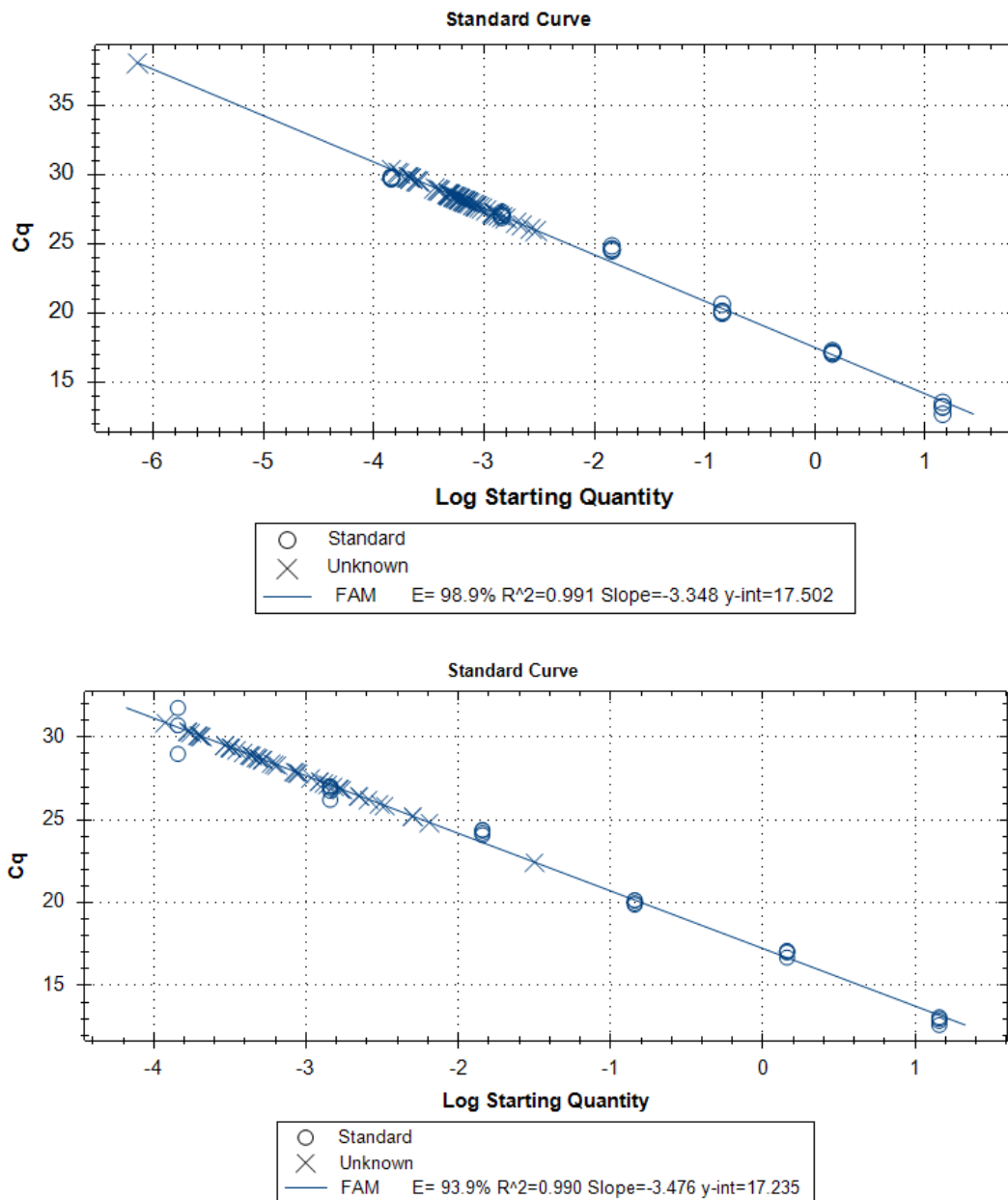
