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Potato (*Solanum tuberosum* L.) response to nitrogen forms and phosphorus sources in different soil types

by

Simon Chege Kiongo

Submitted in partial fulfilment of the requirements for the degree
MSc (Agric) Agronomy
in the Department of Plant and Soil Sciences
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Supervisor: Dr. D. Marais
Co - Supervisor: Prof. J.M. Steyn

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SUPERVISORY COMMITTEE

This dissertation has been approved for submission at the University of Pretoria by:

1. **Dr. Diana Marais**

   Department of Plant and Soil Sciences,

   Faculty of Natural and Agricultural Sciences,

   The University of Pretoria,

   Pretoria, South Africa.

   Signature _____ Date _____

   as the University supervisor.

2. **Prof. JM Steyn**

   Department of Plant and Soil Sciences,

   Faculty of Natural and Agricultural Sciences,

   The University of Pretoria,

   Pretoria, South Africa.

   Signature _____ Date _____

   As the University co-supervisor.

ABSTRACT

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Potato (*Solanum tuberosum* L.) is one of the most important tuber crops globally and is classified amongst the most crucial food crops in Africa. South Africa has a very vibrant potato industry, producing about 2.5 million tonnes every year, with quantities bettered only by Algeria and Egypt.

Potato production is very expensive (R150 000 ha^{-1}), with fertilizers contributing 20%. Potato is highly reliant on steady nutrient supply and any deficiencies result in poor yield. Potato fertilizer demand is higher than that of other crops such as cereals and it has a very unique demand for phosphorus (P), which is vital from its early development to maturity. In addition, potato has a very shallow root system, which compromises P uptake, making most potato cultivars ineffective in nutrient uptake. Therefore, high P fertilizer rates are applied of which <20% is utilized by plants within a few days after application and about a further 4% within the next 10 days, mostly due to fixation.

The production of P fertilizer, such as super phosphate (SP) is energy-consuming, costly and emits fluorine. There is also a risk of cadmium (Cd) accumulation in soils and plants due to the heavy fertilisation, posing a risk to human health, animals and aquatic life. Runoff phosphorus leads to eutrophication of water bodies. In addition, P fertilizer production is severely

threatened by declining rock phosphate (RP) reserves, expected to hit a low by 2200. This will result in a hike in P fertiliser prices as miners move to low concentration ores. The high demand of P in potato, the environmental and human health risks, the high costs and declining reserves, all call for prudent and sustainable management of P in potato production.

Nitrate and ammonium results in contrasting plant metabolism and growth. Most importantly through rhizosphere modification where ammonium supply results in reduced soil pH while nitrate results in increased soil pH. The pH reduction in ammonium supplied soils increases P dissolution and availability while the opposite is noted in nitrate. Most of the studies in this phosphorus-nitrogen interaction have been conducted on tree species, grasses and cereal crops with little done on tuber crops. In addition, the application of RP directly to plants could help cut the emissions, processing costs and environmental contamination associated with chemical P fertiliser production. There is, therefore, an urgent need to develop P fertilizer management systems to effectively manage this finite resource by improving its use efficiency for maximum yield at optimum application rates.

To attain this objective, two experiments were conducted, namely a laboratory study to investigate the interaction between nitrogen forms and phosphorus sources in soil columns without a test crop, and a glasshouse pot trial to investigate the same interaction with potato as test crop.

The column study treatments comprised of two soil types, N supplied as ammonium or nitrate and three P sources (SP, RP and a P_0) to give 12 treatments that were replicated four times to give 48 columns. Mechanical dry packing method was used. The columns were leached with one pore volume over four watering events (1, 21, 42 and 63 days) and terminated on day 90. The leachate was collected in glass bottles at the column bases and analysed for pH, phosphorus, calcium, potassium and magnesium contents.

A glasshouse pot trial was set up at the University of Pretoria Experimental Farm with potato cultivar Mondial as the test crop over two seasons, with a high and low initial soil P in season one and two, respectively. One minituber was planted per 10 litre pot. Watering was done using a pressure compensated drip irrigation system. Data was collected at tuber initiation (TI) and

at the end of the season (ES). Parameters assessed included plant height, dry masses, number of tubers initiated, yield, leaf tissue and soil P status.

Significant phosphorus-nitrogen interactions occurred on most assessed parameters in both trials. The exceptions were pH, potassium, phosphorus, calcium and magnesium levels, at some stages of the column study. Significant phosphorus-nitrogen interactions were noted at all watering events for both soil and leachate pH, phosphorus, potassium, calcium and magnesium concentration. In the pot trial, significant phosphorus-nitrogen interactions were noted for most of the plant measurements at both the TI and ES assessment periods with a few exceptions. Ammonium + SP produced the highest tuber initiation rate and final yield, as well as highest tissue and plant available P levels in both seasons.

In the leachate and soils at the end of the column study, as well as at both stages assessed in the pot trial, ammonium treatments tended to have higher P contents. In the pot trial, ammonium treatments gave taller plants, but with lower dry mass compared to nitrate. Nitrate treatments had higher soil and leachate pH compared to ammonium treatments in both trials. Plants supplied with SP tended to have longer haulms and roots, higher haulm and root biomass and higher yield compared to treatments with RP and P_0.

The findings of these trials indicated that ammonium results in higher phosphorus dissolution (with or without a crop) and uptake by plants due to increased soil acidity. The resulting effect on potato crop is an increase in the number of tubers initiated and higher yields.

However, the positive effect of ammonium was mostly achieved in combination with superphosphate. Rock phosphate, despite the increased yields, compared to treatments without P, gave inferior plant performance and is therefore not a worthy substitute for superphosphate.

Keywords: phosphorus – nitrogen interaction, column study, pot trial, leachate

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DECLARATION

I, Simon Chege Kiongo, declare that the dissertation, which I hereby submit for the degree Master of Science (Agric) Agronomy 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 _____

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DEDICATION

This work is dedicated to my Fiancée Teresia N. Macharia

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LIST OF SYMBOLS AND ABBREVIATIONS

P	- Phosphorus
NO_3^-	- Nitrate
NH_4^+	- Ammonium
HPO_4^{2-}	- Hydrogen phosphate
$H_2PO_4^{2-}$	- Dihydrogen phosphate
Tg	- Teragram
ATPase	- Adenosine Tri- Phosphate monophosphatase
ATP	- Adenosine triphosphate
ADP	- Adenosine diphosphate
N	- Nitrogen
SA	- South Africa
KCl	- Potassium chloride
kg ha^{-1}	- Kilogram per hectare
PSA	- Potatoes South Africa
SP	- Super phosphate
RP	- Rock phosphate
P_0	- Zero phosphorus
ASP	- Ammonium + super phosphate
ARP	- Ammonium + rock phosphate
AP_0	- Ammonium + zero phosphorus
NSP	- Nitrate + super phosphate
NRP	- Nitrate + rock phosphate
NP_0	- Nitrate + zero phosphorus
W1	- watering event 1
W2	- watering event 2
W3	- watering event 3
W4	- watering event 4
TI	- Tuber initiation
ES	- End of season
S1	- Season one
S2	- Season two

CHAPTER 1

GENERAL INTRODUCTION

Potato (*Solanum tuberosum L.*) is the fifth most produced crop (388 190 674 tons) after sugar cane, maize, wheat and rice (FAOSTAT 2017). Potato is also considered amongst the most important food crops in almost all countries in Africa (Tshisola 2014). In South Africa, the crop is produced roughly throughout the year in all provinces under various climatic conditions (Department of Agriculture 2013). The contribution of potato to the South African horticultural sector has experienced an upsurge; rising from R1.2 billion to R5.1 billion in the period between 1996 to 2010 (DAFF 2010).

The sustainability of this increase, however, faces a struggle due to increased nutritional demand with increased productivity. Potato is highly dependent on constant nutrient supply, the disruption of which leads to the production of low quality and quantity of tubers (Stark et al. 2004, Laboski and Kelling 2007). In addition, potato has an inefficient and shallow root system, compromising its uptake of nutrients such as phosphorus (P) (Sattelmacher et al. 1990, Munoz et al. 2005, Pack et al. 2006).

Phosphorus is a fundamental nutrient for plants and plays a major role in most plant metabolic processes such as energy transfer, photosynthesis, signal transduction, respiration, macromolecular biosynthesis (Khan et al. 2010) and leguminous nitrogen fixation (Kouas et al. 2005).

Despite being abundant in most soils and its critical importance for optimal crop growth, P is often unavailable for plant uptake as it is fixed into complex minerals (Rengel and Marschner 2005). Phosphorous is fixed by either aluminium and/or iron in acidic soils and calcium and/or magnesium in alkaline soils (Havlin et al. 2005). Some of the P in soils results from continued injudicious chemical phosphatic fertilizer applications by farmers. Roughly, 0.1% of all P in the soil is accessible by plants for uptake (Zou et al. 1992). Approximately 1 018 million hectares of tropical soils are classified to be of a high P fixation nature (Sharma et al. 2013). A study by Khan et al. (2009) implied that the P already accumulated in farm soils could sustain crop production globally for at least 100 years at maximum production– if only it was available.

Under P deficiency conditions, farmers respond by applying phosphatic fertilizers (Alori et al. 2017) of which less than 20% is utilized by plants within a few days after application. The levels go down further to about 4% within just 10 days. In dry soils, the percentage can be as low as 0.6% because of reduced soil microbial activities (Koopmans et al. 2004).

Phosphatic fertilizer production is a high energy-consuming and costly process. Its production furthermore emits fluorine, a greenhouse gas that is highly volatile and poisonous. The continued cadmium accumulation in both soil and most probably in plants due to plant competition for P and continued P fertilizer application also poses a health risk to humans, animals and aquatic life (Alori et al. 2017). Runoff P leads to eutrophication of water bodies (Alori et al. 2017). Phosphatic fertilizer use is severely threatened by declining rock phosphate (RP) reserves, expected to hit a low by 2200 (Cordell et al. 2009). Prices of P fertilizers will, therefore, increase as miners concentrate on low concentration ores, hence resulting in high processing costs (Elser and Bennett 2011). Therefore, there is an urgent need to develop fertilizer management systems to effectively manage this finite resource by improving its use efficiency for maximum yield at optimum application rates.

Past studies indicate that nitrate and ammonium forms of nitrogen (N) result in contrasting plant metabolism and eventual growth responses varying from one species to another and even within cultivars of the same species (Gojon et al 1998, Matt et al. 2001). The different N forms affect soil pH through acidification under ammonium supply and increased soil pH under nitrate supply (Thomson et al. 1993). This effect on soil pH affects the availability of soil nutrients, including P. Past studies, have suggested a positive interaction between N form and P in the soil (Ruan et al. 2000) where ammonium enhances P dissolution and availability. Ammonium has also been reported to enhance P availability when supplied together with RP (Ruan et al. 2000). Most of these studies are confined to tree species, grasses and cereal crops with little done on tuber crops and even lesser done in potato. Thus, literature is scanty on the recommendations of N form and P source in potato production. This current study was aimed at investigating the effect of the phosphorus- nitrogen interaction and how it affects P availability.

To investigate this interaction, two trials were conducted. The first one was a soil column study using no test crop, but packing two soil types in soil columns and leaching the soils. The

following hypothesis was tested: ammonium will enhance P availability, regardless of soil type and P source, due to increased acidity, while nitrate will reduce P availability regardless of soil type and P source, due to reduced soil acidity.

The second study was a pot trial to investigate the P - N interaction using potato as a test crop. the following hypothesis was tested: ammonium will enhance P availability, regardless of soil type and P source, due to increased acidity as a result of nitrification and H^+ extrusion from the roots, while nitrate will reduce P availability regardless of soil type and P source, due to reduced soil acidity due to uptake of H^+ and the extrusion of OH^-.

The objective of the column study was to determine the effect of different P sources and N forms on P availability in different soil types without a test crop. The objective of the pot trial was, to determine the effect of different P sources and N forms on P availability in different soil types on potato.

CHAPTER 2

LITERATURE REVIEW

2.1 Forms of phosphorus available in the soil for plant uptake

Phosphorus (P) is found in the soil in two distinct forms, i.e., organic and inorganic. In most soils, about 30 to 60% of P is found in the inorganic state. However, this can, range between 5 to 95%. The availability of P is determined by precipitation, solubilisation of inorganic phosphates and the immobilization and mineralization of the organic P fraction (Sims and Pierzynski 2005). Phosphorus is found in the soil in four distinct fractions; the first one is P in its non-labile state, which is part of the soil mineral's crystal structures such as aluminium, iron and apatite compounds; this fraction is unavailable to plants. The second fraction is in the form of labile precipitates of phosphates and P that is adsorbed on particles of clay; this form is unavailable to plants, but only becomes bioavailable gradually over time. The third fraction is in soil microorganisms and organic forms, which make up between 10 to 60% of topsoil P; it is immobilized and temporarily unavailable to plants. The fourth fraction is the P in solution (inorganic and organic forms) (FSSA 2007). Both $HPO4^{2-}$ and H_2PO4^{-} found in solution are the only fractions of P available for plant uptake and are the only forms with quantifiable mobility in the soil (Mundus et al. 2013).

2.2 Phosphorus reserve availability and sustainability

Phosphatic fertilizer is produced directly from rock phosphate (RP). The RP reserves available worldwide are roughly around 67 000 Tg (Jasinski 2013). Despite the relatively large reserves, there is about 16 000 Tg which have the proper quality for economic processing (Butusov and Jernelöv 2013). About 200 Tg are mined annually, which means that the stock available to be processed economically can only last about 80 years. The total 67 000 Tg can only last for about 350 years further. This limited resource, coupled with the increasing world P demand as the population grows and demands more food, means that we might even deplete it earlier. The annual mining of RP is expected to rise to about 250 to 280 million tons by 2050, this could further accelerate the depletion of the existing resources (Mew 2011). In 2013, China (world

leader in RP mining) extracted about 40% of the total P, while the USA and Morocco followed with 13% each (Jasinski 2013). China and the USA have very little resources and are projected to be depleted in about 60 years as reported by Butusov and Jernelöv (2013). According to Jasinski (2013), Morocco has about 77 to 85% of the world's reserves. Phosphorus is a scarce resource as RP deposits are available in very few locations globally and RP is a finite naturally occurring resource that must be utilized sustainably (Mundus et al. 2013).

2.3 Uptake and translocation of phosphorus in plants

Plants absorb P from soil solutions at extremely low P concentrations. Plant roots (xylem sap and root cells) have about 100 to 1000-fold higher P concentrations relative to the soil solution, which by implication means that plants take up P through active transport systems due to the high concentration gradient and mediated by hydrogen ion co-transport. The optimum physiological pH for P uptake by plants ranges between 5 to 6 (Pandey et al. 2013). It is noteworthy that the plant cell plasma membrane is negatively charged and so is the inorganic forms of P and for that reason, they cannot be taken up by the plant with that charge due to the resulting effect of plasma membrane hypersensitization. That withstanding, P uptake leads to plasma membrane depolarization. It is for this reason that the uptake of P entails the hydrogen ion co-transport system, as well as the hydrogen ion-ATPase, pumps to pump hydrogen ions into the apoplast to enhance P carriers (Ullrich-Eberius et al. 1984). Adenosine triphosphate (ATP) is also reported to have an effect on the uptake of P due to the relationship between the uptake of P and plant root respiration (Ullrich-Eberius et al. 1981). Inorganic P reaches the rhizodermal cells and the root hairs through diffusion as the major movement mechanism (Ullrich and Novacky 1990). Inorganic P is uploaded to the xylem vessels through a combination of water potential and hydrostatic pressure. Phosphorus enters specific cells through different routes, the first route is into the cytoplasm as well as the organelles in the cytosol, which is the main P pool. The inorganic P in this pool is specifically for making organic compounds e.g. ATP. The second route is into the biosynthetic pathways where it is used in making nucleic acids as well as phospholipids. The third route is the storage in the cell vacuole with the chief role being the regulation of inorganic P homeostasis (Mimura 1999). The final route is in the event where P goes into the parenchyma cells of the xylem and is then conducted into the xylem vessels (apoplast) ready for the long-distance translocation to other plant organs. The movement of P differs under varying conditions; i.e., in the instance where the soil solution

has high P concentration, inorganic P is taken up into the xylem and subsequently translocated to the young plant leaves and later transported to the roots through the phloem (Mengel and Kirkby 1979). However, under P deficiency conditions, the stored inorganic P is mobilized from the older plant leaves to the growing roots as well as the young leaves. Phosphorus can also be remobilized through the degradation of the stored organic compounds in the cytoplasm (Bouma 1967).

The ability of plants to actively take up P varies from one species to another and can differ amongst cultivars of a similar plant species as Barber and Thomas (1972) discovered for different maize cultivars, which exhibited different rates of P uptake. They argued that it is a genetically fixed trait. The ability of plants to take up P is advantageous in conditions of limited P supply (Brown et al. 1977).

Upon uptake by plants, P is rapidly converted from its inorganic forms into various organic forms. Approximately 30% of P after uptake is assimilated into nucleotides after just 10 seconds while about 70% of the total is assimilated after just 50 seconds (Hall 1976).

2.4 Role of phosphorus in the growth and development of plants

Phosphorus is a plant macro element that is needed in abundance by plants for normal growth (Mengel et al. 2001). Phosphorus is the second most used plant nutrient after N, yet it is the least available resource globally out of all the other plant macro-elements (Hilton et al. 2010).

Phosphorus is an active element in plant cell division, flowering, fruit ripening, root growth, respiration, photosynthesis, maintaining plant genetic identity as well as reproduction (Vance et al. 2003, FSSA 2007). Phosphorus is also a key component of organic compounds, for example in adenosine triphosphate (ATP), phytine, phospholipids and adenosine diphosphate (ADP) (Mundus et al. 2013, Wall et al. 2013). Past studies indicate that P application significantly increased yield (Buresh et al. 1997, Amanullah et al. 2010) through increased root growth and thus increased water and nutrient uptake. Phosphorus deficient soils lead to less adventitious root formation and a lower leaf area index (LAI) resulting in reduced photosynthetic active radiation (PAR) interception (Amanullah et al. 2010). Plants grown under limited P supply exhibit stunted growth, with the stems and underside of the leaves at times developing a deep purple colour.

2.5 Factors influencing phosphorus availability to plants

There are various factors influencing P content and its availability to plants in the soil. These include pH, temperature, profile, texture, depth, absence or presence of various other nutrients, plant species, climate, soil water, organisms and microorganisms present in that particular soil as well as the cultivation practices (Schoenau 1989). It is understood that under conditions of moist soil, increased temperatures consequently increase microorganism and enzyme activity as well as their effectiveness. This increases mineralization and consequently the inorganic P concentrations (Javid and Rowell 2003). Soils with pH < 5.5 are most likely to experience P deficiencies, as the soluble aluminium and iron at this pH are higher and end up forming insoluble complexes with available phosphate. Conditions where high pH prevails also lead to deficiencies in P (Carrow et al. 2001). This indicates that anything that affects soil pH when applied to the soil will definitely influence P levels (McDowell et al. 2002). Past studies indicate that application of various chemicals to the soil can influence rhizosphere pH while the pH of the rest of the soil is left unchanged, which may affect P availability (Armstrong and Helyar 1992). Soils below or at field capacity may have superior levels of inorganic and organic P as a result of microbial activity. Microbial activity deteriorates with decreasing soil moisture. The availability of P also declines in soils that are flooded and poorly drained (Horner 2008).

Soils high in organic matter and clay content have shown an increased ability of P adsorption. Contrastingly, sandy soils are known to contain little organic matter, which in turn reduces their ability to adsorb inorganic P (Beard 1973, Horner 2008). Organic amendments enhance P solubility in sandy/ low clay content soils (Ohno et al. 2006). Biological activity (in the short term) is the main factor affecting P distribution in soils as the largest portion of P available to plants is obtained from organic matter (Cross and Schlesinger1995). Even though organic matter breakdown by soil microorganisms leads to higher available P, this leads to concomitant increases in both iron and aluminium ions that in turn fix the inorganic P, therefore, again rendering it inaccessible to plants (Ohno et al. 2006). Previous studies indicate that earthworms have a huge impact on mineralization and affect the availability of P and its eventual distribution in the soil (Le Bayon and Binet. 2006).

Weather events, for example, freezing and thawing as well as drying and rewetting results in a flushing of soil P. Freezing makes cells lyse and, in the process, cell constituents, P included,

flows into the soil solution and when plant cells intake a lot of water too quickly after a prolonged dry span, they might burst and in the process release P (Turner et al. 2005).

Soil P content reduces with increase in soil depth, as it is immobile in the soil (Beard 1973). Organic P is highly soluble as compared to inorganic and travels much in the soil (Havlin et al. 2005). Various nutrients affect P cycling differently. Magnesium and calcium adsorb P in alkaline soils while aluminium and iron adsorb P in acidic soil conditions. Phosphorus is deemed more available when it is fixed to magnesium and or calcium as compared to when it is fixed to aluminium or iron as the reversal of the process is easier (Foth 1990). Nitrogen concentration affects P availability in the soils by encouraging the growth of plants leading to increased root mass and surface area, which enables plant roots to efficiently explore the soils for P. Nitrogen is also a main component of phosphatases (enzymes taking part in P mineralization) indicating that N must be present for the production of phosphatase enzymes to occur and the N available determines the subsequent enzyme production (Wang et al. 2007).

2.6 Soil phosphorus solubilizing microorganisms

A huge number of soil microorganisms comprising fungi, bacteria, algae and actinomycetes have proven ability to solubilize and mineralize P. Bacteria that are known to mobilize fixed P include, *Bacillus circulans*, *Pseudomonas* spp. as well as *Agrobacterium* spp. (Babalola and Glick 2012), *Azotobacter* spp. (Kumar et al. 2014), *Erwinia* spp. and *Enterobacter* spp. (Chakraborty et al. 2009), and *Paenibacillus* spp. (Bidondo et al. 2011).

Fungi known to solubilize P include various strains of *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Mortierella*, *Paecilomyces*, *Penicillium*, *Rhizoctonia*, *Pythium*, *Sclerotium* and *Trichoderma* (Sharma et al. 2013). Fungi population in the soil are understood to traverse great distances as compared to bacteria and they also produce greater amounts of acids including gluconic, citric, 2-ketogluconic, lactic, oxalic, acetic and tartaric acids (Sharma et al. 2013). Approximately 20% of actinomycetes have the ability to solubilize P. Some cyanobacteria algae are also known to solubilize P (Sharma et al. 2013).

2.7 Plant responses to phosphorus deficiency in the soil

Phosphorus is an immobile nutrient that does not readily translocate from the point of application in the soil. After inorganic P is applied in the form of fertilizer, it literally moves only 3 to 5 cm (Havlin et al. 2005). The P is rapidly adsorbed onto minerals and soil particles, rendering it unavailable to plants despite being within the rhizosphere (Javid and Rowell 2002). As plants deplete inorganic P along the root zone, inorganic P in the surrounding area enters the rhizosphere through simple diffusion. In situations where there is not enough available P in the area surrounding the rhizosphere, soil-living organisms and phosphatase enzymes free the fixed P in the larger soil volume (Stewart and Tiessen 1987). In P-deficient soils, plants develop unique P acquiring ways to thrive under the limited supply. Firstly, by the proliferation of the roots to cover a wider soil surface through basal root development, improved root hair development, while some species develop proteoid roots (Haling et al. 2013, Jin et al. 2015). Secondly, plants initiate associations with mycorrhiza, particularly developing symbiotic relationships with abascular mycorrhizae fungi where the hypha of the fungi increases the P absorption surface area of the plant root (Facelli et al. 2010, Brown et al. 2013). Plants can modify the environment around them (rhizosphere) in order to improve mobilization of P. This is attained through rhizosphere acidification by efflux of protons, carboxylate exudation that dissolves amorphous P through ligand exchange and chelation, and through phosphatase enzyme production into the rhizosphere to release fixed P from the soil (Pang et al. 2010). Finally, plants can also alter their genetic network in response to P starvation as well as altering the amount and concentration of phytohormones including auxins, cytokinin, ethylene and gibberellic acid, which play major roles in both systemic, as well as the local response of plants to P deficiency (Pandey et al. 2013).

2.8 Constraints associated with the use of chemical phosphate fertilizers

Large amounts of phosphatic fertilizers have consistently been applied from the onset of the green revolution in order to realize sustainable agricultural production (Tilman et al. 2001). This fertilization has, however, come with great disadvantages, including greenhouse gas emissions (Haverkort et al. 2014). The greenhouse gas emissions due to synthetic fertilizer application in South Africa stood at 3.0 million tonnes CO_2 - equivalent in 2012 (Tongwane et al. 2016). Synthetic fertilizer use has also led to the contamination of surface water, thereby

affecting ecosystem functioning (Tilman et al. 2001, Ruark et al. 2014). The use of synthetic P fertilizer is also heavily threatened by the declining RP reserves, which are expected to decline markedly by the 21st century (Cordell et al. 2009). Secondly, prices for P fertilizers will tend to increase as the mineral ores continue to be depleted and miners concentrate on low concentration ores, which will be more expensive to process due to low P concentrations, coupled with increased demand (Elser and Bennett 2011). Thirdly, the rising concern due to environmental pollution by P fertilizers will most definitely lead to more regulations to limit this pollution, challenging its use (Thornton et al. 2014). Being a substantial contributor to greenhouse gas emissions through ways such as fertilizer application, agricultural practices will be duly regulated in relation to climate change, both globally and locally in South Africa such as the carbon tax, with the agricultural sector only exempted up to 2020 (Tongwane et al.2016).

Despite these challenges associated with P nutrition in plants, past research findings have indicated that N fertilization has a direct effect on soil P availability as well as the effectiveness of P fertilizers after application. This can be achieved through the application of nitrogen which will increase the plants' roots foraging ability for P. Ammonium as a form of N has been reported to enhance P availability and absorption by plants when compared to nitrate (Thomson et al. 1993). Ammonium has been reported to increase P solubility in the soil due to increased acidity while nitrate raises soil pH (Gahoonia 1992). Despite some of these findings being several decades old (Grunes 1959), there exists no clear crop guidelines on the optimal N – P combination for specific crop.

2.9 Nitrogen (N) forms and mechanism of nitrogen uptake by plants

A study by Brady and Weil (2008) indicated that plants contain about 1 to 5% nitrogen (N) by mass. Nitrogen is absorbed by plants in the form of nitrate (NO_3^-) and ammonium (NH_4^+) through the process of diffusion and mass flow. In moist and aerated soils, NO_3^- is present in high concentrations and its uptake is favoured by low pH. An increase in organic anions has been noted with increased NO_3^- uptake in plants. Generally, NH_4^+ uptake is preferred against nitrate as it saves energy during protein synthesis. Plants supplied with NH_4^+ are inclined to increase their protein and carbohydrate synthesis as compared to NO_3^- (Havlin et al. 2005). The uptake of NH_4^+ is favoured under neutral soil pHs and decreases as the pH decreases.

Ammonium application lowers the soil pH, reducing calcium (Ca_2^+), magnesium and potassium uptake while enhancing P uptake in the form of $H_2PO_4^-$ as well as chloride uptake. Uptake of ammonium also increases the tillering ability of plants but results in retarded growth (Havlin et al. 2005). The preference of plants for ammonium or nitrate is dependent on the crop type, age and the environment (Brady and Weil 2008).

2.10 Effect of different nitrogen forms on phosphorus acquisition by plants

Different forms of N affect cation balance and uptake of anions by plants and consequently alter the pH of the rhizosphere. This change in rhizosphere pH, because of differential anion and cations uptake is more dominant as compared to changes in pH because of plant root exudates e.g. anions of organic acid and protons (Jaillard et al. 2003, Marschner 2012). Ammonium results in preferential cation uptake thus net excretion of protons by the roots resulting in acidification of the rhizosphere. Supply of NO_3^- leads to the secretion of hydroxyl, raising the pH of the rhizosphere (Gahoonia and Nielsen 1992, Tang et al. 2011). In leguminous plants, nitrogen fixation acidifies the rhizosphere because of excess cation uptake as compared to anion uptake (Tang et al. 1997, Jaillard et al. 2003). Nitrogen is among the greatest factors influencing plant P supply. Ammonium supply stimulates the uptake of P through acidification of rhizosphere (Miller 1974, Marschner 2012). Low rhizosphere pH may lead to an increased $H_2PO_4^-$ /HPO_4^{2-} ratio. This will also result in increased calcium phosphates' solubility. The interaction between P and different N forms and the influence of the rhizosphere pH are not well understood. This is because P deficiency may increase, decrease or not be affected when plants are supplied with different N forms (Dinkelaker et al. 1989, Tang et al. 2009) and this indicates that plant response to P deficiency and various N forms greatly vary from one plant species to another and even among cultivars.

2.11 Geographical distribution, value and use of potato crop

Potato crop (*Solanum tuberosum L.*) is the world's fourth most produced and utilized crop ranked after maize, rice and wheat (CIP 2010). Potato was first cultivated in the Andean highlands in South America for its starch-rich tubers well over 7000 years ago. The crop was then produced in Europe in the 1600s (Hawkes 1999). Later, potato production spread all over the world and at present, it is produced nearly in every country (CIP 2006).

Potato is produced due to its rich and quality nutrition, notably its food energy, potassium, protein, vitamin C and dietary fibre (Horton and Sawyer 1985). Potato produces higher food calories on a per hectare basis while at the same time uses substantially less water as compared to both wheat and rice (Horton and Sawyer 1985, CIP 2013). Potato has a relatively brief production cycle and yields food faster than cereals and legumes. Potato is a useful hunger-breaker crop for most of the smallholder farmers as is the case for East Africa (Daniels-Lake 2013). Potato tubers have to be cooked before they are eaten and this can be through boiling, baking or alternatively they can be fried. Potato is a versatile food and can be served as the main dish, as accompaniment, or as an ingredient in mixed dishes (Salunkhe et al. 1991).

Potato tubers can also be processed or be semi-processed into products such as frozen chips, flakes and mashed potatoes. The tubers can also be used in making of snacks such as potato crisps. Potato tubers are rich in starch, which has various industrial uses such as ethanol manufacturing (Daniels-Lake 2013).

2.12 Potato production in South Africa

Potatoes are cultivated in a wide range of environments in Africa, ranging from commercial irrigated farms in countries such as South Africa and Egypt to intensive cultivation in central and eastern Africa in smallholder farms. The top five potato producing countries in Africa are as indicated in Table 2.1.

Table 2. 1. Top five potato producing countries in Africa in 2017 (FAOSTAT 2017)

Country	**Production (tons)**
Algeria	4 606403
Egypt	4 325 478
South Africa	**2 450 541**
Morocco	1 924 871
Tanzania	1 749 213

About 50 000 ha are under potato production in South Africa per year and about 2.45 million tons were produced in 2017 (Table 2.1). Over 92% of South Africa's total potato production is produced under irrigation (Franke et al. 2018). Potatoes are among the most essential

vegetables in South Africa. Potato tubers have a relatively lengthy keeping quality as compared to other cultivated vegetables in the country, with good post-harvest care. The potato industry is continually increasing its contribution to the horticultural sector in South Africa with an increase from R1.2 billion in 1996 to R5.1 billion in 2010 (DAFF 2010). South Africa's potato producing zones are indicated in Figure 2.1.

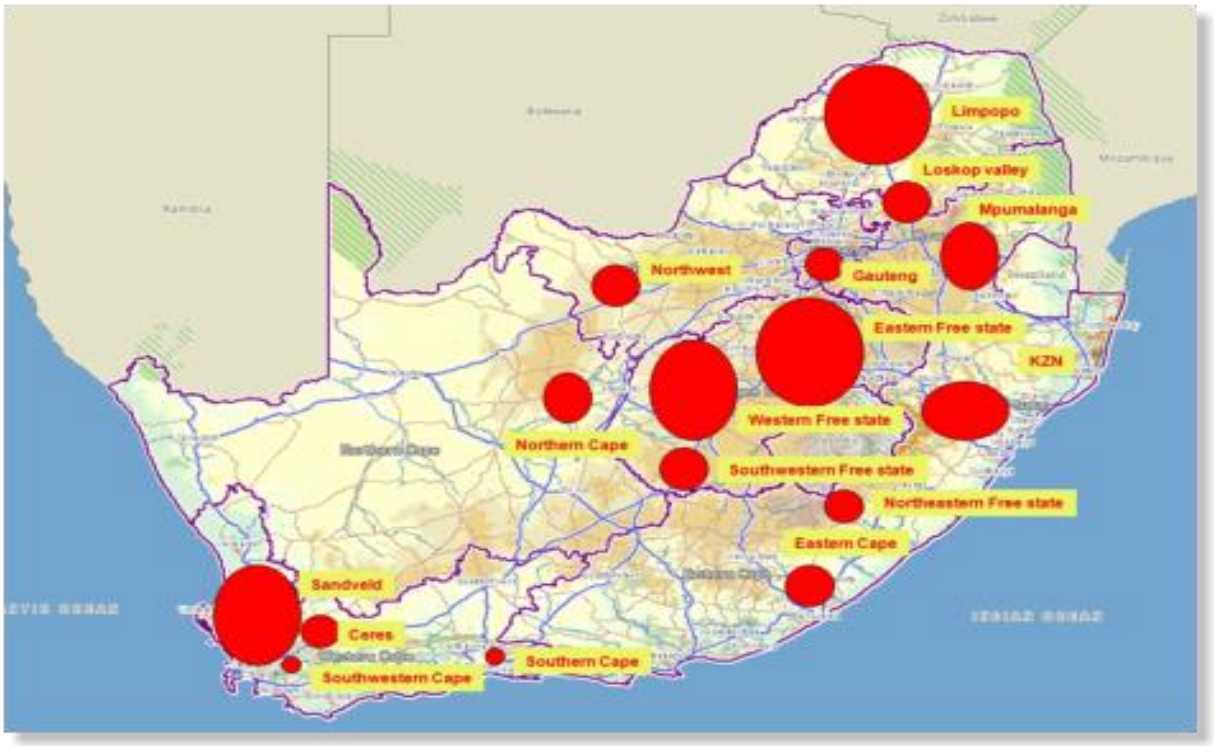


Figure 2. 1. Potato production zones in South Africa (circle size is relative to production area) (PSA 2012)

Three provinces; Free State, Western Cape and Limpopo produce well over 50% of the total amount of potatoes produced in South Africa (PSA 2016). Production in SA is split into two, table and seed potatoes. Most farmers produce table potatoes, which includes processing potatoes, while about 100 registered growers handle seed (certified) potato production with the Potato Certification Service (PCS) supervising the production. The processing and table potatoes constitute 88% of the entire potato crop, with the remaining 12% made up of seed potatoes (PSA, 2012).

2.13 The role of phosphorus in potato

Potato has a high P demand and is classified as a P inefficient crop (Pursglove and Sanders 1981). Phosphorus is actively involved in the metabolism of potato plants by taking part in photosynthesis, energy transfer and respiration (Ozanne 1980, Grant et al. 2001). Phosphorus forms part of the structure of nucleic acids, phospholipids and coenzymes as well as phosphoproteins. Phosphorus also plays a role in nutrient storage in the form of phytic acid in seeds (Bundy et al. 2005). A stable P supply is paramount from the very early growth stages, all the way to maturity (McCollum 1978, Grant et al. 2001). Phosphorus is actively involved in potato growth through the increased growth of literally the whole plant for a number of weeks as soon as it emerges (Dyson and Watson 1971). The addition of P greatly affects leaf area index within eight weeks after shoot emergence, resulting in an increase in the leaf area duration by about 17% (Dyson and Watson 1971). Dubetz and Bole (1975) reported that P deficiency leads to reduced root growth due to its role in the division of cells (Westermann and Kleinkopf 1985). Phosphorus affects tuber starch synthesis (Stark and Love 2003). In some soils, the number of tubers is increased with the addition of P (Jenkins and Ali 2000, Rosen and Bierman 2008). Past research has also indicated that P addition leads to an increase in the number of large tubers (Benepal 1967, Freeman et al. 1998). Mohr and Tomasiewicz (2011) noted an increase in yield but no effect of P on tuber numbers. Phosphorus also fastens potato maturity (McCollum 1978, Stark and Love 2003).

2.14 Unrivalled phosphorus demand by potato

Potato has a unique requirement for phosphorus management due to the limitation of P availability in the soil as a result of iron and aluminium fixation in acid soils and the plant-driven demand for P (Fixen and Bruulsema 2014). Potato production is costly with the costs averaging $4 397 to $6 644 per hectare (Patterson 2012) with 20% of these costs being fertilizer. In South Africa, potato production costs average R150 000 per hectare. Potato fertilizer demand is higher than that of other crops (Stark et al. 2004, Munoz et al. 2005). In fact, potato crop is highly dependent on a steady supply of nutrients and its disruption leads to poor quality tubers (Stark et al. 2004, Westermann 2005, Laboski and Kelling 2007). Adequate P is crucial during early development of the potato crop (Jenkins and Ali 2000), tuber set and thus the number of tubers (Jenkins and Ali 2000, Rosen and Bierman 2008) and tuber maturity

(Stark and Love 2003). Low P supply greatly reduces the size, quality and the yield of tubers (Westermann and Kleinkopf 1985, Stark and Ojala 1989, Hopkins et al. 2010). Phosphorus uptake is challenging in shallow-rooted plants with an inadequate root system, such as potato (Sattelmacher et al. 1990, Love et al. 2003, Pack et al. 2006). Tanner et al. (1982) indicated that most roots of potato plants are found on the top 60 cm, and 90% of their length found in the upper 25 cm, compared to most crops whose roots are much deeper. Potatoes have high nutritional demand, especially for P (Stark et al. 2004, Westermann 2005). Most potato cultivars have ineffective water as well as nutrient uptake structures (Sattelmacher et al. 1990, Love et al. 2003). This means that P fertilizer in potato has to be applied at higher rates (Stark et al. 2004). A study carried out in Idaho, USA, showed that daily P demands are 0.8 to 0.9 kg per hectare per day at tuber bulking (Westermann 2005). To supply in the high potato P requirements, fertilizer recommendations are normally high (Stark et al. 2004), in some instances exceeding 400 kg ha^{-1}. For example, in the USA, the maximum P recommendation for maize stands at 134 kg ha^{-1} (Brown et al. 2010), while the potato demand lies between 252 to 493 kg ha^{-1} depending on soil P status (Stark et al. 2004).

2.15 Phosphorus deficiency in plants and its impact on plant growth

The optimal P required for optimal plant growth ranges between 3 to 5 mg/g dry mass in the vegetative phase. Some plant species growing in low P soils may require an even lower P concentration and still attain optimal growth (Lambers et al. 2010). Phosphorus toxicity in plants is infrequent as plants normally down-regulate their Pi transporters in the event of too high P concentration in the soils than is required by the plant (Dong et al. 1999, Lambers et al. 2010).

Plant responses to P deficiency are regulated through sugar signalling (Karthikeyan et al. 2007) and also involves specific microRNA molecules (Doerner, 2008). Under limiting P conditions, plants suffer reduced expansion of the leaves (Fredeen et al. 1989) and reduced leaf number (Lynch et al. 1991). Assuero et al. (2004) reported reduced cell production and division in maize due to a reduction in the length of the cell division region under limited P supply. Epidermal cell expansion could also be affected by low P due to reduced root hydraulic conductivity. This is solely as a result of reduced expression of aquaporins- encoding genes (Clarkson et al. 2000). Protein concentrations are hardly influenced by low P supply (Rao and

Terry, 1989, Hawkesford et al. 2012). On the contrary, chlorophyll concentration is increased under low P supply (Rao and Terry, 1989). The dark green colouration of the leaves due to P deficiency is due to increased chlorophyll formation and reduced leaf expansion (Hecht-Buchholz, 1967).

In relation to haulm growth, roots tend to be marginally inhibited under low P supply, thus lower haulm to root ratio. This can be attributed to increased carbohydrate allocation to the roots (Hawkesford et al. 2012). The elongation rate of the roots, as well as the individual root cell enlargement, increases under P deficient conditions (Anuradha and Narayanan, 1991). This continued root growth even under low P can be attributed to reduced P transport to the haulm tissues and the translocation of P to the roots from the haulms (Smith *et al.* 1990).

Despite the various plant responses to P starvation (Lambers *et al.* 2006), shoot growth and reproductive organ formation by plants is inhibited. Flower initiation is delayed as well as a reduction in the number of flowers formed (Rossiter, 1978, Bould and Parfitt, 1973). This further inhibits the formation of seeds (Barry and Miller, 1989). Another evidence of P deficiency in the early senescence of plant leaves.

The leaf underside and the stems turn purple as a characteristic P deficiency indicator (Fig 2.2). Yield, however, may be lost due to P deficiency without necessarily identifying these symptoms on the plant (Bennett 1993).

Figure 2. 2. Phosphorus deficiency on potato leaves (Steyn and Du Plessis 2012).

2.16 Rock Phosphate (RP) and its reactivity

Rock phosphate refers to any geological material occurring naturally and has a single or multiple mineral phosphate (Notholt and Sheldon 1986). This forms the raw material for P fertilizer production (Brady 1980, Das 2005). Rock phosphate occurs in four distinct apatite forms: carbonate, hydroxy, fluoro and sulpho (Kumari and Phogat 2008). Apatites of metamorphic and igneous origin are less reactive due to their complex crystalline form as compared to sedimentary rock whose structure is soft microcrystalline, making them fit for application to plants without processing (Narayanasamy and Biswas 1998). About 75% of the total P reserves are sedimentary marine deposits while weathered and igneous deposits account for 15 to 20%. Biogenic reserves (bat and bird) guano forms 1 to 2% (Van Straaten 2002). Different forms of RP differ in texture, chemical composition and mineralogy. About 200 phosphate minerals are known, with the main one being apatite (Van Kauwenbergh 2010).

Rock phosphate has prolonged residual effects, resulting in great P recapitalization in the soil. The solubility or chemical reactivity of RP refers to its ability to release P into the soil for uptake by plants (Ghosal and Chakraborty 2012). Moreover, the reactivity of RP is a

combination of its properties determining the rate at which the RP dissolution occurs in a certain soil type under specific field conditions (Rajan et al. 1996). Minerological and chemical characteristics of RP are the major determinants of its reactivity and the eventual effectiveness (Ghosal and Chakraborty 2012). Studies conducted by Hammond et al. (1986) indicated that RP effectiveness as a P source when evaluated under similar field conditions varied with the changing agro-edaphic and climatic conditions.

2.17. Rock phosphate and factors affecting its dissolution

Soil physicochemical properties

Rock phosphate availability for plant use depends primarily on its properties, the plant species and characteristics of the specific soil as well as the general soil fertilization management (Kumari and Phogat 2008). Similarly, RP efficiency is highly dependent on its reaction and retention when applied, chemical composition and the soil type (Chien et al. 2010). Past studies indicate that the declining pH of the soil enhances the effectiveness of RP (Prochnow et al. 2006, Rivaie et al. 2008, Chien et al. 2010). In addition, RP dissolution decreases linearly or exponentially as soil pH increases (Rajan et al. 1991, Ghosal and Chakraborty 2012). The dissolution of RP in acidic soils is expressed as as $Ca_{10}(PO_4)_6\,F_2 + 12H_20 \rightarrow 10Ca^{2+} + 6H_2PO_4^- + 2F^- + 12OH^-$ (Rajan et al. 1996). The ability of soil to hold P (Chien et al. 1980, Babare et al. 1997) and moisture content in the soil (Kanabo and Gilkes, 1988, Bolland 1994) also affect RP dissolution. Rock phosphate dissolution is enhanced when the soil is adequately wet (Kanabo and Gilkes, 1988). Organic acids, including tartaric, oxalic gluconic and citric acid have been reported to have a positive influence on the dissolution of RP (Rashid et al. 2004, Kumari and Phogat 2008, Khan and Sharif 2012).

Plant species

Plants affect RP dissolution rate through alkaline or acidic secretions as well as citric, 2-ketogluconic and malic acid production (Kumari and Phogat 2008). Different plant species vary in demand, uptake and P absorption from the soil (Helyar 1998, Baligar et al. 2001). Different plant species manifest different abilities to obtain the scarce P that could be available (Hocking et al. 1997, Hasinger 1998, Hocking 2001). Previous studies showed that RPs can be

applied on alkaline media/soil when rapeseed (*Brassica napus*) - an organic-acid producing plant is grown (Ae et al. 1990, Hoffland, 1992, Adams and Pate 1992, Montenegro and Zapata 2002, Chien 2003).

Partial acidulating of RP

Rock phosphate can be acidulated with either phosphoric or sulphuric acid with a lower stoichiometric quantity as that of making single superphosphate or triple superphosphate (Rajan and Watkinson 1992). The effectiveness of low reactivity RP can be enhanced through acidulation using either 40 to 50% sulphuric acid or 20% phosphoric acid (Chien and Menon 1995).

Mechanical activation

Gock and Jacob (1984) conducted a test on a rotary vibrating chamber mill for mechanical activation of sedimentary Egyptian RP. This process reduced the size of RP grains considerably and opened the defect sites of P minerals. This influenced the solubility characteristics of RP relative to the time of milling. A test using infrared light and diffraction using X-ray together with solubility tests using citrate against time proved mineralogy adjustments that improved RP solubility (Gock and Jacob 1984). Rock phosphate obtained from Burkina Faso (Kodjari RP) was mechanically activated and evaluated in several greenhouse trials which showed a significant increase in yield (Kantor and Schwertmann, 1990).

Chemical dissolution of RP

The wet blending of low concentration/grade RP with half the amount of oxalic acid is as efficient as commercially available phosphatic fertilizers (Singh and Ruhal 1993) because oxalic acid solubilizes P and the same time chelates calcium in the form of calcium oxalate. Ammal et al. (2001) combined low P concentration ores with elemental sulphur in a 5:1 ratio and found increased dissolution and a substantial upsurge in P availability because of sulphur microbial oxidation, which leads to H^+ production, hence increased dissolution of RP. Rock phosphate dissolution can be influenced by pyrite addition (Rastogi et al. 1976). Adding iron pyrite to RP decreases P_2O_5 concentration in the RP due to dilution and solubilization. Adding pyrite to RP improves its solubility and increases soil Sulphur content (Mishra et al. 2002).

Ion exchange activity of the soil

As Ca^{2+} is freed during RP dissolution it is sequestered by zeolites, which hastens further RP dissolution (Lai and Eberl 1986, Chesworth et al. 1987). For ion exchange to occur, the zeolites must be charged by NH_4^+, which then reacts with the RP. The already charged zeolite acts as a Ca^{2+} sink in the exchange process, releasing ammonium while taking up calcium ions. This process lowers Ca^{2+} concentration thus increase RP dissolution (Lai and Eberl 1986).

CHAPTER 3

THE EFFECT OF DIFFERENT NITROGEN FORMS AND PHOSPHORUS SOURCES ON PHOSPHORUS AVAILABILITY IN DIFFERENT SOIL TYPES

3.1 Introduction

The long-standing view of Liebig´s Law of the minimum that crop production is only limited by one element at a time has been discredited by a wide range of findings on nutrient co-limitations, where crop growth can be derailed by more than one limiting element at a time (Harpole et al. 2011, Fay et al. 2015). Nutrient co-limitation can be simultaneous or independent (Harpole et al. 2011). Independent co-limitation is depicted by positive influence on crop growth by two or more nutrients individually, while a simultaneous co-limitation is where positive growth response is evident only when two elements are applied together. Hedwall et al. (2017) reported simultaneous N and P limitation in forest vegetation in Sweden. A meta-analysis by Elser et al. (2007) noted a synergistic relationship as the simultaneous application of N and P resulted in higher crop responses than the application of either of the elements alone across various ecosystems.

Potato has been classified as an ‘inefficient utiliser’ of P fertiliser (Hopkins 2015), resulting in over-application by farmers to ensure crop response. This has been attributed to a shallow and ineffective potato plant root system (Peralta and Stockle 2002, Love et al. 2003, Munoz et al. 2005, Pack et al. 2006). Potato has a shallow, less dense and branched root system and a lesser root hair density (Iwama 2008) compared to other crops such as maize.

Plants combat P deficiency via mechanisms such as specialized carrier proteins activation (Schachtman et al. 1998), increased root hairs and cluster root formation (Lamont 2003, Shane et al. 2003), enzyme and acid exudation (Kamh et al. 1999, Dakora and Phillips 2002) and improved mycorrhizal infections (Jayachandran et al. 1992). There has been a wide array of findings indicating varying effects (direct/indirect) of the different N forms (primarily due to their ability to influence soil pH) on P availability as well as different P sources. This study ascertained this interaction using different P sources and N forms in varying soil types with

limited P supply to possibly improve the understanding of P dynamics in the soil. The trial was done in a soil column set up to investigate the chemistry of these elements without a test crop with two soil types with a high and a low P content.

The primary hypotheses of this study were that ammonium will lead to increased P dissolution in different soil types due to its ability to acidify the soil. Secondly, nitrate will result in P adsorption in the two different soil types due to a rise in pH in the soil columns. Finally, it was that superphosphate as a P source will result in increased phosphorus dissolution and availability compared to rock phosphate and the treatments without P application.

Therefore, the objectives of this study were to examine the effect of ammonium and nitrate on the dissolution and adsorption respectively, of P in two soil types in soil columns. In addition, it was to examine the effect of rock phosphate and superphosphate on the dissolution and adsorption respectively, of P in two soil types in soil columns

3.2 Materials and methods

3.2.1 Study site

The study was set up in the Soil Physics Laboratory, Hatfield Campus of the University of Pretoria, South Africa, starting November 2018 and terminated in February 2019.

3.2.2 Experimental setup and treatments

The experiment was set up on wooden laboratory benches with each bench holding exactly 44 columns. The treatments included different sources of P namely superphosphate (SP), rock phosphate (RP) and zero phosphorus (P_0) and different N forms (ammonium and nitrate) and two soil types (clay and sandy soil, table 3.1).

Table 3. 1. Treatment combinations for the soil column experiment

Treatment	Soil type	N source	P source/ level
1	Clay	NO_3^-	Superphosphate
2	Clay	NO_3^-	Rock phosphate
3	Clay	NO_3^-	Zero phosphate
4	Clay	NH_4^+	Super phosphate
5	Clay	NH_4^+	Rock phosphate
6	Clay	NH_4^+	Zero phosphate
7	Sandy	NO_3^-	Superphosphate
8	Sandy	NO_3^-	Rock phosphate
9	Sandy	NO_3^-	Zero phosphate
10	Sandy	NH_4^+	Super phosphate
11	Sandy	NH_4^+	Rock phosphate
12	Sandy	NH_4^+	Zero phosphate

The clay soil was obtained from within zero to 15 cm depth from the University of Pretoria Experimental Farm while the second soil was a sandy soil with ≤ 0.6 mm particle size and was sourced from a supplier of silica sand. The experiment was laid out in a 3×2×2 factorial set up in a completely randomized design (CRD). Each treatment combination was replicated four

times to give a total of 48 columns. The treatments were evenly spread over two benches with 24 columns per bench.

3.2.3 Soil analysis prior to the start of the experiment and fertilisation rates

Soil chemical and physical analyses were done at the soil analysis laboratory of the University of Pretoria. The results of the soil analysis were as shown in Table 3.2.