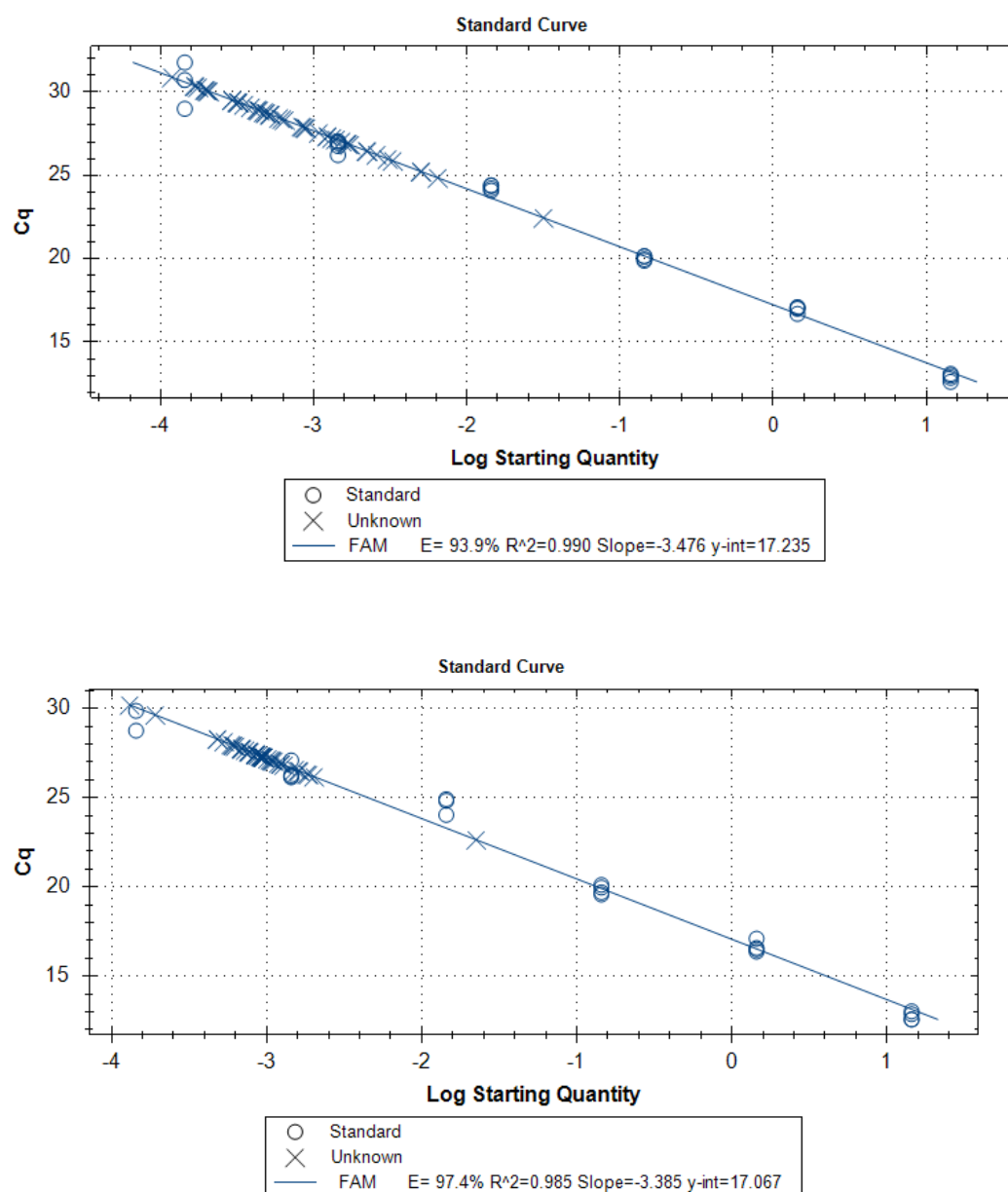