Table 3. 2. Soil nutritional status at the start of the experiment for both soil types

Element (mg/kg)	P (Bray1)	K	Ca	Mg	Na	S	Clay	pH
Clay	9.94	53.60	26.34	4.37	0.00	78.98	22	3.69
Sandy	5.19	3.72	6.73	0	0	0	2.67	4.7

Ammonium was applied as ammonium sulphate, while nitrate was applied as potassium nitrate. Phosphorus was applied as superphosphate, while RP was supplied as Langfos. Potato fertilizer application rates were adopted for a 60 ton ha^{-1} yield potential (Steyn and Du Plesis 2012). Each column received fertilizer quantities (Table 3.3) as per the soil analysis results. The N application was uniformly done in the two soil types despite the variations in the clay content for uniformity purposes for a 60 ton ha^{-1} yield potential

Table 3. 3. Fertilizer application rates for per column

Application rate	Nutrient quantities				
	N	P	K	Ca	Mg
kg/ha	237.5	130	270	805	75
g/column	0.32	0.18	0.36	1.08	0.10

The actual amount of fertilizer applied per pot was calculated as follows:

Soil volume per hectare (top 15 cm) = 100 000 000 cm^2 × 15 cm (Eq. 3.1)

= 1 500 000 000 cm3

The approximate bulk density of soil = 1.5 g/cm^3

$$\text{Soil mass per hectare} = (1\,500\,000\,000\ \text{cm}^3 \times 1.5\ \text{g/cm}^3)/1000 = 2\,250\,000\ \text{kg}$$

$$\text{Fertilizer applied per pot} = (\text{Rate per ha} \times \text{mass of soil per pot})/(2\,250\,000\ \text{kg/ ha})$$

Exactly 1.5 g ammonium sulphate and 2.4 g potassium nitrate and 1.3 g SP and 1.9 g Langfos RP were applied per column. Approximately 0.2 g of magnesium hydroxide was applied per column meet magnesium demands.

The pH of both soils was adjusted to 5.5 using calcium hydroxide. The amount of lime to be applied was determined as follows:

Approximately 10 g of soil was weighed out into a 50 mL centrifuge tube. Calcium hydroxide stock solution (0.02 M) was prepared by weighing 0.37 g of Calcium hydroxide into a 250 mL conical flask and then filled up to the 250 mL mark with deionised water. The ratio of the stock solution to deionised water was added as indicated in Table 3.4.

Table 3. 4. Liming quantity determination using different ratios of Calcium hydroxide to deionised water to determine the desired pH after liming

Sample	Calcium hydroxide	Deionised water	pH
1	25	0	9.80
2	20	5	9.17
3	15	10	8.58
4	10	15	7.49
5	5	20	5.42
6	0	25	3.67

A graph was then plotted for the pH against the volume of the stock solution added to approximate the amount of calcium hydroxide required to raise the soil to pH 5.5 (Fig 3.1)

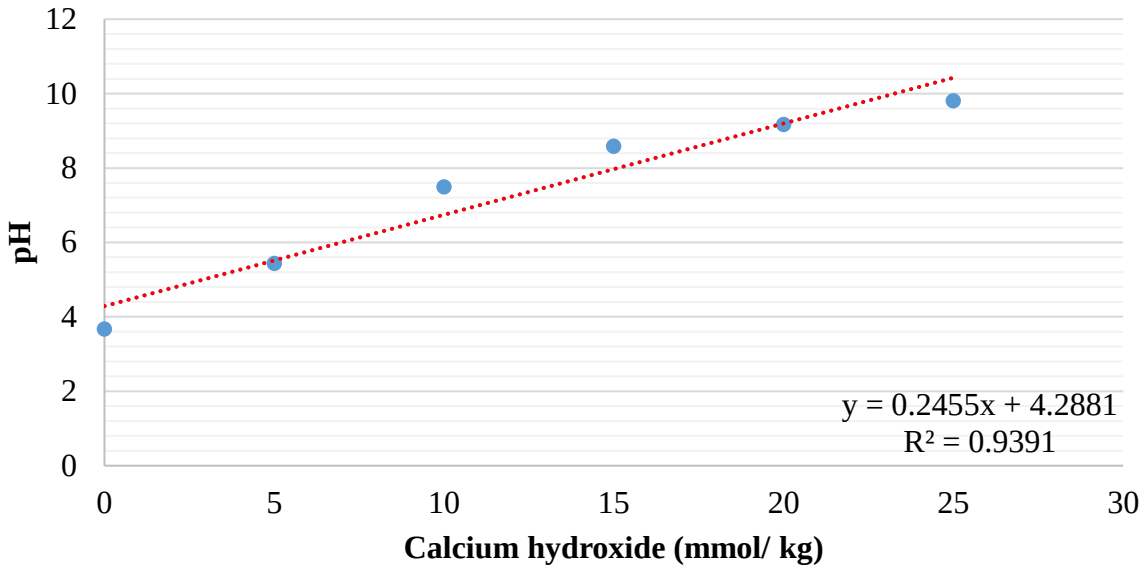


Figure 3. 1. Calcium hydroxide concentration plot against the pH

$y = 0.2455x + 4.2881$ (Eq. 3.2)

where x = the concentration needed to raise the pH to the desired point

while y = the target pH

Therefore,

For pH 5.5 = (0.2455 * X) + 4.2881 (Eq. 3.3)

x = (5.5 - 4.2881)/ 0.2455

x = 4.94 mmol/ kg of Calcium hydroxide was needed

To convert mmol/ kg to mg/kg the (Eq. 3.3) was multiplied by the molar mass of calcium hydroxide (Eq. 3.).

4.94 mmol/ kg × 74 g/mol = 365.56 mg/kg (Eq. 3.4)

To convert mg/kg to kg/kg

= 365.56 mg/kg/1 000 000 (Eq. 3.5)

= 0.000366 kg/kg

The application per hectare based on 15 cm soil depth and a bulk density of 1400 kg/m^3 was;

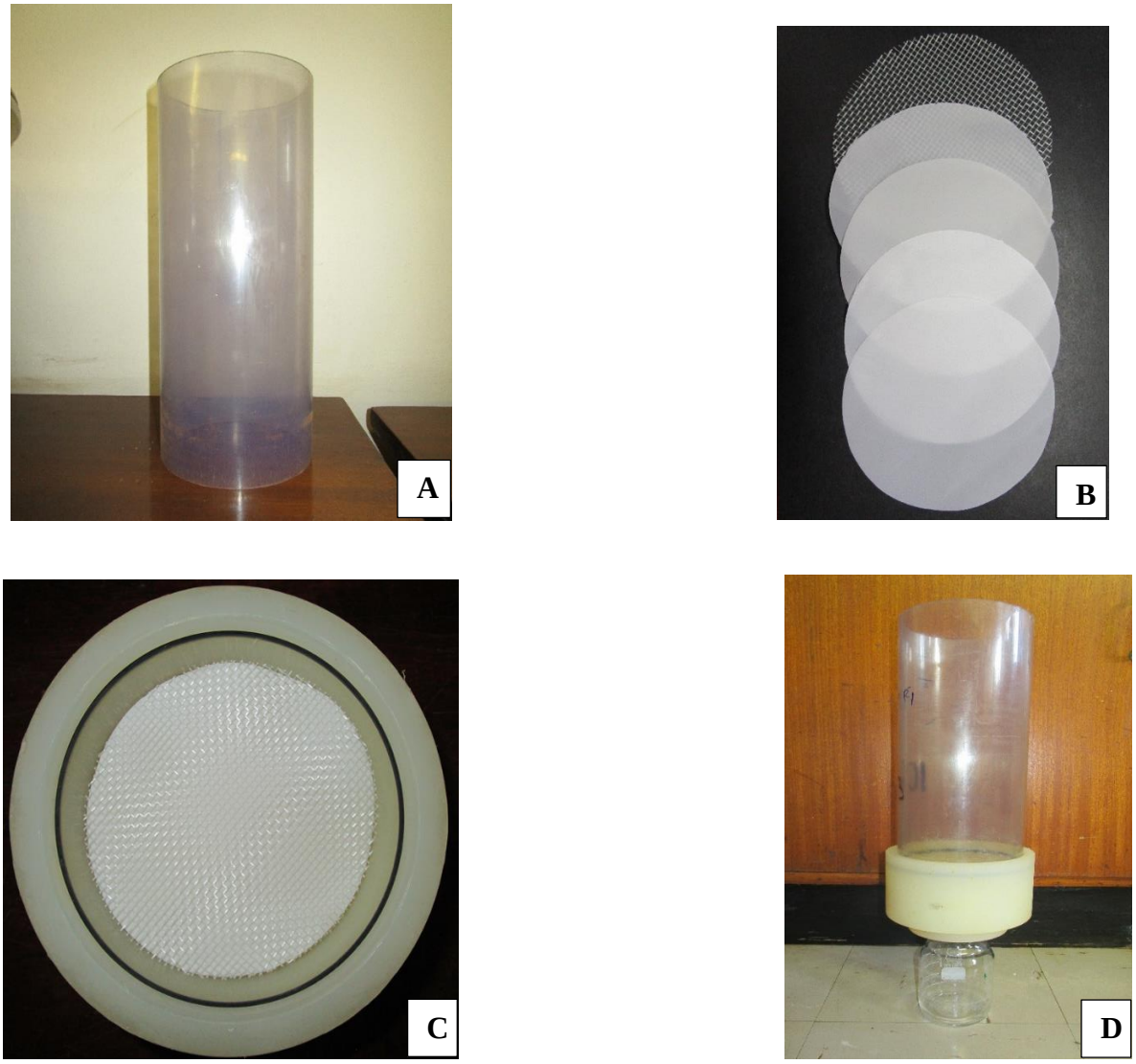
= 0.000366 kg/kg × 2,100,000 (Eq. 3.6)

= 767.676 kg/ha.

The actual amount applied per column was thereafter determined using a similar formula as that of fertiliser determination (Eq. 3.1). Calcium hydroxide was applied to the soil prior to the start of the trial and incubated for three days (Liu et al.2004, 2005). Approximately 2.0 g calcium hydroxide applied per column for liming and supply the calcium requirements.

3.2.4 The components of the full column assembly

The column assembly constituted of a 30 cm long transparent Plexiglas tube with a 10.05 cm internal diameter (Fig 3.2 A).

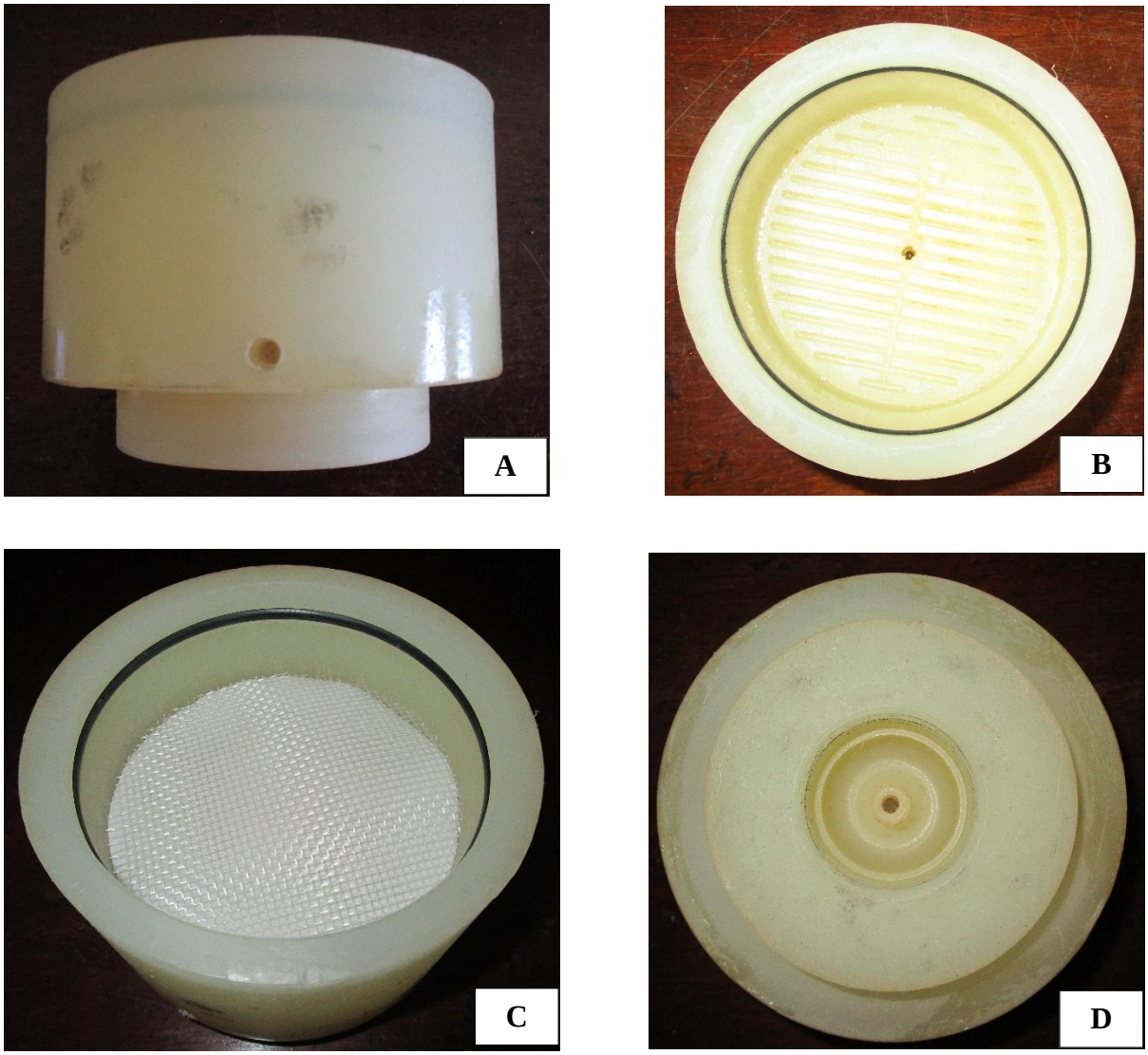


(A) Transparent Plexiglas column (length: 30 cm, internal diameter: 10.5 cm). B) The five-layered mesh placed at the base of the column, C) The depth filter secured on the column base. (D) the Plexiglas at the column base with the Schott bottle attached.

Figure 3. 2. The components used to set up the complete soil column

A five layered mesh was placed at the outflow boundary of the column at the base of the column (Fig 3.2 B) to form a 'depth filter' with three nylon meshes of 20, 10 and 5 µm sandwiched between two 2000 µm polypropylene mesh disks. A 250 mL Schott Duran glass bottle was screwed at the bottom part of the column base (Fig 3.2 D).

The column base was made of polypropylene material with an internal diameter of 11.1 cm and 4.5 cm internal depth (Fig 3.3 A - D).



A) The column base side view, B, C) Top view of column base showing grooved 'floor', drainage outlet and embedded O-ring and D) Bottom view of column base showing drainage outlet and the threaded section for Schott Duran glass bottle screwing.

Figure 3. 3. The pictorial view of the column base used in the column set up

The complete column assembly with all the stated components was assembled on two laboratory benches in a completely randomised design (CRD) (Fig. 3.4).

Figure 3. 4. An illustration of the complete column set up showing the two soil types

3.2.5 Determination of the soil porosity

The amount of water to be added to each column was approximated by calculating the pore volume of the mixture (Tan, 2005) prior to the start of the experiment (Eq. 3.7).

Total porosity = $1 - \rho b \; / \; \rho s$ (Eq. 3.7)

Where:

Dry bulk density (ρb) = $M\ solids \; / \; V\ total$

Particle size density (ρs) = $M\ solids \; / \; V\ solids$

Pore volume = $(1 - \rho b \; / \; \rho s) \times 100$

To determine the porosity, the particle density of each soil was first established. The soil was air-dried and sieved through a 2 mm sieve. Approximately 50 g of soil was then weighed out in duplicates per soil type. The soil was then heated in an oven at 105 ^{o}C till dry and the gravimetric water content (Θ_w) determined. Distilled water was gently boiled to remove any trapped air and subsequently cooled to room temperature and the corresponding density (D_w)

at the noted temperature determined. The distilled water was then poured into a volumetric flask up to the 100 mL mark and weighed (W_w). The water was then poured out (50 mL) and reweighed. Approximately 50 g (W_a) of air-dried soil was poured into the flask. The difference between the 50 mL filled flask and after the soil is added was the amount of air-dried soil added. The mass of the oven-dried soil was determined using the following formula (Eq. 3.8):

$$Ws = Wa/(1 + \theta w) \quad \text{(Eq. 3.8)}$$

The flask was refilled and weighed again to determine the mass of the soil and water (W_{sw}) and the particle density determined as follows (Eq. 3.9);

$$D_P = DwWs/(Ws\ -(Wsw - Ww)) \quad \text{(Eq. 3.9)}$$

To estimate the porosity, the equivalent dry bulk density was taken to be 1.4 g/ cm^3 for both soils as the columns were packed to a specified bulk density because the set up involved the use of disturbed soil columns (the column packing density) and the particle density was determined to be 2650 kg $m^{-3}$for both soils (Eq. 3.10).

Porosity = (1- (1400 kg/m^3 / 2650 kg/ m^3) × 100 (Eq. 3.10)

= (1- 0.566) × 100

= 0.434 × 100

= 47.2% was determined to be the porosity of both soils after packing.

3.2.6 Column packing calculations

The soils were packed to a dry soil bulk density of 1.4 g cm^{-3} (Dry bulk density (ρb) = M_{solids} / V_{total}). Approximately 0.25 m of the column was filled with soil to leave adequate space for water application and inhibit air entrapment. Therefore, based on this, the amount of soil to be packed was determined as follows;

Volume = $\pi r\text{^}2\ h$ (Eq. 3.11)

r = radius

h = height of the column

Column volume (cm^3) $= \pi \times (5.25\ cm)^2 \times 25\ cm$

$= 2164.75\ cm^3$

Total soil mass per column $= M\ solids\ /\ V\ total) \times soil\ volume$ (Eq. 3.12)

$= 1.4\ g/\ cm^3 \times 2164.75\ cm^3$

$= 3030.65\ g$

$= 3.03\ kg$ of soil per column

3.2.7 Column packing

The dry packing method was adopted for the column packing. The soil was weighed out and thoroughly mixed with the fertilizer prior to packing in the columns, with each treatment mixed individually. The soil was poured into the column in distinct portions and then mechanically packed on a vibrating table for 15 seconds to ensure that the soil was deposited in small-sized lifts to the required column height of 25 cm to maintain the column packing density. The soil surface was then lightly scarified prior to the introduction of another portion to enable hydraulic connectivity between the various portions.

3.2.8 Column maintenance

The soil columns were maintained under unsaturated conditions to allow the redox process to occur freely. One pore volume deionised water was applied to the columns at day 1 (W1), 21 (W2), 42 (W3) and 63 (W4) and terminated on day 90. After every watering event, the water was allowed to drain freely until no more draining was evident. The experiment was maintained at room temperature.

3.3. Data collection

The leachate was collected 12 hours after each water application event and analysed. The leachate was analysed using the ICP - AES method for P, K, Ca, Mg and Na. The columns were disassembled at day 90 and soil sampled, dried and sieved through 2 mm sieve and then analysed.

3.3.1 Plant available phosphorus determination

Plant available soil P analysis was done using the P-Bray 1 method. Exactly 4 g of soil was weighed into a 50 mL tube and 30 mL Bray 1 solution was added, followed by handshaking for 60 seconds. Exactly 1 mL of super flock solution was added using a pipette, followed by an approximately five-second shaking of the solution. This was then followed by filtration using 'Whatman nr 2' filter paper. Extractable P content was determined using Inductively Coupled Argon Plasma Emission Spectrometry (ICP-AES).

3.3.2 Soil potassium, calcium and magnesium content determination

The ammonium acetate method was used to determine soil potassium (K), calcium (Ca) and magnesium (Mg). Approximately 3 g of the ground and sieved soil was weighed into a 50 mL centrifuge tube and 30 mL of the ammonium acetate solution added. The mixture was then placed on a mechanical table shaker for 60 minutes after which each sample was filtered into 15 mL centrifuge tubes using 'Whatman nr 2' filter paper within three minutes after the shaking. The concentration of each element was determined using the ICP-AES method.

3.3.3 Rhizosphere pH (KCl)

The soil pH was determined both at the leaching events and end of the trial. The potassium chloride (KCl) method was used, where KCl masked the variations in the salt concentration due to fertilizer residues, water used for irrigation and any microbial decomposition. Exactly 10 g of the soil was weighed out, followed by addition of 25 cm^3 KCl solution (1 mol dm^{-3}). The mixture was then shaken rapidly for 5 seconds and allowed to stand for 30 minutes and shaken again, then allowed to stand for 10 minutes and the pH readings were done using a calibrated pH meter.

3.4 Data analysis

Data were analysed using Statistical Analysis Systems (SAS) Version 9.4. Two-way ANOVA was used to identify P-N interactions using the PROC general linear model (GLM) procedure at $P<0.05$ level. The differences in means were quantified using the Tukey honestly significant difference (HSD) test, controlling for overall experiment wise error rates. The soils were analysed separately (clay soil and sandy soil separately). This was to assess the performance of the treatments against each other in different soil types. A similar approach in the pot trial (Chapter 4) where no inter-season comparison was done, was followed.

3.5 Results and discussion

3.5.1 Effect of different phosphorus sources and nitrogen forms on leachate and soil pH

Two-way ANOVA revealed a significant interaction between P source and N form for the soil pH at all watering events and for both soil types, apart from first watering event (W1) for the sandy soil. At W1 in the sandy soil, P source had no effect (P = 0.8415) on soil pH. However, N form had a significant effect (P = < 0.0001) on soil pH (Appendix A).

At W1, sandy soil supplied with either of the phosphorus sources and P_0 showed no significant difference in pH (Fig 3.5A). Sandy soil treated with nitrate gave a significantly higher soil pH at W1 compared to ammonium treated soil (Fig 3.5B).

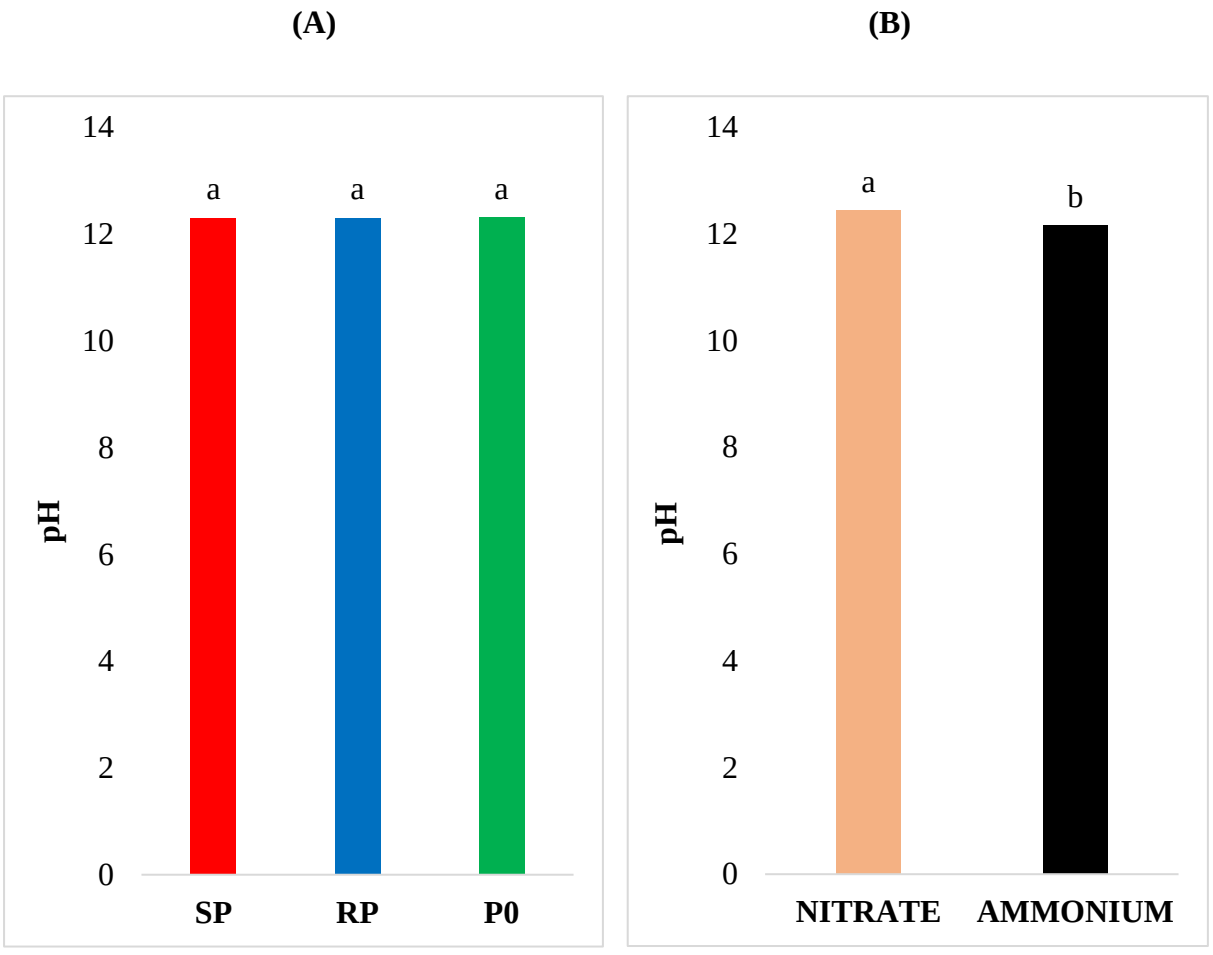
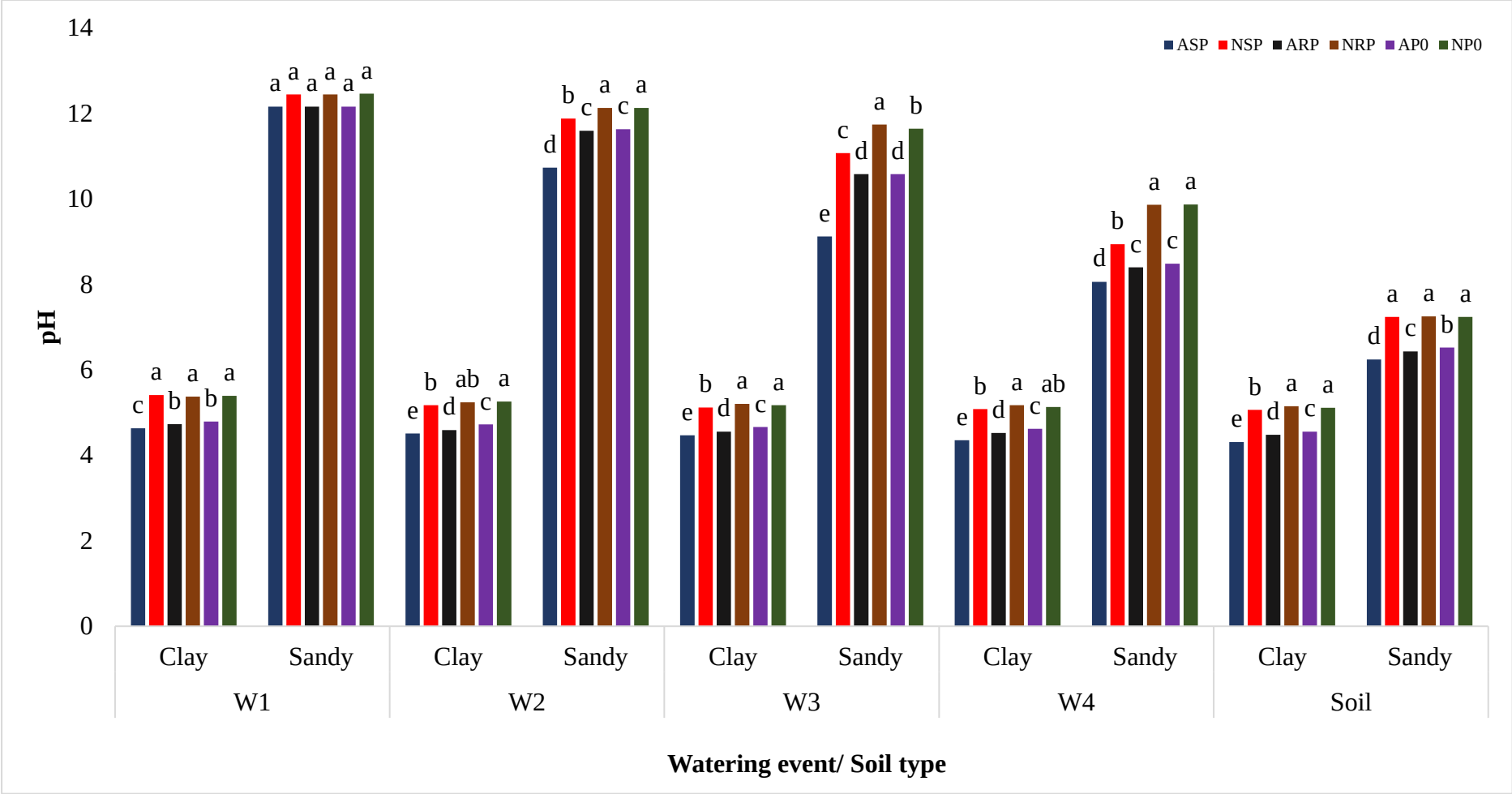


Figure 3. 5. Leachate pH at the first watering event in the sandy soil as affected by phosphorus source (A) and nitrogen form (B).