Figure S9.2 Standard curve for sensitivity and linearity of the real-time PCR quantification. Standard curve of quantification cycle number (C_q) plotted versus the log of the initial template concentration (logQ) in ng/μl. The six points are ten-fold serial dilutions that are linear over five logs. Data represent four replicates of each *R. solani* AG 3-PT DNA concentrations (14.4–0.000014 ng). Efficiency (E) = $10^{-1/\text{slope}}$. The plotted symbol (X) represents Season 2016/2017 samples and the open circles represent ten-fold serial dilution points.



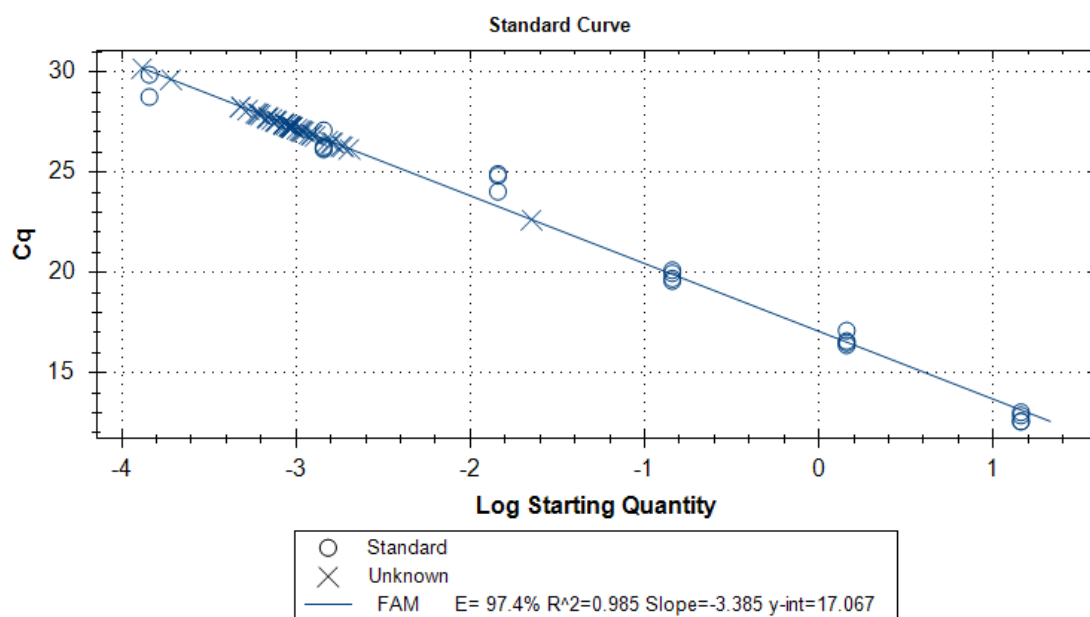


Figure S9.4 Standard curve for sensitivity and linearity of the real-time PCR quantification. Standard curve of quantification cycle number (C_q) plotted versus the log of the initial template concentration (logQ) in ng/μl. The six points are ten-fold serial dilutions that are linear over five logs. Data represent four replicates of each *R. solani* AG 3-PT DNA concentrations (14.4–0.000014 ng). Efficiency (E) = $10^{-1/\text{slope}}$. The plotted symbol (X) represents season 2018/2019 samples and the open circles represent ten-fold serial dilution points.

Supplementary Tables

Table S9.1 Fertilization regime (N – nitrogen, P – phosphorus, K – potassium) for the crop rotation field experiment from season 2016/17 to season 2018/19

Crop	N	P	K	Row spacing	Population	Variable cost	Over-heads [¥]	Returns
	Kg/ha			m	Plants/ha	Rands/ha		Rands/tonne
Maize 2017	90	22.5	6	0.91	28000	6408	3434	1977
Maize 2018	100	24	6	0.91	28000	5755	6317	1808
Sugar bean 2018	45	27	27	0.91	120000	10693	1808	13000
Soybean 2018	45	27	27	0.91	240000	4002	4438	4800
Sunflower 2018	100	24	6	0.91	45000	4002	4438	4494
Maize 2019	120	20	6	0.91	32000	6317	5755	2300
Teff 2019	83	8	0	Broad-cast	7 kg/ha	2800	1500	R50/25kg bale
[¥] hire, administration, labour, salary								

Table S9.2 Pearson's r_{16} correlation coefficients of *R. solani* AG 3-PT DNA quantities among cropping seasons. The correlations were calculated using $\ln(x + 1)$ transformed pathogen DNA data. N = 16.

	Season 2015/16	Season 2016/17	Season 2017/18	Season 2018/19
Season 2015/16	—			
Season 2016/17	0.414	—		
Season 2017/18	0.039	0.493	—	
Season 2018/19	-0.392	-0.278	-0.223	—
Note. * $p < .05$, ** $p < .01$				