The leachate from the sandy soil tended to have higher pH compared to the clay soil across all treatments regardless of the N form or P source (Fig. 3.6). The pH tended to decrease gradually after every watering event in both soil types but was much more evident for the sandy soil. All nitrate treatments had significantly higher pH than ammonium treatments in both soils over the four watering events, apart from the sandy soil at W1. Of all ammonium treatments in the clay soil, AP_0 had the highest pH, followed by ARP and ASP over the four watering events. Ammonium treatments resulted in significant variations over the four watering events with the exception of first watering where ARP and AP_0 had no significant differences. There were no clear-cut differences in nitrate treatments in the clay soil and at the various watering events, the treatments did tend to have minimal significant variations except between W2 to W4 where NP_0 had higher pH that NSP and from W3 NRP hand higher pH than NSP.

For the sandy soil, a trend where ASP had significantly lower pH, compared to ARP and AP_0 was noted from the second to the fourth watering events. Nitrate P_0 and NRP both had significantly higher pH than NSP at the second watering event in the sandy soil. A similar trend was observed for the ammonium treatments. All nitrate treatments had a significantly higher pH than ammonium.

The three nitrate treatments at the third watering event all showed significant differences for the sandy soil. Ammonium RP and AP_0 had no significant differences, however, they both had significantly higher pH than ASP. The fourth watering gave similar findings to the second, but with a lower pH.



N = nitrate, A = ammonium, SP = superphosphate, RP = rock phosphate, P0 = zero phosphorus. Bars with by the same letter within a column group have no significant differences. N =4, LSD: Clay =0.04, Sandy =0.04, W1 clay = 0.10, W1 sandy = NS (not significant), W2 clay = 0.07, W2 sandy = 0.10, W3 clay = 0.05, W3 sandy = 0.10, W4 clay = 0.05, W4 sandy = 0.06.

Figure 3. 6. Effect of different phosphorus sources and nitrogen forms on leachate and final soil pH for clay (A) and sandy (B) soils

Two-way ANOVA for soil analysis at the end of the trial revealed a significant interaction between P source and N form in both soil types on soil pH (Appendix A). In both the clay and sandy soils, nitrate treatments resulted in significantly higher soil pH, compared to ammonium. There were no significant differences across all nitrate treatments in the sandy soil. However, NSP had significantly lower pH than NRP and NP_0, while NRP and NP_0 showed no significant differences in the clay soil (Fig 3.6 A and B). Ammonium treatments differed significantly in both soils with a trend observed where P_0 had the highest pH, followed by RP and SP respectively.

In both soil types, there was a tendency for ammonium to lower pH compared to nitrate treatments. This indicates that ammonium supply results in a reduction in soil and leachate pH, even in the absence of a test crop. Pedersen et al. (2019) also reported a similar result in maize plants, with and without a test crop. Jarvis and Robson (1983) also reported increased acidity in an uncropped field supplied with ammonium. Researchers have previously proposed these effects of N fertilisers and their sources on soil pH through various mechanisms (Marschner 1997). First, by the displacement of H^+ or OH^- ions (depending on N form) adsorbed to the soil colloids and secondly, via the nitrification (Eq. 3.13 and 3.14) and denitrification (Eq. 3.15) processes in the soil (Jarvis and Robson 1983).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \qquad \text{(Eq. 3.13)}$$

$$NO_2^- + O_2 \rightarrow 2NO_3^- \qquad \text{(Eq. 3.14)}$$

$$2\ NO_3^- + 10\ e^- + 12\ H^+ \rightarrow N_2 + 6\ H_2O \qquad \text{(Eq. 3.15)}$$

Another mechanism involves the influx (under nitrate) or efflux (under ammonium) of H^+ by plant roots. The first and second processes are not in relation to any crop and influence the whole soil volume, while the third mechanism is as a result of plant nutrient uptake which is restricted to the rhizosphere (Marschner and Romheld 1996, Dotaniya and Meena 2015). This indicates that even without a test crop, the effect of ammonium compared to nitrate on soil pH was still evident.

The lower pH of the SP treatments, compared to RP and P_0 could be due to the acidic nature of single superphosphate fertiliser (Rahmatullah et al. 2006), which could have slightly lowered the pH, regardless of the soil type.

Soils high in sand particle content are known to quickly acidify as a result of their low buffering capacity and consequently show a rapid rise in pH when limed. The pH of sandy soils can consequently be corrected faster by liming as compared to soils with high clay content, which have a higher buffering capacity and when acidic, raising the pH is also difficult.

This phenomenon best explains the differences observed in this current study. The findings of this trial also raise a question on the effectiveness of liming on sandy soils as was the case with soil in this current study where the soil pH went up way beyond the expected pH of 5.5 after liming, despite following a proper lime requirement determination method. These findings agree with the hypothesis of this trial that ammonium will result in reduced pH, regardless of the P source. However, the effectiveness of liming procedures on sandy soils might need to be elaborated further through actual field trial to determine the direct effects of these practices under field conditions

3.5.2 Effect of phosphorus sources and nitrogen forms on the phosphorus content of the leachate and the soil at the end of the trial

A two-way ANOVA revealed a significant interaction between P source and N form at all the watering events except at W2. At W2, the N form and the P source had a significant effect on the leachate P content (Appendix A). Clay soil supplied with SP had a significantly higher leachate P content at W2 than both RP and P_0, both of which did not differ significantly (Fig 3.7A). On the other hand, ammonium treated clay soil at W2 gave a higher leachate P content than nitrate (Fig 3.7B).

In contrast, for the sandy soil, P could only be detected in the leachate from the first water event, giving a significant P source and N form interaction effect. For the subsequent watering events, no detectable P was recorded in the leachate of the sandy soil (Fig 3.8). For the clay soil, on the other hand, detectable P levels were recorded after each leaching event (Fig 3.8).

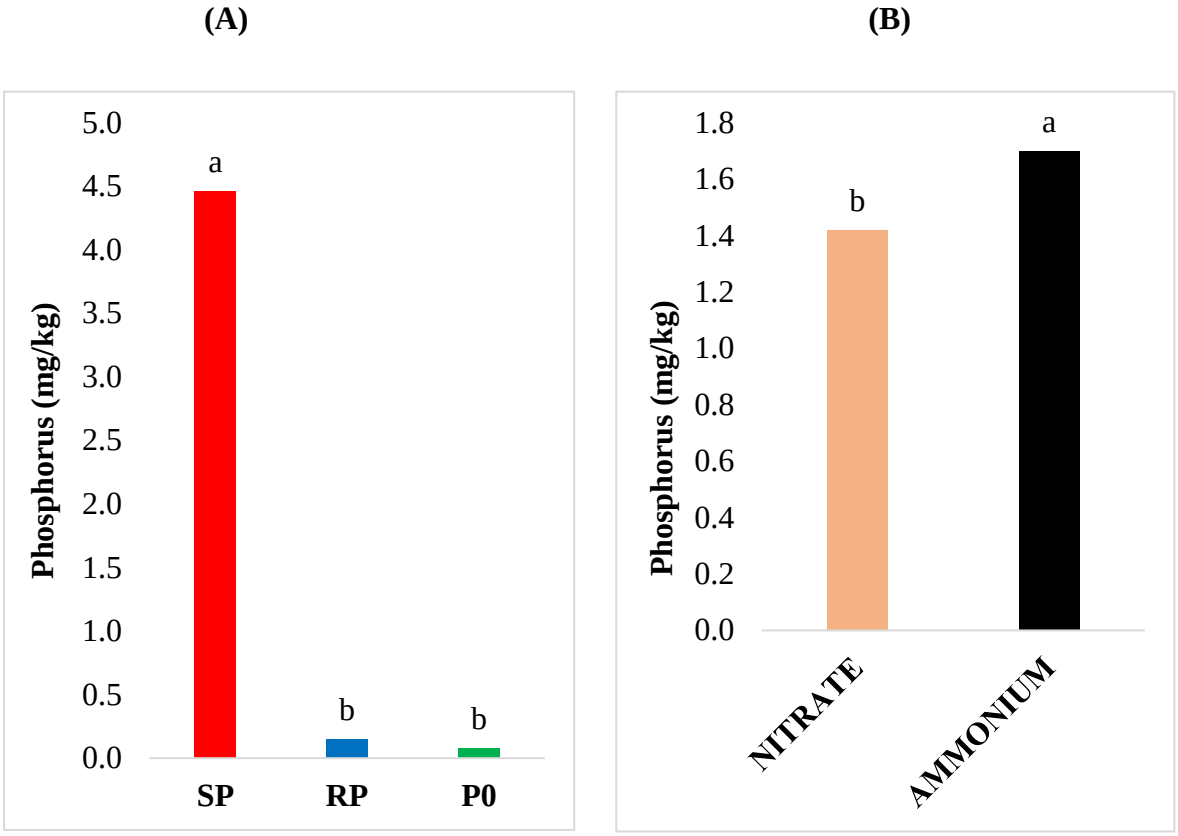
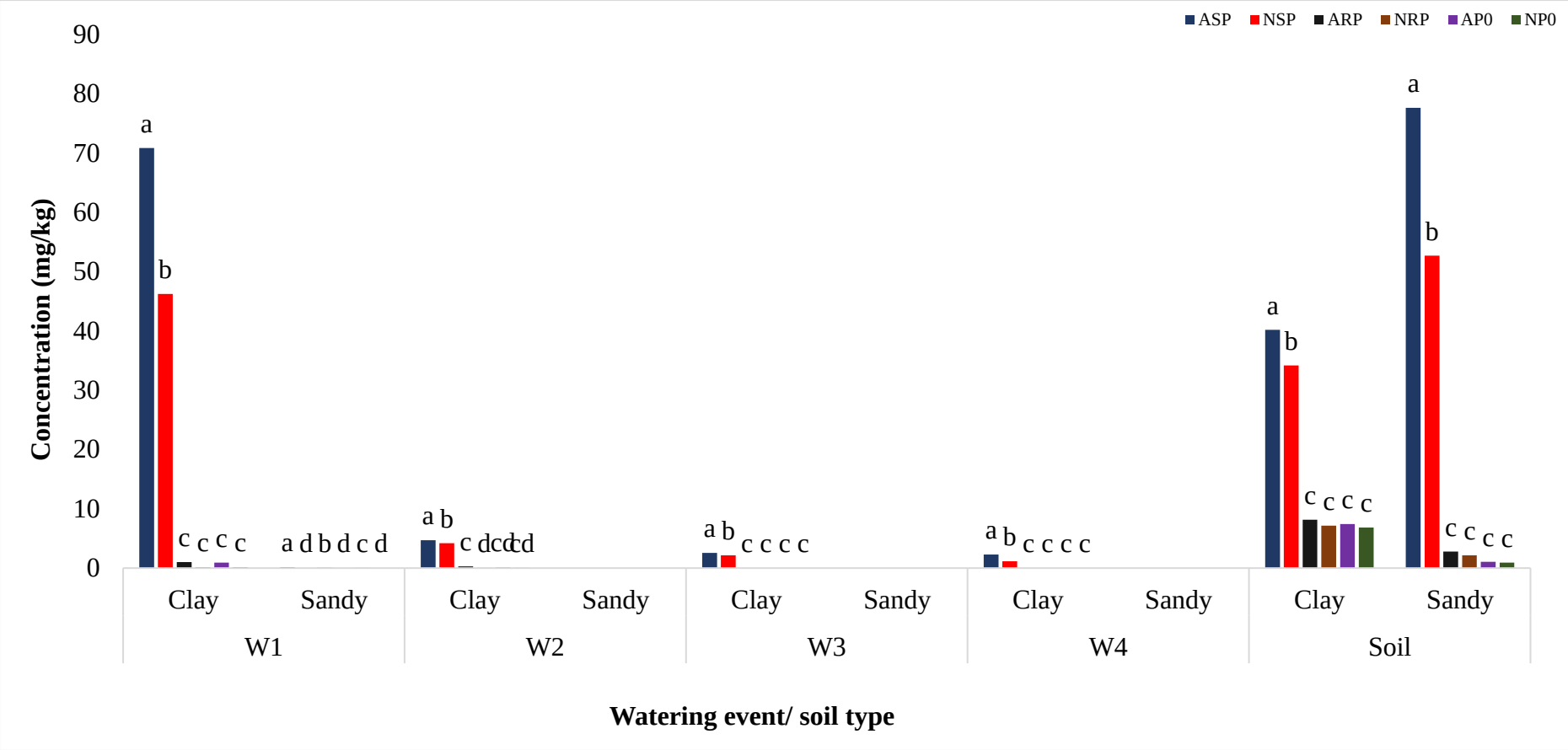


Figure 3. 7. Leachate phosphorus content at the second watering event in the clay soil as affected by phosphorus source (A) and nitrogen form (B).

A trend where P concentration in the leachate was highest after the first watering event and lowest at the last event was noted (Fig.3.8).

Phosphorus leachate concentration was highest in columns supplied with SP (Fig 3.8) followed by RP and P_0 (Fig 3.8), with either N form. Clay soil recorded higher leachate P content compared to the sandy soil. Ammonium treatments also did tend to have higher P compared to nitrate.

Over the four watering events, ASP had significantly higher P than all other treatments followed by NSP which had significantly higher P in the leachate than RP and P_0, regardless of N form and soil type (Fig 3.8).



N = nitrate, A = ammonium, SP = superphosphate, RP = rock phosphate and P_0 = zero phosphorus. Bars with the same letter within a column group have no significant differences. N =4, LSD: W1 clay = 2.01, W1 sandy = 0.02, W2 clay = NS (not significant), W2 sandy = NA (not applicable), W3 clay = 0.21, W3 sandy = 0, W4 clay = 0.16, W4 sandy = NA (not applicable), clay = 1.78 and sandy = 3.44.

Figure 3. 8. Effect of different phosphorus sources and nitrogen forms on leachate phosphorus concentration: A) effect of ASP and NSP and B) effect of ARP, NRP AP_0 and NP_0.

After W1, ASP recorded significantly higher P concentration than all other treatments, followed by NSP, which also had higher P concentration than all RP and P_0 treatments (Fig 3.8 A). There were no significant differences in RP and P_0 treatments, regardless of the N form and despite the fact that ammonium supplied soils recorded higher P concentration than soil with nitrate treatments (Fig 3.8).

At W2, there was a drastic reduction in P concentration in the leachate in the sandy soil. There were also no differences in P concentration for RP and P_0 treatments, except between ARP and NRP (Fig 3.8). After W3 and W4, only ASP and NSP in the clay soil had detectable P levels in the leachate. There was no detectable P found on both RP and P_0 treatments regardless, of the N form and soil type, except for NRP which had negligible P content. None of the RP and P_0 treatments differed statistically from each other.

At the end of the trial, Two-way ANOVA revealed a significant interaction between P source and N form for both soil types (Appendix A).

Analysis of both soils, at the end of the trial indicates that ammonium combined with SP had significantly higher P concentration than the corresponding nitrate supplied soils regardless of the P source (Fig 3.8). The two soils types showed a similar trend, where SP combined with either N form had the highest plant-available P followed by RP and P_0. Interestingly, the sandy soil had higher plant-available P in the soil at the end of the study compared to the clay soil.

Analysis of both soils, at the end of the trial indicates that ammonium treatments had higher P concentration than nitrate supplied soils regardless of the P source (Figure 3.8). The two soils types showed a similar trend, where SP had the highest plant-available P followed by RP and P_0. Interestingly, the sandy soil had higher plant-available P in the soil compared to the clay soil (Fig 3.8). For both soil types, ASP had significantly higher soil P content than NSP and these two treatments had significantly higher P content than all other treatments. Rock phosphate and P_0 combined with either N form did not give significant differences in soil P concentration in either soil type.

In a column leaching trial, Fernández-Sanjurjo et al. (2014) also noted a high level of P in the first leaching event, while in later watering events they noted that even the P supplied

treatments tended not to differ from treatments without P. They also reported a high amount of P in the soil at the end of the leaching, compared to the controls, which strongly agree with the findings of this current trial. The leaching of P was much higher in clay soil indicating higher availability of the element compared to the sandy soil. This was repeated across all P sources, indicating that there was low P dissolution and availability in the sandy soil. This unavailability of P in sandy soil can be strongly attributed to variations in soil pH, with the alkaline pH in the sandy soils resulting in low availability of $H_2PO_4^-$. The low P content in the sandy soil could also be due to the Ca reacting in the soil with P forming insoluble complexes that make P unavailable.

Phosphorus availability in solutions is driven by the pH (Schachtman et al. 1998). The effect of pH alteration on P chemistry has been studied since the early 1950s, with varying findings noted, for example, raising soil pH was reported to increase (MacLean and Cook 1955, Paton and Loneragan 1960), decrease (Neller 1953, Ensminger and Pearson 1957) or have no effect (Shoop et al. 1961, Abruña et al. 1964) on P concentration. Sumner and Farina (1986) also reported discrepancies in the effect of pH on P availability and uptake by plants. Further studies have also reported that raising the soil pH decreases soluble P concentration due to increased sorption (Curtin and Syers 2001, Gustafsson et al. 2012), while others reported increased P availability due to decreased P sorption (Chen et al. 2003, Penn and Bryant 2006, Scanlan et al. 2015, Penn et al. 2018, von Tucher et al. 2018).

Nitrification is optimal at pH 8.5, but N uptake by plants is optimal at pH 6 (Wortman, 2015). Zou et al. (2016) reported that a pH of 6 was optimum for N utilisation efficiency and crop growth. In aqueous solutions, P is dominantly available to plants as $H_2PO_4^-$ (Becquer et al. 2014). Phosphorus availability and subsequent uptake decrease with an increase in solution pH due to reduced H_2PO_4 availability. In plants, H_2PO_4 is the substrate of the proton-coupled phosphate symporter in the plasma membrane at a pH of 5.6 to 8.5; equally, a reduction in pH increases the proton-coupled solute transporters activity and boosts anion uptake (White 2012). According to Sentenac and Grignon (1985), lowering the external pH from 8 to 4 increased phosphate uptake by a factor of 3 in maize roots and this could support the increased P availability in the clay soil with its lower pH compared to the sandy soil which had an alkaline pH.

A rise in pH to >7 in aqueous solutions results in the dissolved P reacting with Ca to form calcium phosphates, rendering it unavailable to plants (Siebielec et al. 2015). This reaction forms dibasic calcium phosphate dihydrate, octocalcium phosphate and hydroxyapatite, resulting in low P availability (Siebielec et al. 2015). This scenario could best explain the undetectable P concentration in the leachate from sandy soil over the four watering events due to the high pH and the high Ca concentration. The approximately two-fold high P concentration in ASP and NSP at the end of the trial in sandy soil, compared to clay soil could be as a result of the initial optimal P fertiliser application, coupled with the minimal P observed in the leachate, as well the release of the loosely adsorbed P ions on the soil particles.

3.5.3 Leachate and soil cation concentration as affected by phosphorus sources and nitrogen forms in two soil types

3.5.3.1 Leachate and end of trial soil potassium concentration

Two-way ANOVA revealed a positive interaction between P source and N form in both clay and sandy soil at all the watering events except in the clay soil at W2 and W3. However, at W2, N form had a significant effect on potassium concentration in the leachate ($P = < 0.0001$) while at W3, both the P source ($P = 0.0036$) and the N form ($P = < 0.0001$) had significant effect on leachate K concentration. (Appendix A).

At W2 in the clay soil, ammonium supply resulted in significantly higher K compared to nitrate (Fig 3.9B). On the other hand, nitrate supply in the clay soil at W3 and end of the study resulted in a significantly higher K concentration than ammonium supply (Fig 3.9B). At W3 in the clay soil, SP supply resulted in a higher K concentration than RP and P_0, both of which had no significant difference between them (Fig 3.9A). The K contents at W2 and end of the trial followed a similar trend as that of W3, but were not significantly impacted by the P source.

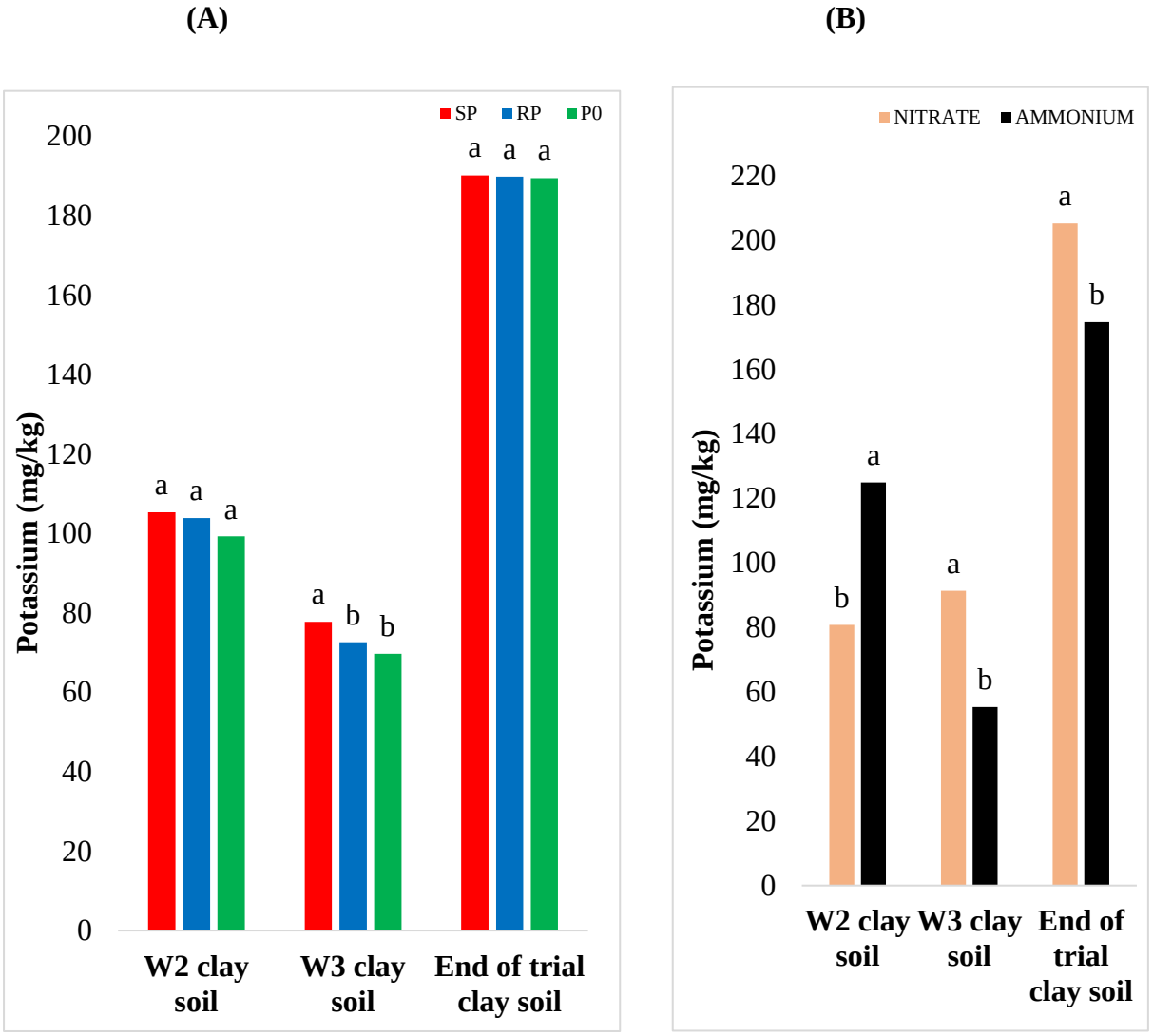


Figure 3. 9. Leachate potassium content at the second and third watering event and at the end of the trial in the clay soil as affected by phosphorus source (A) and nitrogen form (B).

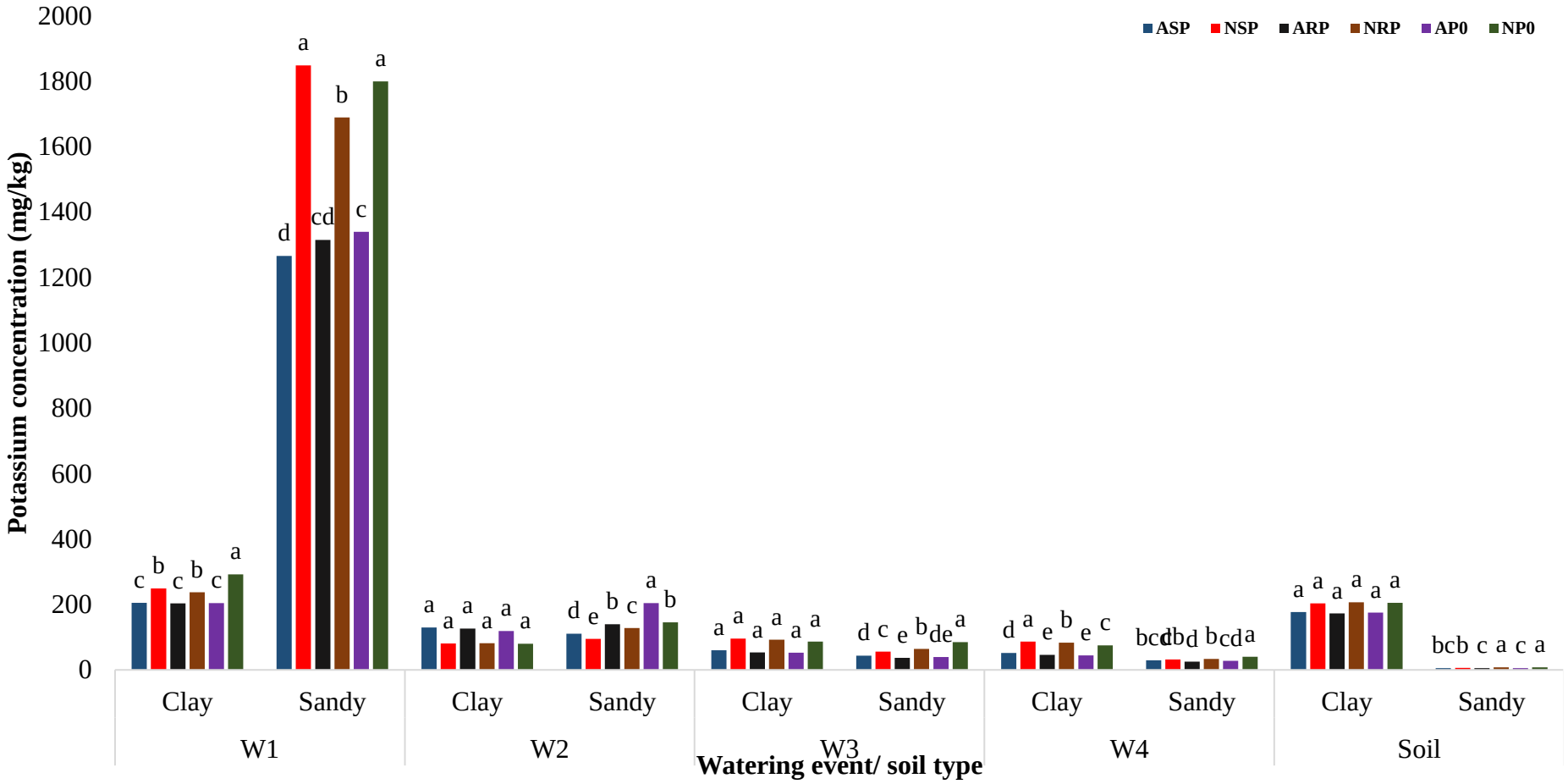
The sandy soil did tend to have a higher leachate K concentration over the first two watering events (Fig 3.10) than the clay soil, especially at W1. For W1, K concentration in the sandy soil leachate was significantly higher in nitrate treatments compared to ammonium. Nitrate SP did not differ significantly from NP_0 but both had significantly higher leachate K content than all other treatments. All nitrate treatments had significantly higher K content in the leachate than all ammonium treatments. Ammonium P_0 had the highest leachate K content of all ammonium treatments but did not differ significantly from ARP, while ASP had the lowest K content.

Similar to the W1 results of the sandy soil, nitrate treatments in the clay soil had significantly higher K than ammonium, but were up to 7-fold lower than in the sandy soil. Nitrate P_0 had significantly higher K than all other treatments. Nitrate SP and NRP did not differ significantly. All ammonium treatments had no significant variation.

In the sandy soil at W2, ammonium treatments had higher K concentration than nitrate treatments. A trend where P_0 had the highest K content followed by RP and SP respectively was noted, regardless of the N form. All treatments showed significant differences, except for ARP and NP_0. Nitrate SP had the lowest K content, significantly lower than all treatments. Ammonium P_0 had significantly higher K concentration than all other treatments.

The leachate of nitrate treatments had higher K content than ammonium in the sandy soil at W3. Nitrate P_0 had significantly higher K content than all other treatments in the sandy soil. Nitrate SP had the lowest K content of all nitrate treatments, which were all significantly different. Ammonium SP and AP_0 did not differ significantly, but ASP had significantly higher K content than ARP.

The clay soil had higher K content in the leachate than sandy soil at W4. All clay soil nitrate treatments had significantly higher K content than ammonium treatments. All nitrate treatments differed significantly from each other. Ammonium SP treatments had significantly higher K content than ARP and AP_0, which showed no significant differences. A trend where SP had the highest K followed by RP and P_0 was noted under either N form. Nitrate P_0 had significantly higher K content than all other treatments in the sandy soil. There were no clear variations between all other treatments in the sandy soil.



N = nitrate, A = ammonium, SP = superphosphate, RP = rock phosphate, P_0 = zero phosphorus. Bars with by the same letter within a column group have no significant differences. N =4, LSD: Clay = NS (not significant), Sandy = 0.88, W1 clay = 25.78, W1 sandy = 68.67, W2 clay = NS (not significant), W2 sandy = 8.06, W3 clay = NS (not significant), W3 sandy = 5.31, W4 clay = 2.86, W4 sandy = 4.82.

Figure 3. 10. Effect of different phosphorus sources and nitrogen forms on soil and leachate potassium concentration in the clay and sandy soil.

At the end of the trial, the two-way ANOVA revealed a significant interaction between P source and N form in the sandy soil (P = 0.0080) but no significant interaction in the clay soil (P = 0.1468). Phosphorus source had no significant effect (P = 0.9276) on soil K in the clay soil, whereas N form had a significant effect (P = < 0.0001) (Appendix A).

In the clay soil, P source had no effect on end of trial K content (Fig 3. 9A). However, clay soil supplied with nitrate gave a significantly higher K content compared ammonium treated soil (Fig 3.9B).

Nitrate treatments had higher K concentration than ammonium in the sandy soil, where NP_0 had the highest K content followed by NRP and NSP respectively (Fig 3.10). Both NRP and NP_0 had significantly higher K content than NSP. Ammonium SP had the highest K content of all ammonium treatments, but there were no significant differences across all ammonium treatments in the sandy soil. Both SP treatments did not differ significantly when combined with either N form.

Marschner and Rengel (2012) reported higher K replenishing ability in a soil high in clay, supposedly due to its higher cation exchange capacity (CEC) in a study comparing high (21%) and low (4%) clay content soils. This affirms the fact that K concentration in the soil is largely a factor of the clay content and the clay mineral composition of the soil. The findings of the current study corroborate findings from the previous study, with a clear variation in K concentration between the two soil types especially at W1 due to the high leaching of applied K while the high clay soil did seem to retain considerable amounts of the applied K. The K concentration in the leachate did tend to stabilise from the second to the last watering, but the soil analysis at the end of the trial gave a contrasting picture, where the sandy soil had very little K content.

According to Aulakh and Malhi (2005), N interaction with K is the second most important nutrient interaction in crops. Potassium and nitrate leaching have been noted to have a very significant and strong correlation (Lucas et al. 2011) and this could serve to explain the higher K content in the leachate of nitrate treated soils, compared to ammonium especially, at W1.

There was no obvious effect of the P source on K concentration in the leachate and in the soil. This evident non-interaction between P and K as depicted in this study where P supply or no supply had minimal to no effect on K concentration has also been reported in other studies (Aulakh and Malhi 2005, Rietra et al. 2017). The high K concentration in nitrate treated soils could be partly explained by the fact that nitrate was supplied as potassium nitrate which has 38% K_2O.

3.5.3.2 Leachate and end of trial soil calcium concentration

Two-way ANOVA revealed a significant interaction between P source and N form at all watering in both soils except at W1 in the sandy soil and at W2 and W4 in the clay soil. W1 sandy soil (P = 0.2612), W2 clay soil (P = 0.7973) and W4 clay soil (P = 0.0528). However, P source (P = 0.0044) and N form (P = < 0.0001) at W2 and P source (P = < 0.0001) and N form (P = < 0.0001) at W4 had a significant effect on the leachate Ca content. At W1 in the sandy soil, P source had no effect on leachate Ca content but N form had a significant effect (P = <0.0001) (Appendix A).

In the sandy soil at W1, there was no effect of P source on leachate Ca concentration (Fig 3.11A). In contrast, leachate of sandy soil at W1 supplied with nitrate gave a significantly higher calcium content than ammonium (Fig 3.11B).

At W2 in the clay soil, SP application resulted in significantly higher calcium compared to P_0 but did not differ significantly with RP. There was no significant difference in calcium content between soil supplied with RP or P_0 (Fig 3.11A). Ammonium supply in the clay soil at W2 resulted in significantly higher calcium content compared to nitrate treated soil (Fig 3.11B).

The Ca content in the leachate of W4 clay soil, followed a similar trend as the W2 clay soil in terms of P source, but 1) were lower and 2) with P_0 resulting in significantly the lowest Ca content in the leachate (Fig 3.11A). The Ca content in the leachate from the W4 clay soil were much lower than that of the W1 sandy soil, with nitrate similarly resulting in significantly more Ca in the leachate than ammonium (Fig 3.11B).

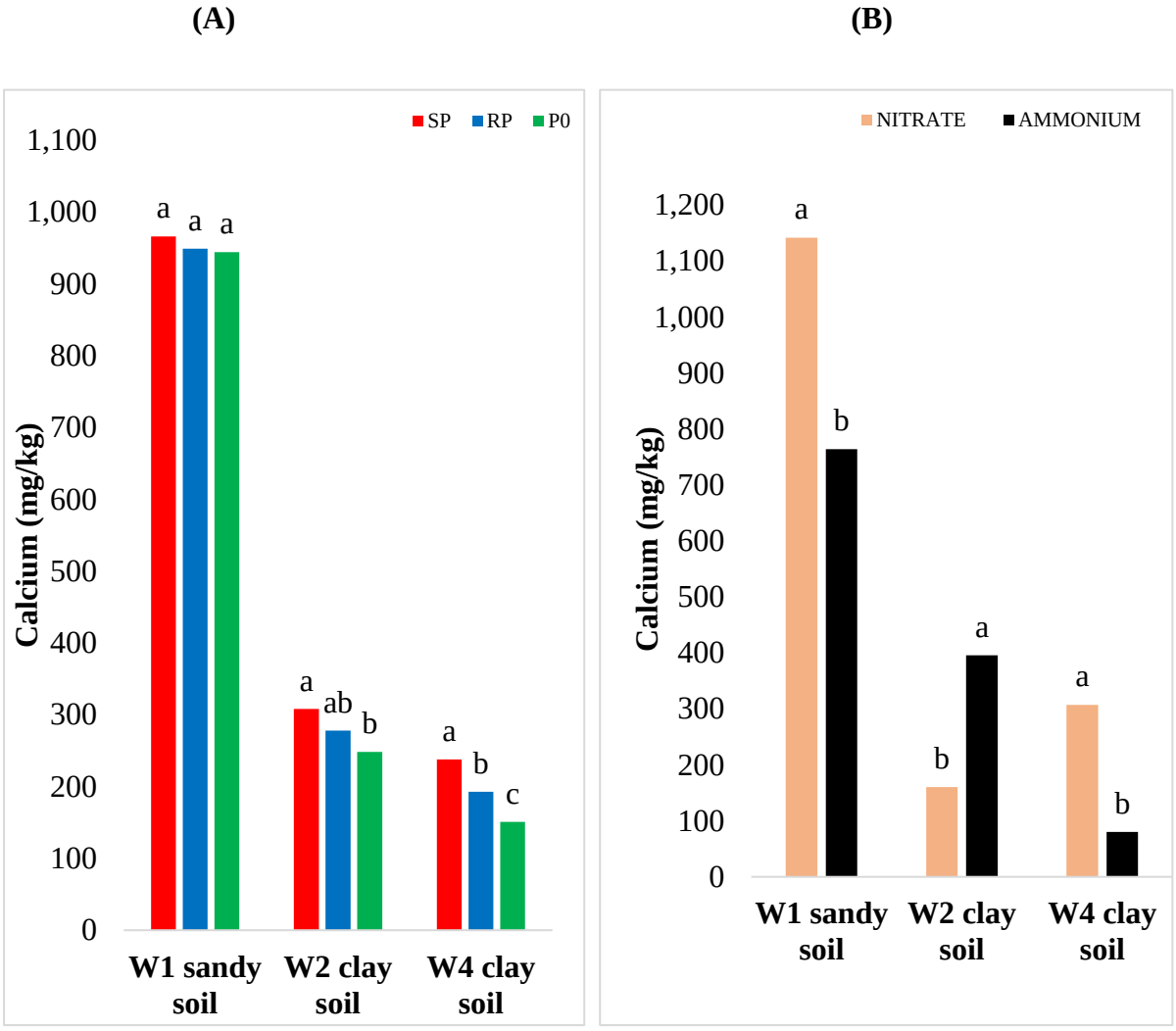
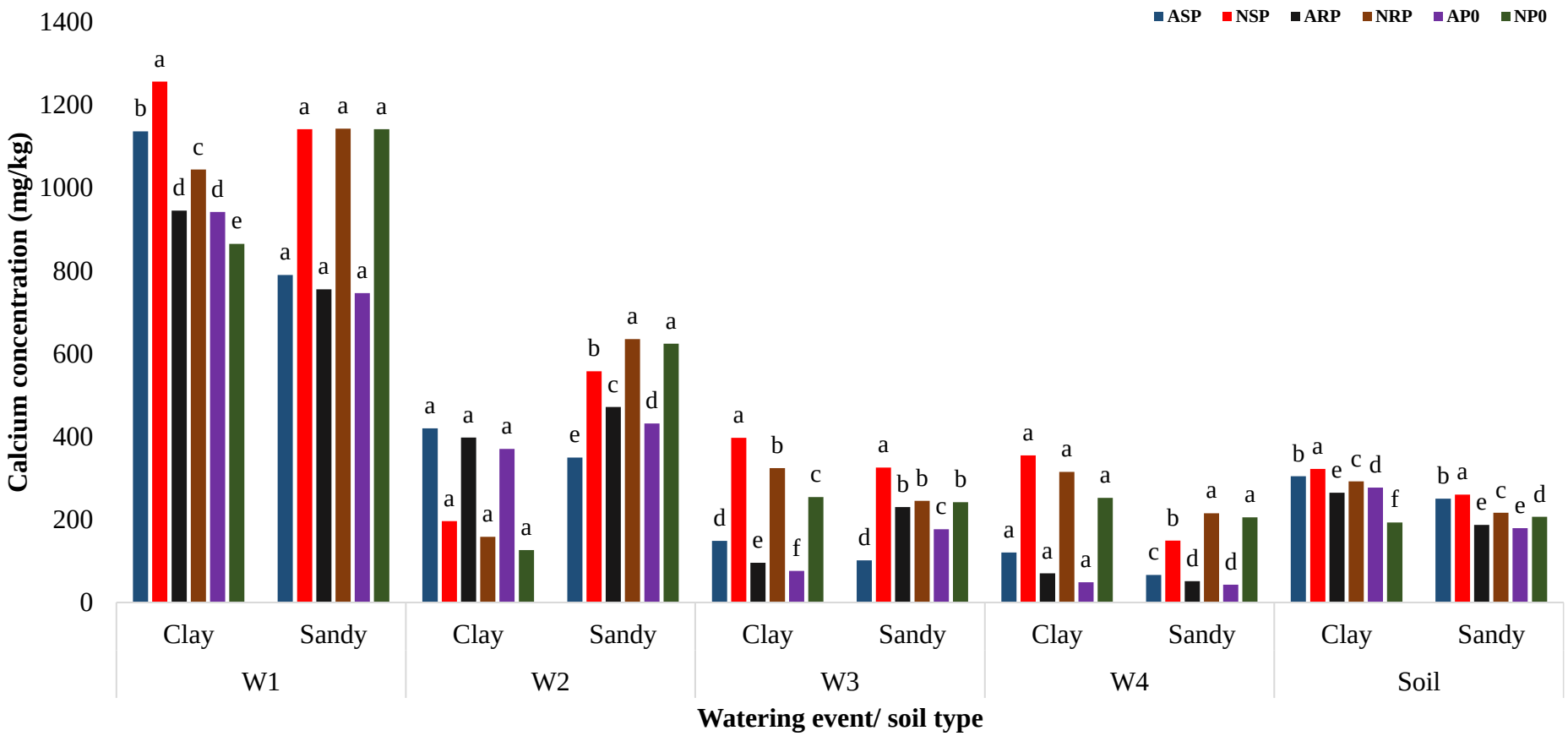


Figure 3. 11. Leachate calcium content at first watering event in the sandy soil, second and fourth watering events in the clay soil as affected by phosphorus source (A) and nitrogen form (B).

Calcium leaching was most significant at the first watering event and decreased gradually from the first to the last watering (Fig 3.12). Nitrate SP gave the highest leachate Ca content in the clay soil at W1, followed by ASP. Apart from ARP and AP_0, all treatments showed significant differences in the clay soil. A trend where SP had the highest Ca concentration followed by RP and P_0 respectively in either N form was observed for the clay soil.



N = nitrate, A = ammonium, SP = superphosphate, RP = rock phosphate, P_0 = zero phosphorus. Bars with by the same letter within a column group have no significant differences. N =4, LSD: Clay = 10.33, Sandy = 8.04, W1 clay = 43.03, W1 sandy = NS (not significant), W2 clay = NS (not significant), W2 sandy = 13.49, W3 clay = 17.36, W3 sandy = 22.77, W4 clay = NS (not significant), W4 sandy = 13.28.

Figure 3. 12. Effect of different phosphorus sources and nitrogen forms on soil and leachate calcium concentration in clay (A) and sandy (B) soil.

Nitrate application resulted in significantly higher Ca content in the sandy soil at W1, compared to ammonium. All nitrate treatments did not differ significantly in the sandy soil. In the ammonium treatments, only ASP had significantly higher Ca content than AP_0.

At W2 in the sandy soil, all nitrate treatments had significantly higher Ca content than ammonium treatments. Nitrate P_0 and NRP did not differ significantly but both had significantly higher Ca content than NSP. Ammonium RP had the highest Ca concentration of all ammonium treatments in the sandy soil, while ASP had the lowest Ca content. All ammonium treatments showed significant differences.

Nitrate treatments had a higher Ca concentration in the leachate at W3 compared to ammonium in both soil types. A trend where SP had the highest Ca content followed by RP and P_0 was noted in the clay soil for both N forms. All treatments showed significant differences. Nitrate SP had the highest Ca content followed by NRP and P_0 in the clay soil. Nitrate SP had significantly higher Ca content than all other treatments in the sandy soil. All RP and P_0 treatments gave no significant differences in the sandy soil, except for AP_0, which had significantly lower Ca concentration. Ammonium RP had the highest Ca content of the ammonium treatments while ASP had the lowest and all ammonium treatments showed significant differences in the sandy soil.

Nitrate supply gave higher Ca content in the sandy soil compared to ammonium treatments. Nitrate RP and P_0 had significantly higher Ca content than all other treatments in the sandy soil. Ammonium treatments showed a similar trend to clay soil, where SP gave the highest Ca content followed by RP and P_0. Ammonium SP gave significantly higher Ca content than both ARP and P_0, which did not differ significantly.

At the end of the trial, there was a significant interaction between P source and N form in the clay ($P = < 0.0001$) and sandy soils ($P = 0.0030$) (Appendix A).

Soil analysis at the end of the trial indicated that SP resulted in the highest Ca content in both soil types, where NSP had the highest Ca concentration in both soils (Fig 3.12). Nitrate treatments gave higher Ca than ammonium in both soils with all other P sources except for RP_0 in the clay soil. All treatments gave significant differences in the clay soil. Similarly, in the

sandy soil, all treatments showed significant differences, except for, ARP and AP_0 which did not differ significantly.

Past studies in crops have indicated that Ca concentration in plants is higher in nitrate supplied plants compared to ammonium; Mengel and Kirkby (1987) in tomato, Van Beusichem et al. (1988) in castor oil plants, and Tabatabaei et al. (2006) in strawberry. These changes are attained due to the ability of nitrate ammonium to influence soil pH which in turn influences nutrient availability. In a study conducted on acidic soil, Sharpley (1991) noted an increase in Ca concentration with increasing pH. This agrees with the findings of this current trial where the sandy soil (with a higher pH) gave the highest Ca content in the leachate, especially in the nitrate treatments, which consequently resulted in higher Ca content than ammonium treatments. Calcium concentration in the soil did seem to be a factor of the pH and the clay content as the clay soil had a higher Ca than the sandy soil. This could also be due to the substantial Ca leaching over the four watering cycles, resulting in low soil Ca. Fixation of P by Ca could also have resulted in the low Ca availability in the sandy soil.

Saturation of the soil solution with dissolved calcium and P leads to calcium phosphate precipitation (Penn and Camberato 2019). According to the Le Chatelier's principle, Ca phosphate formation is amplified under high P and Ca concentration in solution and an increase in pH. Single superphosphate fertilizer is made up of mono-calcium phosphate, which rapidly dissolves, leading to Ca and P saturation, which later precipitates to form meta-stable complexes and subsequently get transformed into less soluble complexes e.g. hydroxyapatite (Essington 2015). The Ca content was also enhanced by lime application in this current study.

Various researchers have also suggested that calcium influences P availability in acidic soils (Curtin and Syers 2001, McDowell et al. 2002b, McDowell and Sharpley 2003, Prietzel et al. 2013, Eriksson et al. 2015). Raising soil pH can reduce P availability via Ca phosphate precipitation as a factor of Ca availability while reducing the pH will dissolve Ca phosphates. Curtin et al. (1987) reported that Ca affected P solubility in 11 different acidic soils, further highlighting the role of Ca in P availability in acid soils. In calcareous soils, the consumption of Ca by carbonate results in enhanced Ca phosphate solubility with further pH rise. This is due to carbonate and Ca competition for P (Penn and Camberato 2019).

3.5.3.3 Leachate and soil magnesium concentration

There was no interaction between P source and N form in the clay soil at W1. However, both P source (P = < 0.0001) and N form (P = < 0.0001) had a significant effect on the Mg leachate content. In the clay soil, watering events 2, 3 and 4 did result in a significant P source and N form interaction. For the sandy soil, there was no detectable Mg in the leachate after watering event one, and even for the other three watering events, there was very low or no detection. Despite this, there was a significant interaction effect after water events 2, 3 and 4. (Appendix A).

At W1, leachate of clay soil supplied with SP had significantly higher magnesium content than that of RP and P_0, both of which were not significantly different (Fig 3. 13A). On the other hand, ammonium supplied clay soil at W1 resulted in significantly higher magnesium content than nitrate (Fig 3.13B).

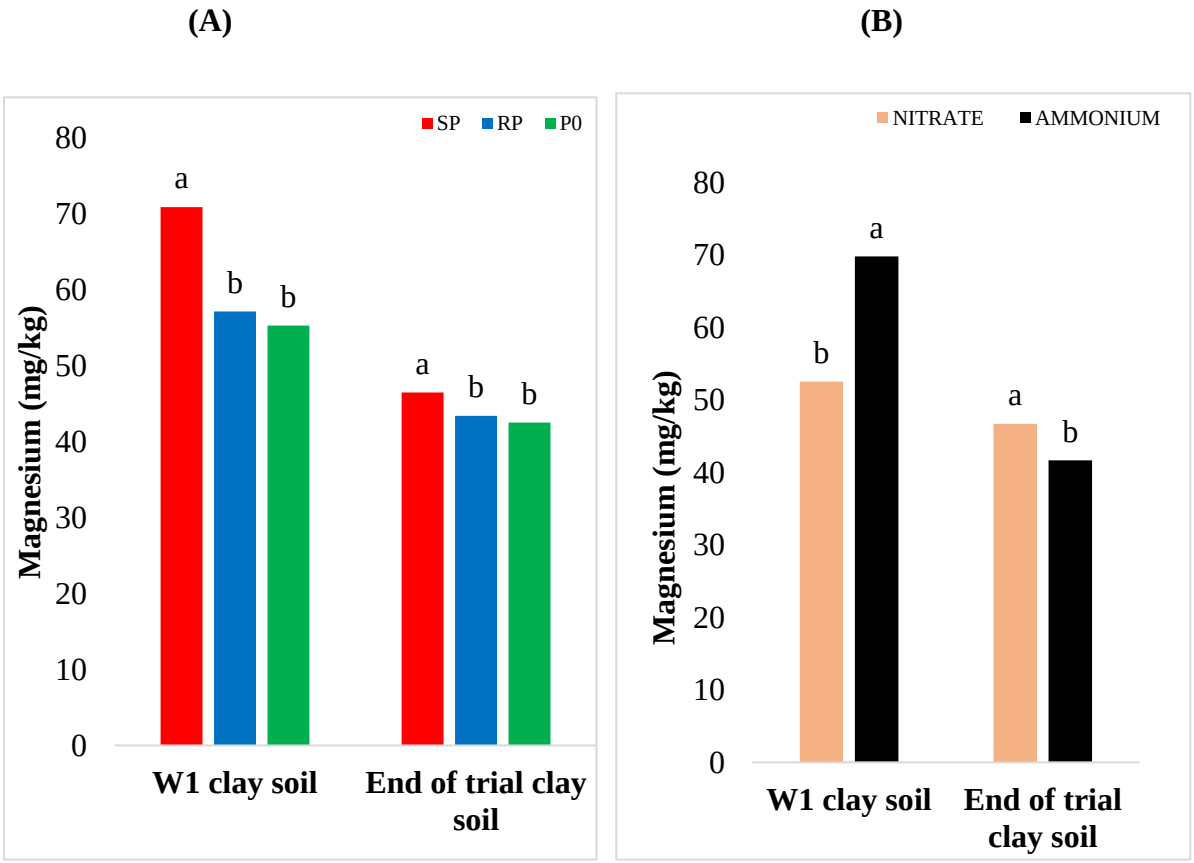
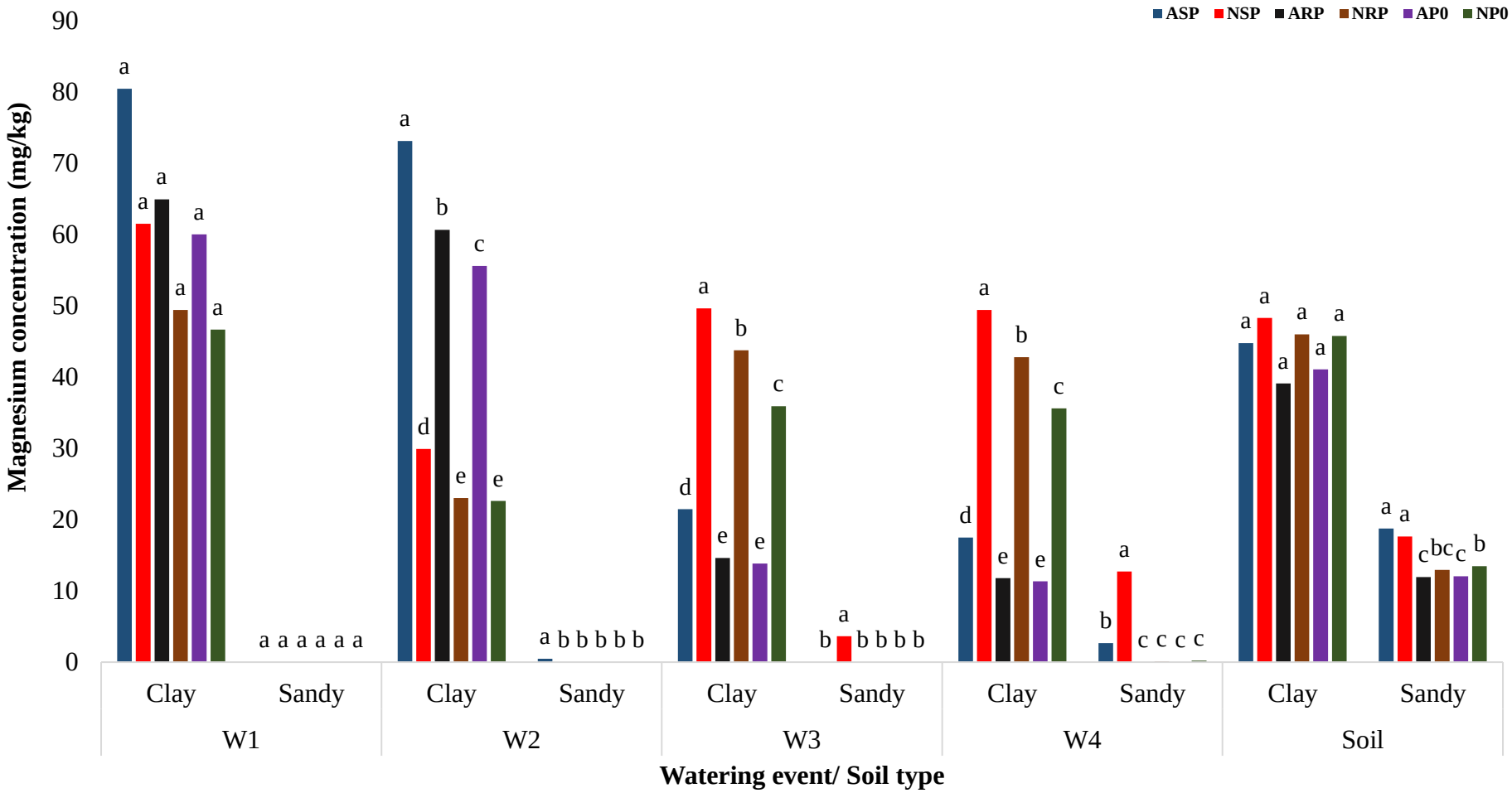


Figure 3. 13. Leachate magnesium content at the first watering event and at the end of the trial in the clay soil as affected by phosphorus source (A) and nitrogen form (B).

The magnesium content in leachate and in the soil was higher in the clay compared to sandy soil on all occasions (Fig 3.14). Magnesium content in the sandy soil leachate was not detectable over most of the four watering events, with only marginal amounts detected for ASP at W2 and a higher content at W3 for NSP. At W4, Mg was detected in the leachates of NSP, ASP and NP_0, with that of NSP being significantly higher than both ASP and NP_0.

At W2 in the sandy and clay soil, ammonium treatments had higher Mg content in the leachate than nitrate, while nitrate treatments had a higher Mg concentration at the third and fourth watering. All ammonium treatments showed significant differences at W2. Superphosphate supplied with either N form had the highest Mg concentration, followed by RP and P_0.

Nitrate treatments had higher Mg contents in the clay soil at W3 and W4. Both watering events showed identical trends, where SP had the highest concentration, followed by RP and P_0, when supplied together with either of the N forms. Over the last two watering events, all nitrate treatments showed significant differences between them in the clay soil, while for the ammonium treatments, ASP had significantly higher Mg concentration than ARP and AP_0, which did not differ significantly from each other at W3 and W4. The increased Mg concentration in the sandy soil at W3 and W4 could be in response to the gradual decline in soil pH from W1 to W4. Magnesium availability in soil is known to decrease under extremely alkaline pH conditions.



N = nitrate, A = ammonium, SP = superphosphate, RP = rock phosphate, P_0 = zero phosphorus. Bars with by the same letter within a column group have no significant differences. N =4, LSD: Clay = NS (not significant), Sandy = 1.29, W1 clay = NS (not significant), W1 sandy = 0.00, W2 clay = 3.74, W2 sandy = 0.05, W3 clay = 3.25, W3 sandy = 0.20, W4 clay = 3.10, W4 sandy = 1.40.

Figure 3. 14. Effect of different phosphorus sources and nitrogen forms on soil and leachate magnesium concentration.

At the end of the trial, there was a significant interaction between P source and N form in the sandy soil (P = 0.0211) but had no significant interaction in the clay soil (P = 0.2705). However, P source (P = 0.0025) and N form (P = < 0.0001) had a significant effect on soil Mg content in the clay soil (Appendix A).

In the clay soil at the end of the trial, when supplied with SP gave significantly higher magnesium leachate content than RP and P_0, both of which did not differ significantly (Fig 3.13A). On the other hand, nitrate supply resulted in significantly higher Mg than ammonium supply in the clay soil at the end of the column study.

In the sandy soil, both SP treatments did not differ significantly from each other regardless of the N form. Nitrate with RP and P_0 showed no significant differences and neither did ARP and AP_0. Nitrate P_0 had a significantly higher Mg concentration than ARP and AP_0 (Fig 3.14).

Curtin and Smillie (1983) also reported a profound decrease in the Mg concentration in the soil upon application of lime, a scenario that agrees with this trial where Mg concentration was negligible in the sandy soil (high pH) indicating that the Mg availability is largely a factor of the soil pH. Simard et al. (1998) also reported a similar scenario.

In general, high clay content in soils have been perceived to offer greater binding surfaces for a higher concentration of exchangeable base cations, compared to coarse soils low in clay content (Beldin et al. 2007). Other studies have also reported that coarse and sandy soils have low soil organic matter and consequently have a low capacity for nutrient retention (Zhao et al. 2006, Zhou et al. 2008). This could best explain the greater leaching in the sandy soil. Some authors have also attributed the significantly higher leaching, to low carbon in sandy soils, which is the greatest component of soil organic matter (Oorts et al. 2003). Lü et al. (2016) also reported a high positive correlation between soil carbon and base cations. Antisari et al. (2013) also noted that humified organic compounds in soil play a role in the retention of base cations. This results in a decline in leaching, though it was not assessed in this trial, sandy soils are known to be low in humified organic compounds. The coarseness of soils has also been reported to have an effect on soil microbial activities resulting in lower organic matter (Wang et al. 2015) thus decreasing the available functional groups to bind with the base cations. Therefore, this could account for the greater base cation concentration in the clay soil for all

assessed elements presumably due to its higher retention capacity by offering more binding sites for the cations.

Magnesium and ammonium are known to work in synergy where Mg has been reported to reduce the volatility of ammonium in the soil through the formation of magnesium salts (Von Rheinbaben 1987). This could result in higher Mg concentration in ammonium treated soils as was noted in the first two leaching events more so in the clay soil.

The greatest determinant of Mg content appeared to be a split between the pH and clay content of the soil initially, Mg was not detected in the sandy soil, a trend that seemingly continued despite fertilization, whose effect was only noted in the soil analysis at the end. The clay soil tended to have higher Mg concentration regardless of the P source.

Lime induced Mg fixation could have strongly resulted in the minimal Mg leachate content, especially in the sandy soil. Magnesium deficiency in soils has been reported to be adverse at near-neutral soil pH (Farina et. al 1980).

3.6 Concluding remarks/Synopsis

As expected, ammonium enhanced P availability, regardless of soil type and P source. This was mainly due to its ability to increase soil acidity, while on the other hand nitrate resulted in reduced P availability regardless of soil type and P source, due to increased soil pH despite the absence of a test crop. This lend credence to the working hypothesis of this column study.

The ability of both nitrate and ammonium to influence soil pH plays a major role in influencing nutrient availability. As per the hypothesis in this study, ammonium influenced the pH by increasing soil acidity while nitrate influenced nutrient availability by decreasing soil acidity. The sandy soil as compared to the clay soil, showed a greater propensity for a rise in pH despite a similar amount of lime being applied, raising a fundamental question on the effectiveness of lime application as a way of correcting pH in various soils.

It was also evident that the clay content in soils plays a major role in nutrient availability in the soil judging from the clear differences in cation balance in the two soil types and in their respective leachate. Cumulatively, the leachate and final soil P content of the clay soil compared to the sandy soil was between 1.8 - 7.7-fold higher.

Ammonium-treated soils had higher cumulative P compared to nitrate-treated soils in either soil type. This was indicated by the fact that P concentration in ammonium treatments compared to nitrate treated soils was between 1.5-1.3-fold higher. The pH in the ammonium treated soils (leachate and soil average) was between 0.5-1.1-fold lower than the corresponding nitrate treatments. Thus, the hypothesis that ammonium had indeed affected soil P concentration through soil pH adjustments even in the absence of a test crop could be accepted.

The sandy soil tended to have a higher soil pH compared to the sandy soil, regardless of the P source or N form supplied. However, ammonium treatments in either soil had a lower pH compared to nitrate.

Potassium concentration tended to be higher in the sandy soil in the initial watering stages but the concentration went down from the second to the fourth watering event and eventually, the clay soil gave a higher K concentration than the sandy soil. No specific trend was noted regarding K concentration in the leachate and soil. There was generally minimal interaction

between P source and N form, especially in the clay soil. Nitrate combined with either P source tended to increase potassium concentration, which was most striking at W1 in the sandy soil.

Higher calcium concentration was detected in the nitrate treated soils compared to ammonium in the leachate, especially in the sandy soil indicating a substantial improvement in calcium availability upon nitrate fertilisation. This could also have been due to the potassium nitrate fertiliser used in all nitrate treatments. Despite the significant interactions between P and N in the various watering events. A specific trend was not observed in this regard. However, at the end of the trial, the combination of SP with either ammonium or nitrate did tend to increase the Ca concentration in the soils

Lastly, the clay soil gave higher magnesium concentration at all stages observed. Ammonium tended to favour Mg availability in the first and second watering event in the clay soil, however, nitrate treated clay soil gave higher Mg content from the third and fourth watering event and at the end of the trial. There was minimal Mg leaching in the sandy soil and the soil at the end of the trial similarly had lower Mg compared to the sandy soil. These findings suggest that there is an advantage in ammonium fertilisation in clay soils compared to nitrate in regard to Mg availability in the soil.

CHAPTER 4

THE EFFECT OF DIFFERENT NITROGEN FORMS AND PHOSPHORUS SOURCES ON POTATO GROWN IN TWO SOILS VARYING IN PHOSPHORUS CONCENTRATION

4.1 Introduction

Crop plants are affected by numerous biotic and abiotic stresses during their growth cycle. Their ability to negate these challenges ensure that the crops survive and attain optimal productivity. Of these abiotic stresses, phosphorus (P) limitation is a major constraint to crop production. It plays an indispensable role in vital plant metabolic processes such as cell division, root growth, flowering, fruit ripening, respiration, photosynthesis and the maintenance of plant genetic identity (Vance et al. 2003). Phosphorus is also involved in the making of organic compounds, such as ATP, ADP, phytine and phospholipids (Wall et al. 2013).

However, P is very limited in most soils as it largely exists in a fixed state with the available P being <10 mg/kg (George at al. 2011). The concentration of P in the root depletion region is meagre, meaning that plants have to obtain and similarly buffer cytosolic P, which is three-fold that of the rhizosphere concentration (Baker et al. 2015). The resulting P deficiency impedes plant growth through increased root development (proliferation), stunted haulm growth, resulting in an increased root to haulm ratio (Rodriguez et al. 1994).

The limitation of P in most soils has resulted in overdependence on phosphatic fertilizers. The fertilisers are manufactured from rock phosphate (RP), a finite resource which is bound to be depleted in a few centuries (Ashley et al. 2011, Baker et al. 2015). The continued injudicious use of these fertilizers results in environmental pollution and poses human health risks. There is, therefore, a dire need to efficiently use this resource to produce even more food for the increasing demand due to population increase. This leaves farmers constrained by the available options, which are to either use more P fertilizer to increase production, or more efficient use of this finite resource. The efficient use stands supreme and can be achieved by enhancing both the acquisition and utilization of P (Veneklaas et al. 2012). Mass flow provides insufficient P

to supply the needed P in sufficient quantities for optimal plant growth (Kirkby and Johnston 2008). Plants are known to remodel their physiology to increase P acquisition and use (Pang et al. 2015) by developing a large root network through investing greater biomass to the root structures at the expense of the haulms (Lynch and Brown 2008). Physiological mechanisms include organic acid exudation (Pang et al. 2015), enhanced acid phosphatase activity (Gaume et al. 2001) and the adoption of high-affinity transporters (Jia et al. 2011).

In addition to the nature of P and its fixation, other elements, such as nitrogen (N) affect plant P nutrition, both directly and indirectly (Jouany et al. 2011). Phosphorus and nitrogen have been reported to produce a brawny synergistic interaction in nearly all kinds of habitat (Elser et al. 2007). Storia et al. (2007) reported a great P and N interaction in a grassland with soil low in P and Vitousek et al. (2010) reported that excess N induced P limitation in the soil. Under limited P, optimal distribution of resources is dependent on a healthy P and N balance in plants, such that the acquisition of photosynthetic carbon gets limited by these two elements (Chapin et al. 1987).

The number of studies detailing the interaction of these elements is limited and more inclined to forests and grasslands. Even fewer studies have been conducted to unravel the effects of the various N forms on P availability when plants are supplied with varying P sources and the eventual crop growth response. Based on current knowledge, P and N interaction is either absent, negative or positive and widely varies from one species to another and between cultivars of the same species.

Ground rock phosphate has in the past been considered as an alternative to chemical P fertiliser (Sharma and Prasad 2003, Sharma et al. 2009). In paddy, maize and wheat, RP supply has been reported to show no significant differences with superphosphate application and also showed a greater residual effect (Motsara and Datta 1971). In potato, contrasting reports have been found with RP, with some indicating that it does not support optimal growth (Motsara and Datta 1971) and other reports indicating increased yields (Shivay 2010). These studies have been conducted widely with the use of P solubilizing bacterial strains or initial acidification. This trial, therefore, seeks to explore the possibility of soil acidification or alkalinisation by the various N forms on the efficacy of RP as an alternative to chemical P fertiliser.

It is, therefore, important to do species and cultivar specific studies to best understand the P - N interaction. The interaction has also varied widely from one soil type to another with varying initial P concentration and this formed the basis of this current study. The main objective was to unravel the effect of ammonium and nitrate as N forms and superphosphate and rock phosphate as P sources on the growth and yield of potato in soils varying in P concentration.

The hypotheses of this pot trial were, therefore, firstly, that ammonium would result in enhanced P dissolution and availability in the soil and uptake by potato due to increased soil acidification, regardless of the P source and the soil type. Secondly, nitrate would result in reduced P dissolution and availability in the soil and uptake by potato due to increased soil alkalinity. The third hypothesis was that superphosphate P source will result in increased P dissolution and availability in the soil and uptake by potato under either N form. The fourth hypothesis was that rock phosphate will enhance P dissolution, availability in the soil and uptake by potato plants when applied together with ammonium, compared to nitrate. The final hypothesis was that treatment without P application will result in increased P dissolution, availability in the soil and uptake by potato plants when supplied with ammonium, compared to nitrate.

4.2 Materials and Methods

4.2.1 Study site

The experiment was conducted in a glasshouse at the University of Pretoria (UP) Experimental Farm, South Africa, on a rotating table to reduce any bias by ensuring that the plats receive relatively equal amounts of sunlight. The site was located at 23°45' S and 28°16' E, at 1372 m above sea level.

4.2.2 Soil analysis prior to the start of the trial

Five samples per soil type were collected from fields on the Hatfield Experimental farm in a zig-zag pattern for analysis. The soils were first air-dried, milled and passed through a 2 mm sieve before analysis. The analysis was conducted at the Soil Science laboratory of the University of Pretoria. The pH (KCl) values for the first and second season soils were 5.29 and 3.31 respectively. The two soil types were selected on the basis of their initial P concentration (high and low) so as to test the effectiveness of the treatments on a deficient and relatively high soil P concentration. Five samples were collected for each soil type and the average for the samples was used to estimate the P status for each soil. The soil chemical analysis results prior to the commencement of the trials are indicated in Table 4.1.

Table 4. 1. Soil nutritional status at the start of the experiment for season one and two

Season	Element (mg/kg)					
	P (available)	K	Ca	Mg	Na	S
1	24.7	61.3	388.7	94.3	4.3	1.4
2	9.9	53.6	26.3	4.4	0	79.0

Particle size distribution for the soils used in the two seasons was determined using the hydrometer method and results are presented in Table 4.2.

Table 4. 2. Soil particle size distribution prior to the experiment

Particle size	Distribution (season one)	Distribution (season two)
Sand	67.4 %	74.5 %
Clay	28.0 %	22.0 %
Silt	4.6 %	3.5 %

4.2.3 Experimental setup, treatments and growth media

The study was conducted in a glasshouse trial over two seasons. The first season trial was planted in May 2018 and harvested in August 2018 and the trial for the second season was planted in November 2018 and harvested in February 2019. The treatment combinations were: two P sources and a treatment without P, combined with two N forms (Table 4.3).

Table 4. 3. Treatment combinations for the glasshouse trial over the two seasons

Treatment	Nitrogen form	Phosphorus source
1	NO_3^-	Superphosphate (SP)
2	NO_3^-	Rock phosphate (RP)
3	NO_3^-	Zero phosphorus (control; P0)
4	NH_4^+	Superphosphate (SP)
5	NH_4^+	Rock phosphate (RP)
6	NH_4^+	Zero phosphorus (control; P0)

Two soils from the UP Experimental Farm were used as planting media. The soil was collected from the 0 to 15 cm soil layer of two different fields. The soil was then poured into a bunker and steam sterilized for 3 hours at 100 ^oC to kill any harmful microorganisms that might have been present and allowed to cool down. Pots of a 10-litre capacity were sterilised overnight in a jik solution and then rinsed three times with running water.

Fertilisation was based on a yield potential of 60 t ha^{-1} as per Potatoes South Africa guidelines (Steyn and Plessis 2012). Nitrogen application level of 200 kg N per ha was based on the soil clay content (>20%), for both soils and in both seasons (Table 4.4).

Table 4. 4. Nitrogen application level as per soil clay content (Steyn and Plessis 2012)

Clay content (%)	**Yield potential (ton ha^{-1})**					
	30	**40**	**50**	**60**	**70**	**80**
< 10	170	220	250	275	300	320
10 – 20	150	190	220	240	260	280
>20	130	160	180	200	220	240

Based on the soil analysis (Table 4.1), 100 kg ha^{-1} P was applied in season one and 130 kg ha^{-1} P in season two, as per the recommendations for the Bray 1 analysis method and yield potential (Table 4.5).

Table 4. 5. Phosphorus application as per soil phosphorus content (Steyn and Plessis 2012)

Extractable P (mg kg^{-1})	**P fertilization at different yield potentials (ton ha^{-1})**							
	10	20	30	40	50	60	70	80
0-5	100	115	130	145	160	175	190	205
6-10	80	90	100	110	120	130	140	150
11-19	60	70	80	90	100	110	120	130
20-25	50	60	70	80	90	100	110	120
25-30	40	50	60	70	80	90	100	110
30+	30	30	30	30	30	30	35	45

The actual amount of fertilizer applied per pot was calculated according to the method described in *chapter 3* (Eq. 3.1).

The amount of 0.44 g and 0.58 g of P was applied per pot in seasons one and two, respectively. The SP treatment was supplied with single superphosphate (SSP). Rock phosphate was supplied as Langfos RP, a sedimentary rock obtained from Cape Town, South Africa. Ammonium was supplied as ammonium sulphate (21% N) at 4.24 g per pot and nitrate as potassium nitrate (13% N), at 6.8 g per pot in both seasons (Table 4.6). Nitrate treatment did not receive potassium fertiliser as the requirements were met by the potassium content in the nitrate fertiliser used. Potassium chloride was used to supply potassium requirements in ammonium treated soils. Calcium, magnesium and lime were not applied in the first season.

This is because the soil analysis results (Table 3.1) were well within the recommended ranges for a yield potential of 60 t ha^{-1} (Steyn and Plessis 2012).

In the second season, the soil was very acidic and needed pH correction. The soil had to be limed to raise the pH to 5.5, therefore, the amount of lime to be applied was determined according to the method described in *chapter 3* of this current study (Eq 3.2- 3.6). Lime was applied as calcium hydroxide to lower the soil pH and also to supply calcium needs, while Mg was supplied as magnesium oxide.

The nutrient type and actual amount of fertiliser applied per pot as indicated in Table 4.6.

Table 4. 6. Fertilizer application rates per hectare and per pot for season one and two

Season	**N**	**P**	**K**	**Ca**	**Mg**	**Lime**
Season 1 (kg/ha)	200	100	270	-	-	-
Season 1 (g/pot)	0.89	0.44	1.20	-	-	-
Season 2 (kg/ha)	200	130	270	805	75	850
Season 2 (g/pot)	0.89	0.58	1.20	3.58	0.33	4.1

Approximately 10 kg of soil was weighed out and thoroughly mixed with the fertiliser (each treatment separately). The soil-fertiliser mixture was then transferred into the sterilised 10-litre pots. Nitrogen application was split into two dressings with 50% applied at planting and 50% after tuber initiation

The experiment was laid out in a completely randomised design with 8 replicates per treatments so as to provide enough plants for destructive sampling at tuber initiation stage (4 replicates) and the remaining four replicates were retained for data collection at the end of the season.

4.2.4 Planting material

Certified Mondial minitubers (pre-treated with a fungicide) that were pre-sprouted in diffused light at 25 ^{o}C were used as planting material. Mondial is a medium-maturing cultivar and matures within 90 to 110 days after emergence. This cultivar has a relatively short dormancy

period, ranging between 50 to 60 days. The cultivar grows relatively tall, has semi-erect shoots and grows relatively vigorous.

4.2.5 Planting and culture work

Planting was done by placing one minituber at a depth of 5 cm in the centre of each pot. Soil water content was monitored using capacitance probes. Irrigation was done using a pressure compensated drip irrigation system. Temperature and relative humidity were observed using a HOBO Pro v2 U23-001 logger.

4.3 Data collection

Data collection was done in two phases: during tuber initiation (TI), which was at 35 days after emergence and at the end of the season (ES), at 105 days after emergence. Four plants of the eight replicates were used for assessment at TI while the remaining four plants per treatment were used for assessment at ES. At both stages, destructive sampling was done by uprooting the plants and separating the haulms from the roots and also the tubers from the roots. The parameters assessed were similar across the two stages, except for the number of tubers initiated at TI and tuber fresh and dry mass at ES.

4.3.1 Haulm and root length

The haulms were detached from the roots and their lengths determined using a measuring tape by measuring from the point of separation with the roots to the tip of the main growing shoot. Soil adhered to the roots was washed off and root length determined by measuring the length of the longest roots.

4.3.2 Haulm and root dry mass

Haulms and roots were oven-dried separately in brown paper bags to a constant mass at 70 ^{0}C. These were then separately weighed to determine the dry mass of each component.

4.3.3 Haulm to root dry mass ratio

The haulm to root dry mass ratio was determined according to (Eq. 4.1):

$$Haulm\ to\ root\ ratio = \ (Haulm\ dry\ mass)/(Root\ dry\ mass)$$

(Eq. 4.1)

4.3.4 Number of tubers initiated

At approximately five weeks after emergence, four pots per treatment were randomly sampled and the plants were carefully uprooted, soil adhered to the roots was washed off and the number of daughter tubers/ stolons initiated was recorded.

4.3.5 Tuber fresh and dry mass

After uprooting the plants and separating the tubers from the roots and stolons, soil adhering to the tubers was washed off and the tubers were weighed to determine the tuber fresh mass. The tubers were then diced into small pieces and spread evenly inside brown paper bags and oven-dried at 70 ^{o}C to a constant mass. The dried tubers were subsequently weighed to determine the dry mass of each treatment.

4.3.6 Plant available phosphorus determination

Plant available soil P analysis was done both at TI and ES using the P-Bray 1 method as described in *chapter 3* of this current study.

4.3.7 Total inorganic phosphorus analysis in plant leaves

Total inorganic P content of the leaves was determined at TI and ES via hydrolysis by nitric acid. The fourth fully expanded leaf from the top of the plant in each treatment was sampled, put in an envelope and dried to a constant mass. The TI samples were stored in zip lock bags after drying and analysis was done simultaneously with the ES samples, but separately in each season. The leaves were then ground using a mechanical leaf milling machine. This was followed by weighing exactly 0.3 g of the dried leaf sample into a 50 mL centrifuge tube, 9 mL of 65 % nitric acid added and the samples put into a microwave reaction system (Multiwave 3000) for 45 minutes. The solution was then topped up to 30 mL with deionized water and the P concentration was determined using the ICP-AES method.

4.3.8 Rhizosphere pH (KCl)

The soil pH was determined both at TI and ES in each season using the potassium chloride method as described in *chapter 3* of this current study.

4.4 Data analysis

Data were analysed using Statistical Analysis Systems (SAS) Version 9.4. Two-way ANOVA was used to identify P-N interactions using the PROC general linear model (GLM) procedure at $P<0.05$ level. The differences in means were quantified using the Tukey honestly significant difference (HSD) test, controlling for overall experiment wise error rates.

4.5. Results and discussion

4.5.1 Soil pH as influenced by the form of nitrogen and phosphorus source

Two-way ANOVA revealed a significant interaction between P source and N form at all the four stages observed (Appendix B).

The ammonium SP combination resulted in the lowest soil pH, while NSP gave the highest soil pH at TI and ES for both seasons (Table 4.7). In the first season at TI, all ammonium treatments showed significant variations in pH, where ASP resulted in the lowest pH, followed by AP_0 and ARP. Nitrate SP resulted in a significantly higher pH than all other treatments. While NRP and NP_0 treatments did not result in significant differences, the pH's were significantly higher than any of the pH's recorded in any of the ammonium treatments. At ES1, a similar trend to TI1 was observed, but some of the pH values were lower than at TI.

Table 4. 7. Soil pH at tuber initiation (TI) and end of the growing season (ES) in two seasons (1 or 2) as affected by different nitrogen forms and phosphorus sources.

	Soil pH (KCl)			
TREATMENT	TI1	TI2	ES1	ES2
ASP	4.91e	4.07c	4.24e	4.04d
NSP	5.64a	4.74a	5.85a	5.83a
ARP	5.16c	4.11c	4.80c	4.10d
NRP	5.56b	4.72a	5.65b	5.58b
AP_0	5.04d	4.17c	4.62d	4.13d
NP_0	5.54b	4.46b	5.67b	5.25c

N = nitrate, A = ammonium, SP = superphosphate, RP = rock phosphate and P_0 = zero phosphorus. TI= tuber initiation, ES = end of season. Figures followed by the same letter within a column have no significant differences ($P < 0.05$). N= 4. LSD for TI1 = 0.07, TI2 = 0.15, ES1 = 0.06 and ES2 = 0.14.

In the second season at both stages, all the treatments responded similarly, with NSP resulting in the highest pH, followed by NRP and NP_0. Ammonium SP treated soil was the most acidic, followed by ARP and AP_0 except at TI2 where there were no significant differences across the three ammonium treatments. At TI2, NSP and NRP were not significantly different from each other, but both resulted in a significantly higher pH than NP_0. All nitrate treatments resulted in

significantly higher pHs than for ammonium. There were no significant differences across all ammonium treatments. At ES1, all the treatments showed significant differences except between ARP and P_0. At ES2, with NSP having the highest pH, followed by NRP and NP_0, all nitrate treatments had significant differences between them. All nitrate treatments resulted in significantly higher pHs than all ammonium treatments. Similar to TI2, there were no significant differences in pH between the ammonium treatments for ES2.

Different N forms have a known effect on soil pH, where ammonium tended to reduce soil pH, while nitrate application results in a decrease in soil acidity (Thomson et al. 1993, Monsant et al. 2008). Gahoonia et al. (1992) also reported a decrease of over one pH unit and an increase of over 0.5 pH units on ryegrass supplied with ammonium and nitrate, respectively. Ruan et al. (2000) reported increased acidification when tea plants were supplied with ammonium as compared to nitrate. This, therefore, indicates that the form of N can effectively regulate pH increase or reduction. Phosphorus sources also appeared to influence pH, especially SP, which together with ammonium resulted in the most acidic soils across all stages. This could be due to the acidic nature of the single superphosphate fertiliser.

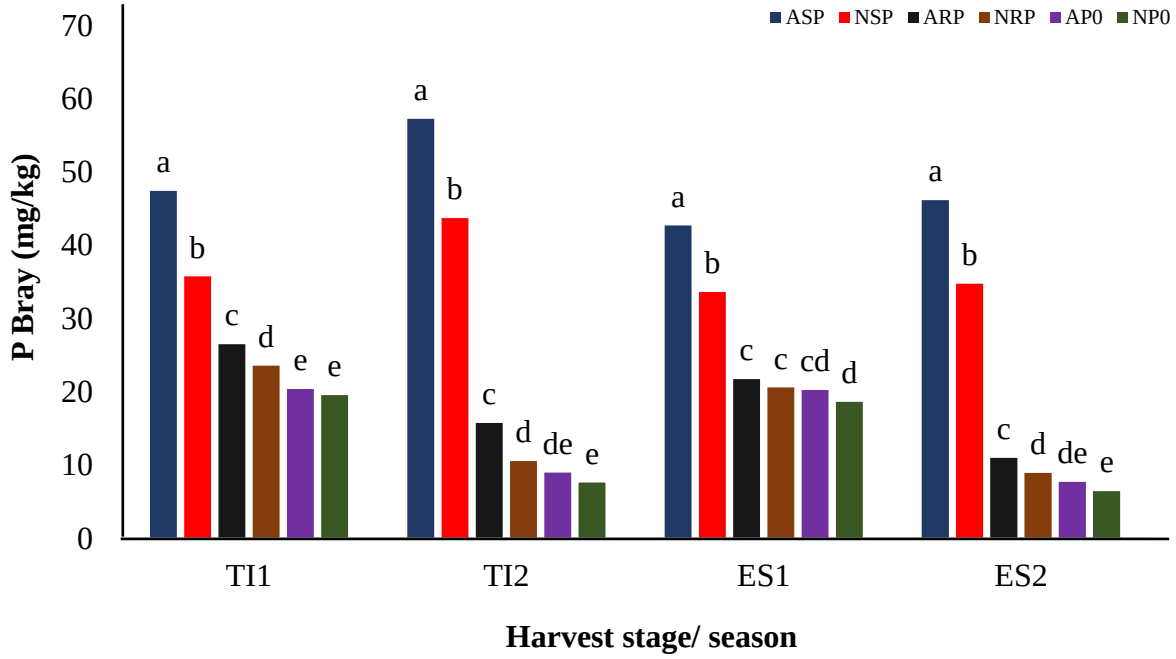
These variations in pH are mainly as a result of the fundamental fact that the N form supplied to plants has a direct effect on the uptake of both cations and anions by plants (Rollwagen and Zasoski 1988). Nitrate results in the net uptake of protons and excess uptake of anions over cations. Assimilation of nitrate also causes the release of OH^-, resulting in an increase in soil pH. Ammonium nutrition, on the other hand, causes H^+ extrusion, which results in increased soil acidity. Plants also modify the rhizosphere through the secretion of weak acids (Pandey et al. 2013). Low rhizosphere pH may lead to increased $H_2PO_4^-$ / HPO_4^{2-} ratios. This will also result in increased solubility of calcium phosphates, and thus increased P availability. This influence on soil pH has a subsequent influence on plant nutrient availability in the soil and the eventual uptake of nutrients.

4.5.2 Effect of nitrogen form and phosphorus source on plant-available phosphorus

Two-way ANOVA analysis showed that a significant interaction between P source and N form on plant-available P at all the four stages observed (Appendix B).

Across the two seasons, a trend was noted where ASP gave the highest plant-available soil P, followed by NSP and ARP, with significant differences between them. Nitrate P_0 gave the lowest available P, followed by AP_0 and NRP (Fig 4.1).

Ammonium SP, NSP, NRP and ARP recorded significant differences between them at both TI1 and TI2, as well as at ES2. At all stages assessed, the P_0 treatments showed no significant differences. A notable increase in P content can be seen at TI2 in the ASP treatment. A general trend of higher available P at TI stage than at ES was noted. At TI1, all treatments showed significant differences, except for both P_0 treatments. Phosphorus content at TI2 was lower than at TI1 for RP and P_0 treatments.



N = nitrate, A = ammonium, SP = superphosphate, RP = rock phosphate and P_0 = zero phosphorus. TI= tuber initiation, ES = end of the season, 1= season one and 2 = season 2. Bars with the same letter within a graph do not differ significantly ($P < 0.05$). N= 4. LSD for TI1 = 1.18, TI2 = 1.64, ES1 = 1.96 and ES2 = 1.52.

Figure 4. 1. Plant-available phosphorus at TI and end ES in two seasons as affected by nitrogen forms and phosphorus sources.

At TI2, similar to TI1, ASP gave significantly higher available P level than all other treatments, followed by NSP. Ammonium RP gave significantly higher P than NRP and both P_0 treatments. Nitrate RP resulted in no significant difference in P, compared to AP_0, but resulted in

significantly higher available P than NP_0. The two P_0 treatments did not differ significantly from each other.

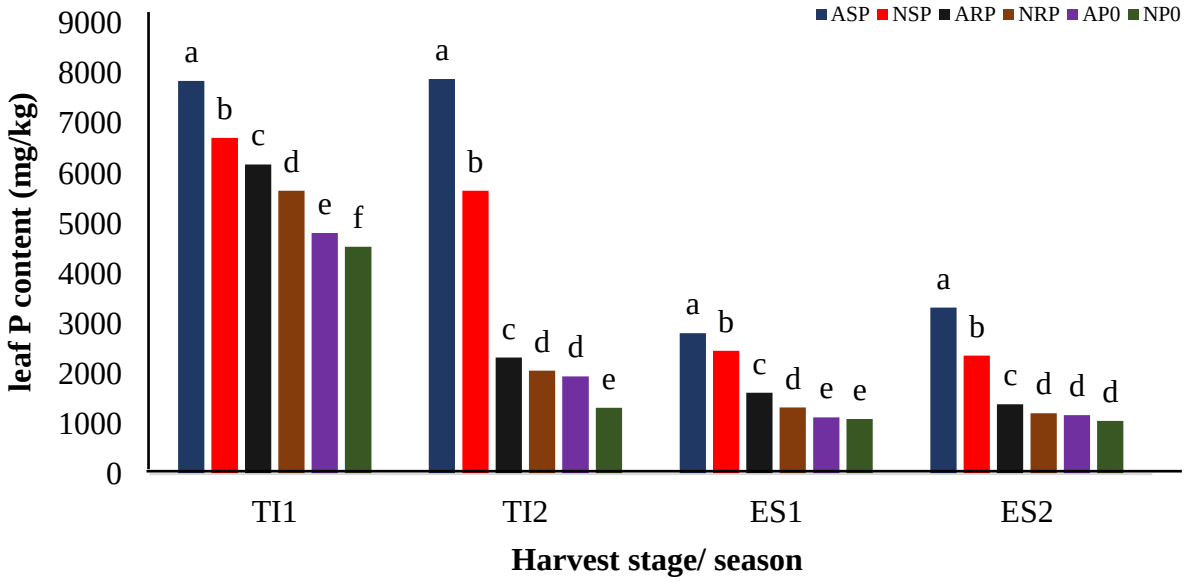
At ES2, a similar trend to TI2 was observed. However, the P contents at ES2 were generally lower compared to TI2. The more distinct differences amongst treatments in S2 than S1 was primarily due to the initial low soil P content for S2. Similarly, Ortas and Rowell (2005) reported limited response to P fertilisation due to a high initial soil P content for a sorghum crop.

Over the two seasons, a trend was noted that SP gave the highest plant-available P, followed by RP and P_0. Ammonium supplied soils tended to have a higher plant-available P, compared to nitrate supplied soils with a similar P source, indicating that ammonium could have favoured P dissolution, which is in agreement with the first hypothesis of this trial. This could be due to the reduction in pH when using ammonium nutrition as observed in this trial, which increased P availability (Gahoonia et al. 1992). Ruan et al. (2000) reported similar findings in *Camellia sinensis* when supplied with ammonium and nitrate as N forms and varying P sources (soluble fertiliser and RP). They reported that ammonium supplied soils had higher P content, especially for the RP treatment. This could, therefore, explain the marginal plant-available P variations between RP and P_0 supplied plants. The increased P availability from RP source was probably driven by acidification during ammonium nutrition, which resulted in increased P dissolution. Hinsinger and Gilkes (1996) in a study on clover and Zoysa et al. (1997) on tea, also alluded that root exudates could affect P dissolution and hence its availability due to pH change.

4.5.3 Leaf phosphorus content as affected by nitrogen form and phosphorus source

Two-way ANOVA analysis showed that a significant interaction between P source and N form for leaf P content at all the four stages observed (Appendix B).

As was the case with plant-available P, ammonium supplied plants tended to have higher leaf P concentration, compared to plants that received nitrate at both TI and ES in both seasons. (Fig 4.2).



N = nitrate, A = ammonium, SP = superphosphate, RP = rock phosphate and P_0 = zero phosphorus. TI= tuber initiation, ES = end of the season. Figures followed by the same letter within a column have no significant differences ($P < 0.05$). N= 4. LSD for TI1 = 97.61, TI2 = 213.37, ES1 = 160.91 and ES2 = 153.04.

Figure 4. 2. Potato leaf phosphorus content at tuber initiation (TI) and end of the growing season (ES) in two seasons (1 or 2) as affected by different nitrogen forms and phosphorus sources.

Ammonium SP gave the highest leaf P content, followed by NSP and ARP, with significant differences over the two stages across the two seasons. Nitrate P_0 gave the lowest leaf P content, followed by AP_0 and NRP across the two seasons, both at TI and ES. Ammonium P_0 had significantly higher leaf P than NP_0 at both TI stages. However, both P_0 treatments did not differ significantly at both ES stages.

In S1 at both TI and ES, all treatments showed significant differences, apart from both P_0 treatments at ES1 which did not significantly differ from each other. The P concentration in the leaves at TI1 was over two-fold higher than that at ES1.

In S2 at TI, there was a sharp contrast between SP supplied plants, compared to RP and P_0. All treatments showed significant differences, with the exception of NRP and AP_0. At ES2, both SP treatments and ARP recorded significant differences, while NRP, AP_0 and NP_0 all showed no significant differences, despite NP_0 having the lowest P content.

A trend was noted, as was the case with plant-available P, that SP gave the highest leaf P content, followed by RP and P_0, which agrees with Soratto et al. (2019), who reported an increase in leaf P content with increased P application. Ammonium treatments tended to record higher leaf P contents than nitrate treatments, with either P source. There is a linear interaction between increased rhizosphere acidification and P uptake (Johnson et al. 1984, Ortas et al. 2004), as was the case in this current study. Maier et al. (2002) reported greater P concentration in potato when supplied with ammonium compared to nitrate, as was noted in this current trial.

Early works by Grunes (1959) also reported that ammonium supplied plants had higher P uptake levels. De Graaf et al. (1998) also reported higher leaf P content in *Cirsium* heathland species. Spiers (1978), in a study on blueberry as well as Schwamberger and Sims (1991) on tobacco, reported higher P uptake with ammonium compared to nitrate fertilization. This suggests that ammonium enhances P uptake by plants. Marschner (2012) defined 3-5 mg g^{-1} (3000-5000 mg kg^{-1}) on a dry mass basis as the optimal leaf P content for sufficient growth and development of a crop's vegetative phase. This, therefore, indicates that at TI1, the plants had sufficient P for growth, while in S2 both RP and P_0 treatments showed P deficiency at the TI stage, as compared to the SP treatments. Adequate P in the plant shoots at TI1 can be attributed to the higher initial P content (24 mg kg^{-1}), which was within the critical value of P (26 mg kg^{-1}) (George at al. 2012). With regards to the former, the observed P limitation was indicative of P deficiency in the leaves (Fig 4.3), where plants from the NP_0 treatment showed the deepest purple colouration, confirming the very high P deficiency observed in the analysis. Limited P supply has also been reported to inhibit ammonium (Alves et al. 1996) and nitrate (Araújo and Machado 2006) uptake and metabolism in plants. This P-N interaction could further explain the reduction in growth for RP and P_0 plants, despite sufficient N being supplied, signifying an effect of P deficiency on N nutrition.

The increased P, both in the soil and in plants, could be attributed to ammonium supply and its subsequent uptake that lower soil pH. This pH change directly effects P availability, its uptake and eventually on plant growth and development (Gahoonia et al. 1992, Ortas et al. 2004).

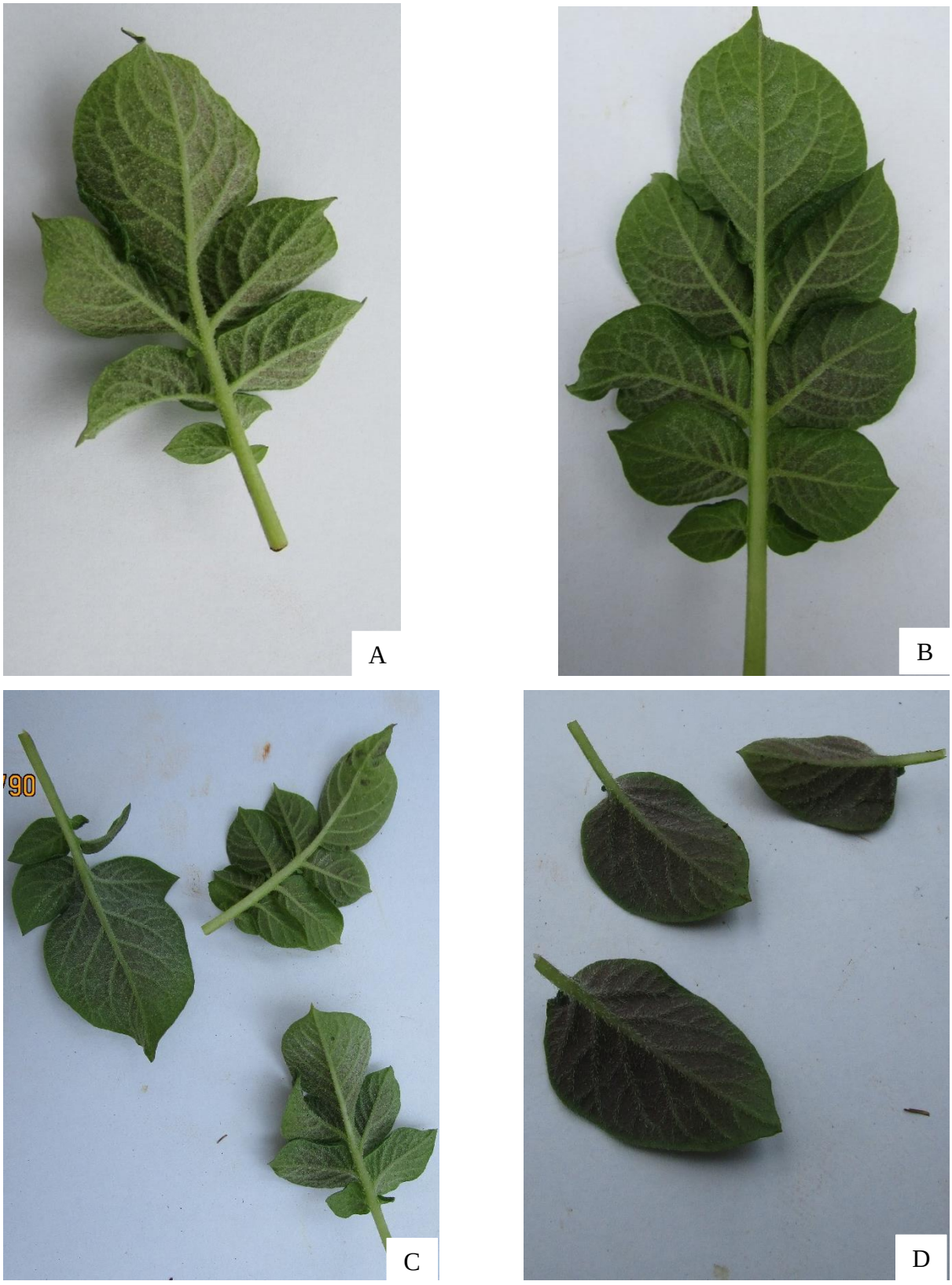
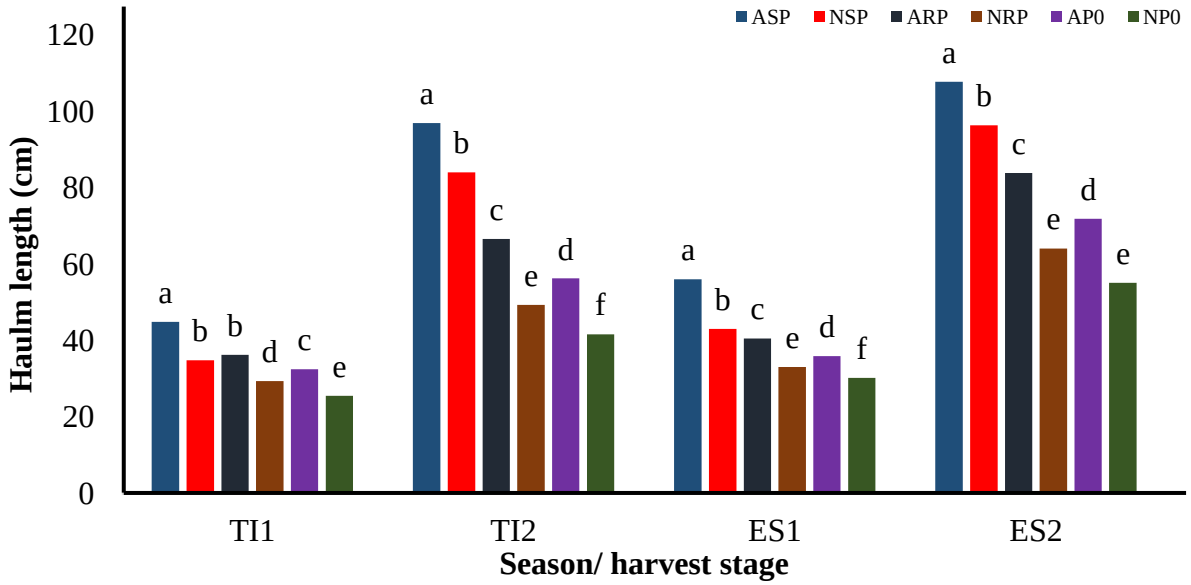


Figure 4. 3. Phosphorus deficiency symptoms on leaves. A = ARP, B = AP_0, C = NRP, D = NP_0 at ES2

4.5.4 Haulm length as affected by different nitrogen forms and phosphorus sources

Two-way ANOVA analysis showed that a positive interaction between P source and N form for haulm length at all the four observation times (Appendix B).

Across the two seasons and at both TI and ES, plants treated with ammonium and superphosphate (ASP) grew significantly longer haulms than all other treatments (Fig 4.4.). Ammonium SP had the tallest haulms, followed by nitrate SP (NSP) and ammonium RP (ARP) over the two seasons, except for TI1 where ARP, despite having no significant difference, tended to have longer haulms than NSP. Nitrate P_0 resulted in the shortest haulms, followed by nitrate RP (NRP) and ammonium P_0 (AP_0). Following the stated order in haulm length, all treatments showed significant differences, except between ARP and NSP at TI1 and NRP and NP_0 at ES2. Ammonium favoured haulm growth, compared to the corresponding nitrate supplied plants, to the extent that plants that received AP_0 developed significantly longer haulms than NRP. Plants treated with SP grew the longest haulms of the P treatments, followed by RP and P_0 respectively when supplied with either N form (Fig 4.5). There was a notable increase in haulm length from TI to ES in both seasons. In the second season, at both TI and ES, the plants tended to grow taller than in the first season. Razaq et al. (2017) reported longer haulms on *Acer mono* supplied with sufficient N and P. Firew et al. (2016) and Girma et al. (2017) also reported increased haulm length in potato plants supplied with optimal P.



N = nitrate, A = ammonium, SP = superphosphate, RP = rock phosphate and P_0 = zero phosphorus. TI= tuber initiation, ES = end of the season. 1 = season 1 and 2 = season 2. Bars with the same letter within a column group have no significant differences. N=4. LSD for TI1 = 1.67, TI2 = 2.28, ES1 = 1.56 and ES2 = 4.06.

Figure 4. 4. Haulm length at tuber initiation (TI) and end of the growing season (ES) in two seasons (1 or 2) as affected by different nitrogen forms and phosphorus sources. A= TI 1, B = ES1, C= TI 2 and D = ES2

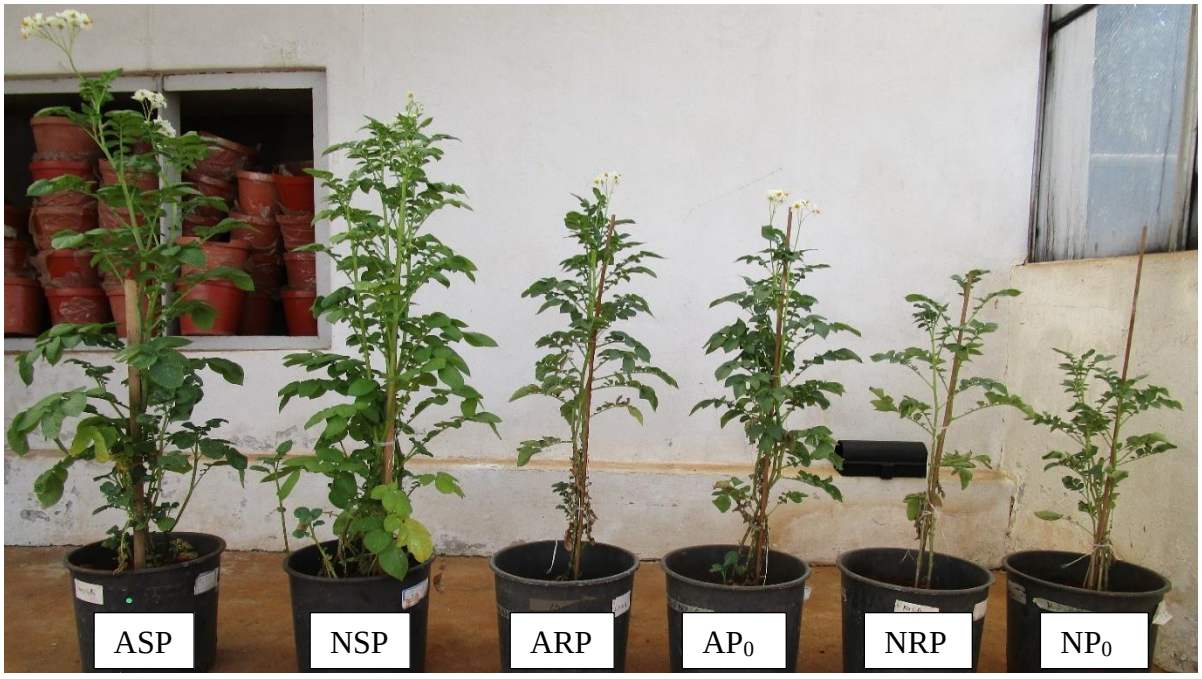


Figure 4. 5. Potato haulm length as affected by nitrogen forms and different P sources at TI in the second season.

The general increase in haulm lengths in ammonium supplied plants compared to nitrate supplied plants indicates that the plants may have favoured ammonium uptake, that resulted in increased haulm growth over the two growing seasons (Hawkesford et al. 2012). This preferential ammonium uptake has been reported previously, especially on acidic soils (Lee 1999), as was the case in this trial. The difference in the haulm length of plants treated with SP compared to RP and P_0 could indicate the induction of P deficiency in the RP and P_0 treatments as indicated in Figure 3.4, which hampered growth. This could be due to the reductions in the length of the cell division zone, coupled with decreased cell division under P starvation (Assuero et al. 2004). Another reason could be decreased epidermal cell expansion under low P supply as a result of low hydraulic conductivity of the roots (Clarkson et al. 2000). The varying climatic conditions during the two seasons could have resulted in the relatively longer haulms in the second season, compared to the first season at both stages, where the warmer weather during the second season could have favoured plant vegetative growth (Appendix C/D).

The increased growth of the plants supplied with both sufficient P and N strongly agrees with the findings of Elser et al. (2007) and Harpole et al. (2011) that there exists a co-limitation of the two elements, meaning optimal effect is noted when the elements are supplied together (Göran et al. 2012). Other literature also supports the theory that P fertilisation enhances plant height, growth and development (Pandey et al. 2006, Waraich et al. 2015). This was clearly indicated by the reduced haulm length the plants supplied with RP and P_0, despite being supplied with an optimal level of N in either form. It is, therefore, clear that RP as a source of P does not match the performance of SP treated crops. This could be due to the high available P content in SP fertiliser, compared to the slow release of calcium-bound P in RP. The P_0 treatments, especially in S2 did show clear effects of P deficiency, gauging from the purpling at the bottom of leaves. This is due to the low P in the soil and the non-application of P at planting. Both N forms did not alleviate P deficiency in P_0 to support optimal haulm growth, meaning that N fertilisation with either RP and P_0 could not alleviate P deficiency barely by influencing soil pH.

4.5.5 Haulm dry mass as influenced by nitrogen forms and phosphorus sources

Two-way ANOVA analysis revealed a significant interaction between P source and N form for haulm dry mass at TI2, ES1 and ES2. No positive interaction was noted at TI1 (P = 0.1574). However, P source (P = <0.0001) and N form (P= <0.0001) had significant effect on haulm dry mass (Appendix B). Therefore, the main effects of N and P were discussed for TI1.

During the first season at TI1, a trend was noted where SP treated plants had the highest haulm dry mass (HDM) followed by RP and P_0 (Fig 4.6A). Moreover, the plants treated with ammonium were significantly higher in haulm dry mass than the nitrate treated plants in TI1 (Fig 4.6B).

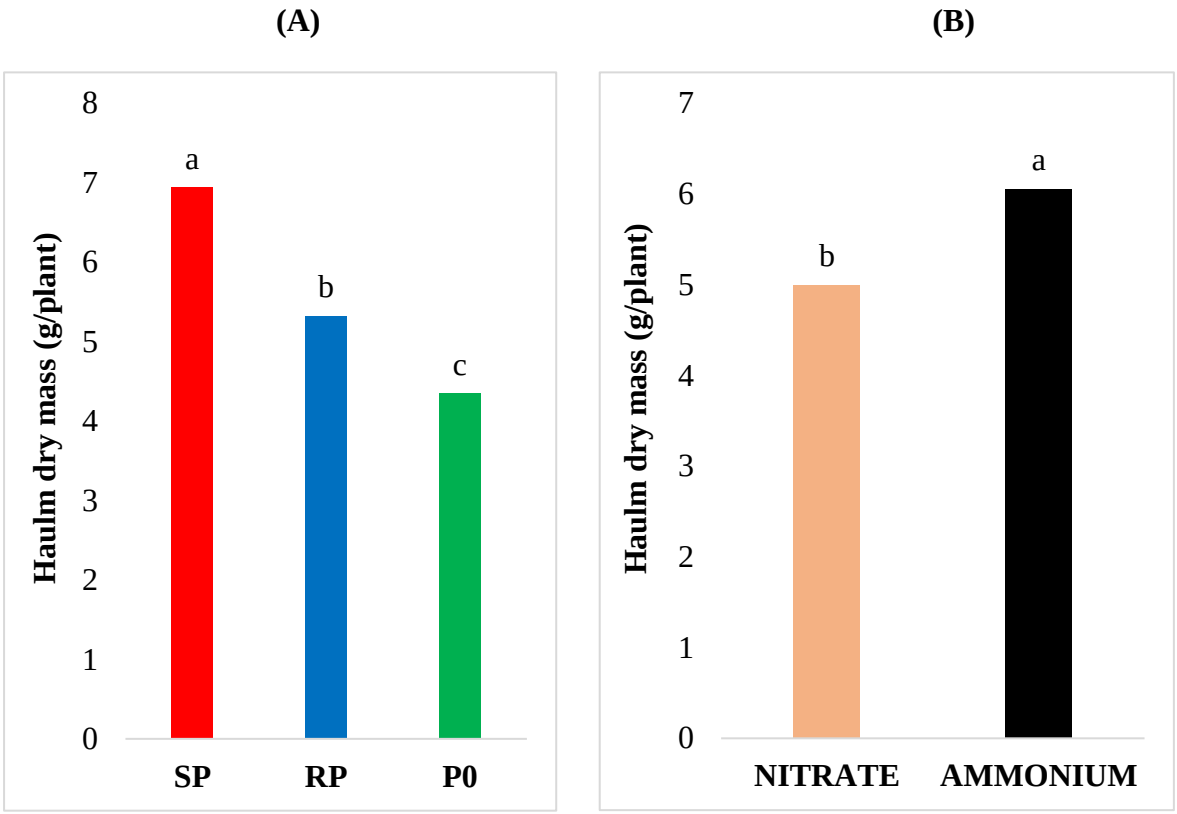


Figure 4. 6. Haulm dry mass at tuber initiation in the first season as affected by different phosphorus sources (A) and nitrogen forms (B).

Ammonium SP produced significantly higher HDM than all other treatments while NP_0 recorded the lowest HDM at ES1 (Table 4.8). Ammonium RP and NSP gave no significant differences, while NRP produced higher HDM than AP_0 at ES1. Ammonium P_0 had

significantly higher HDM than NRP and NP_0 at ES1. All ammonium treatments had significantly higher HDM than the corresponding nitrate treated plants at ES1.

In the second season, both at TI and ES, a similar trend was observed, where NSP produced the highest biomass, followed by ASP and ARP, all with significant differences. Nitrate P_0 produced the lowest HDM followed by NRP and AP_0, all with significant differences. Once again, SP supplied plants produced the highest HDM followed by RP and P_0 when combined with either N form.

Table 4. 8. Haulm dry mass at tuber initiation (TI) and end of the growing season (ES) in two seasons (1 or 2) as affected by different nitrogen forms and phosphorus sources

TREATMENT	TI1	TI2	ES1	ES2
ASP	7.69a	29.45b	12.49a	29.91b
NSP	6.19a	35.93a	8.34b	39.37a
ARP	5.68a	11.00c	8.12b	13.35c
NRP	4.95a	3.87e	5.71d	8.50e
AP_0	4.82a	7.23d	6.71c	10.76d
NP_0	3.88a	2.25f	5.69d	6.69f

N = nitrate, A = ammonium, SP = superphosphate, RP = rock phosphate and P_0 = zero phosphorus. TI= tuber initiation, ES = end of season, 1 = season one and 2= season two. Figures followed by the same letter within a column have no significant differences. N= 4. LSD for TI1 = NS (not significant), TI2 = 1.34, ES1 = 0.55 and ES2 = 0.72.

Nitrate combined with SP dominated over ASP regarding the HDM recorded in the second season. All treatments showed significant variations at both TI and ES. This scenario, where nitrate supplied plants produced greater HDM than ammonium (second season), has been reported in wheat (Haynes and Goh 1978) and oat (Ming-Shou et al. 2009). The reduced HDM in both RP and P_0 corroborates the findings of Balemi (2009), who noted reduced HDM under limited P supply in potato. Jenkins and Mahmood (2003) reported that with all the elements supplied in optimal quantities but without P, 33% reduction in HDM was noted, cementing the importance of P in potato haulm development. The co-limitation of both P and N could explain the reduced HDM for RP and P_0, despite having an optimal N supply. The reduced HDM in plants with limited P supply is a clear indication of the effect of P starvation on haulm development. One of the P deficiency symptoms in potato is stunted growth, accompanied by shorter internodes and this can be observed even during the early vegetative growth stages

(Assuero et al. 2004). This is mainly due to the increased allocation of assimilates for root respiration at the expense of haulm development (Hawkesford et al. 2012). The outcome is a general reduction in haulm growth and development, as compared to the plants with adequate P supply (Vance et al. 2003). The dominant performance of ammonium over nitrate in the first season could partly be attributed to the relatively low temperatures (Appendix C), which could have resulted in preferential ammonium uptake as it is the prevailing inorganic N source taken up under low temperatures (Gigon and Rorison 1972). The increased HDM in nitrate supplied plants could be due to increased vegetative growth due to increased nitrate uptake and the reduced haulm length could be due to P limitation (nitrate treated plants had lower P content, compared to ammonium), which inhibits haulm length development. The second season tended to have warmer temperatures (Appendix D), which could have enhanced nitrate uptake at the expense of ammonium. This could partly explain the increased HDM in the second season for the nitrate treatments, compared to ammonium due to the increased N uptake, though it was not quantified in this current study. Firstly, the favoured ammonium uptake under low temperature and seemingly enhanced nitrate uptake under warmer temperature could be due to the inactivity of the soil chemoautotrophic nitrifying bacteria under cold weather. In addition, another plausible explanation could be the rapid oxidizing of ammonium to nitrate through nitrification in warmer climates, favouring crop growth (Below 2002).

4.5.6 The effect of different nitrogen forms and phosphorus sources on root length

Two-way ANOVA analysis showed a significant interaction between P source and N form for root length at TI1, TI2 and ES2. No positive interaction was noted at ES1 ($P = 0.3596$). However, P source ($P = <0.0001$) and N form ($P= <0.0001$) had a significant effect on root length (Appendix B). The main effects of N and P were significant and were discussed for ES1.

Plants supplied with the three P sources gave significant differences in root length with SP treatments growing the longest root followed by RP and P_0 at ES1 (Fig 4.7A). Similarly, N form gave significant differences in root length with ammonium treatments growing longer roots than nitrate treated plants (Fig 4.7B).

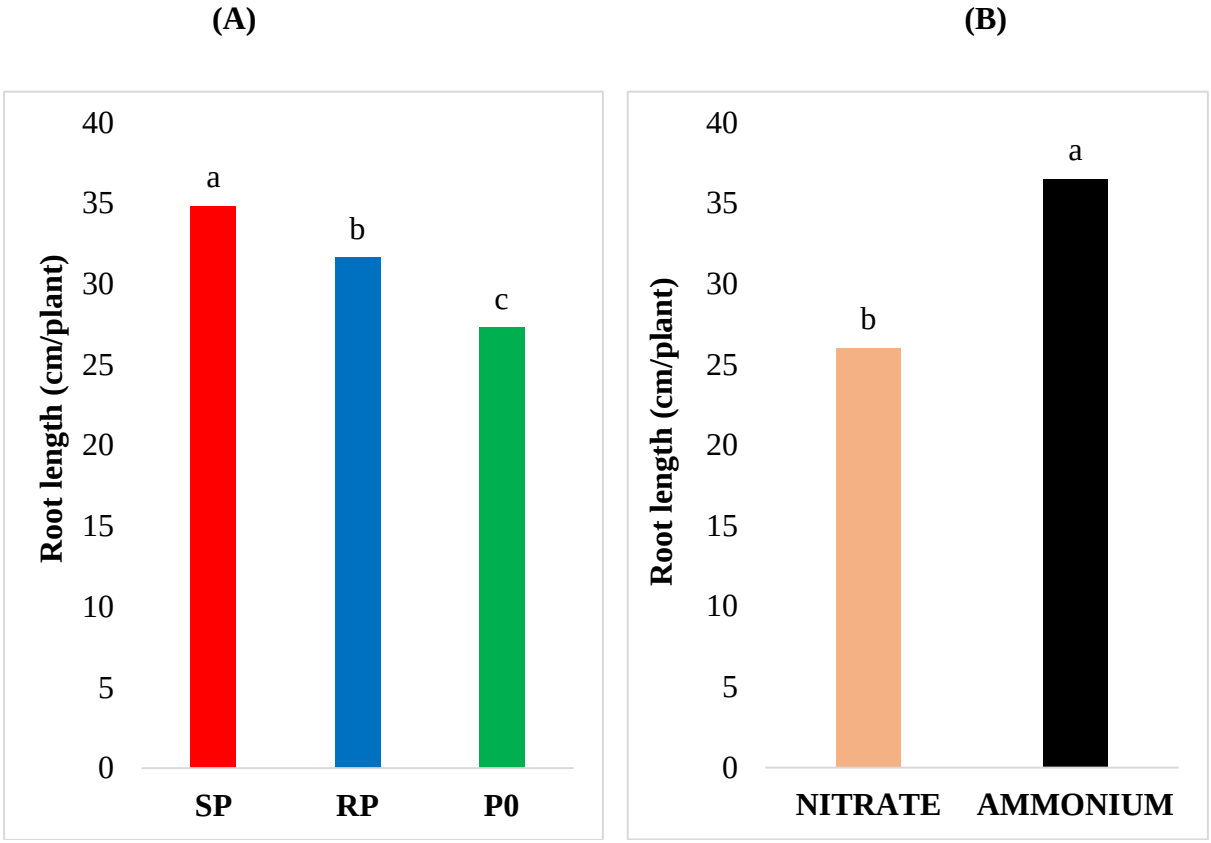
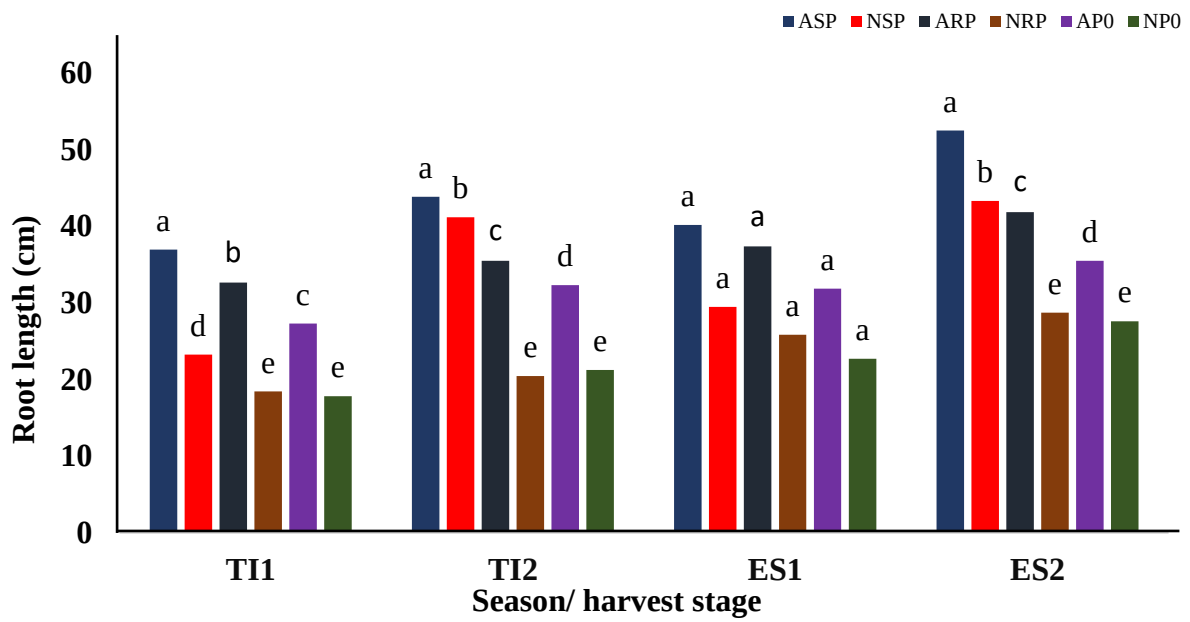


Figure 4. 7. Root length at the end of the first season as affected by different phosphorus sources (A) and nitrogen forms (B).

Ammonium SP resulted in significantly longer roots than all other treatments across the two stages and the two seasons, regardless of the P source (Fig 4.8). Superphosphate treatments gave the longest roots, followed by RP and P_0 across the two seasons at both TII when combined with either of the two N forms. All ammonium treatments had significantly longer roots than the corresponding nitrate treatments within the same P source. For ammonium treated plants, SP had the longest roots, followed by ARP and AP_0. Nitrate SP had significantly longer roots than NRP and NP_0 across the two seasons at TI1. Nitrate RP and P_0 showed no significant differences at all stages in the two seasons.



N = nitrate, A = ammonium, SP = superphosphate, RP = rock phosphate and P_0 = zero phosphorus. TI= tuber initiation, ES = end of the season. Bars with the same letter within a column group have no significant differences. N= 4. LSD for TI1 = 2.07, TI2 = 1.45, ES1 = NS (not significant), and ES2 = 2.30.

Figure 4. 8. Root length at tuber initiation (TI) and end of the growing season (ES) in two seasons (1 or 2) as influenced by different nitrogen forms and phosphorus sources.

In the first season, all ammonium treatments had longer roots than nitrate at TI, while in the second season, NSP gave longer roots than ARP and AP_0. Interestingly, AP_0 had longer roots than NRP and NP_0 in both seasons.

The longer roots for ammonium treatments in the first season, compared to nitrate, despite the high P content agrees with the findings of Schjørring (1986), who found that P deficiency, combined with nitrate as the sole N source, reduced nitrate uptake by 58 %. The reduction did occur even before growth was significantly hampered by limited P supply. When ammonium was supplied as the sole N source, ammonium uptake was higher even under P deficiency. Schjørring (1986) suggested that nitrate was reduced under P deficiency, causing negative feedback on nitrate. This could also explain the minimal difference in haulm length between plants supplied with NSP and those supplied with ARP and AP_0.

The variations in root length were more pronounced in the second season, especially for the RP and P_0 treatments, indicating a probable induction of P deficiency. There was increased root growth in S2 for all treatments at both stages. Lynch and Brown (2008), in a study with

common beans, noted a better PUE in plants that developed a greater quantity of shallow roots, compromising on the primary growth while favouring branching. This was to ideally explore the topsoil where much of the plant available P is found. Nitrogen supply has also been reported to alter root architecture in the soil (Marschner et al. 1986, Zhang et al. 2009), whereby nitrate stimulates the elongation and branching of lateral roots while ammonium tended to favour primary root development (Lima et al. 2010). Nitrogen form has also been reported to affect root surface area, with ammonium resulting in higher root surface area compared to nitrate (Marschner et al. 1986, Lynch et al. 2012). This could explain the reduced root length in NRP and NP_0. High N supply has been reported to inhibit root branching (Marschner 2012). This scenario did not apply in the current study, as the same optimal N was applied across treatments, thus any proliferation was probably induced due to P limitation. This can be supported by the fact that plants with optimal P and N developed the longest roots.

Phosphorus has been largely documented as a salient element, influencing root growth and development in plants. These ranges from playing vital roles in the formation, development and elongation of plant roots, including root hairs' length and density, cortical organisation, plant root branching and formation of adventitious roots (Lynch and Brown 2008). It is this assertive influence of P on root growth that makes it vital in the altering of roots under limited P supply. Similarly, Razaq et al. (2017) also reported that sufficient P supply resulted in increased root length, as was the case in this current trial where SP treatments grew the longest roots. The reduced root length for RP and P_0 is, therefore, a response to P starvation as was confirmed by the leaf P analysis, as limited plant P supply resulted in a considerable reduction in the growth of primary roots (López-Bucio et al. 2003).

4.5.7 Root dry mass as influenced by nitrogen forms and phosphorus source

Two-way ANOVA analysis showed that a positive interaction between P source and N form for root dry mass at TI1 ($P = <0.0001$), TI2 ($P = <0.0001$), ES1 ($P = 0.0017$) and ES2 ($P = <0.0001$) (Appendix B). Therefore, P source and N form interaction significantly influenced the root dry mass in at TI and ES in both seasons.

Nitrate supplied plants accumulated higher root dry mass (RDM) than ammonium supplied plants in both seasons (Table 4.9).

Table 4. 9. Root dry mass at tuber initiation (TI) and end of the growing season (ES) in two seasons (1 or 2) as affected by different nitrogen forms and phosphorus sources

	Root mass (g/plant)			
TREATMENT	TI1	TI2	ES1	ES2
ASP	0.95a	7.71b	1.15b	10.77b
NSP	1.02a	10.81a	1.31a	13.70a
ARP	0.36d	2.18e	0.88d	4.86e
NRP	0.84b	4.66c	1.07bc	7.72c
AP_0	0.32d	2.10e	0.50e	4.76e
NP_0	0.46c	3.53d	0.99cd	6.56d

N = nitrate, A = ammonium, SP = superphosphate, RP = rock phosphate and P_0 = zero phosphorus. TI= tuber initiation, ES = end of season. Figures followed by the same letter within a column have no significant differences ($P < 0.05$). N= 4. LSD for TI1 = 0.08, TI2 = 0.51, ES1 = 0.15 and ES2 = 0.80.

Nitrate SP had the highest RDM at TI1 and ES1 and was significantly higher than all other treatments, except ASP at TI1. Both NRP and NP_0 had significantly higher RDM than ARP and AP_0 in TI1, but NP_0 and ARP did not differ significantly. Nitrate RP had significantly higher RDM than NP_0 at TI1, but not statistically different at ES1. Ammonium P_0 had a significantly lower RDM than all other treatments at both stages in S1 but did not differ significantly from ARP at TI1. There was an increase in RDM from TI towards ES.

In S2, a trend was noted where SP produced the highest RDM, followed by RP and P_0. Nitrate SP had significantly higher RDM than all treatments, followed by ASP, with both treatments having significant differences at TI2 and ES2. Across TI2 and ES2, a trend where both NRP and NP_0 had significantly higher RDM than ARP and AP_0 was noted. Ammonium RP and AP_0 did not differ significantly. At both TI2 and ES2, NRP had significantly higher RDM than NP_0.

Nitrogen deficiency, as well as P limitation, are known to affect plant assimilate distribution within the plant. For both elements, a linear decrease in assimilate allocation to the roots with increasing N and P supply was shown, while their deficiency has resulted in an increased allocation of assimilates to the roots (De Groot et al. 2003). In the event of limited P and N supply, plants tend to alter their carbon allocation in favour of the roots to enhance their foraging potential (Hermans et al. 2006). This scenario results in increased root dry mass as the plant seeks to acquire more of the limiting elements. The reduction in the root length in

favour of root branching in nitrate RP and P_0 treatments (Fig 4.9) is also a response to P deficiency, judging from tissue P levels. The higher RDM at NRP and NP_0 treatments, compared to the corresponding ammonium treatments with similar P sources, indicates that nitrate could have increased root branching at the expense of root length in order to increase the surface area for P exploration and absorption. This could be due to increased carbohydrate allocation to the roots (Hawkesford et al. 2012) under nitrate supply and P deficient conditions. This continued root growth even under low P can be attributed to reduced P transport to the haulm tissues and the translocation of P to the roots from the haulms (Smith et al. 1990). This has been suggested to enhance K uptake and water use efficiency under nitrate supply, which resulted in enhanced growth by Lu et al. (2005).

Thus, there is a clear indication that under P deficiency, nitrate treated plants had better root proliferation, compared to ammonium. This, however, did not reflect in the P concentration in the leaves, suggesting that despite seemingly poor root growth, ammonium fertilisation enhanced P availability, as was confirmed by the plant-available P determination, where ammonium treatments had higher P. The low RDM could also support the fact that the P deficiency was higher for nitrate treatments, which increased root proliferation in search for the scarce P.

Figure 4. 9. Plant root growth as influenced by different forms of N and different P sources at the second season. A = nitrate SP, B = nitrate RP, C = nitrate P_0, D = ammonium SP, E = ammonium RP and F = ammonium P_0

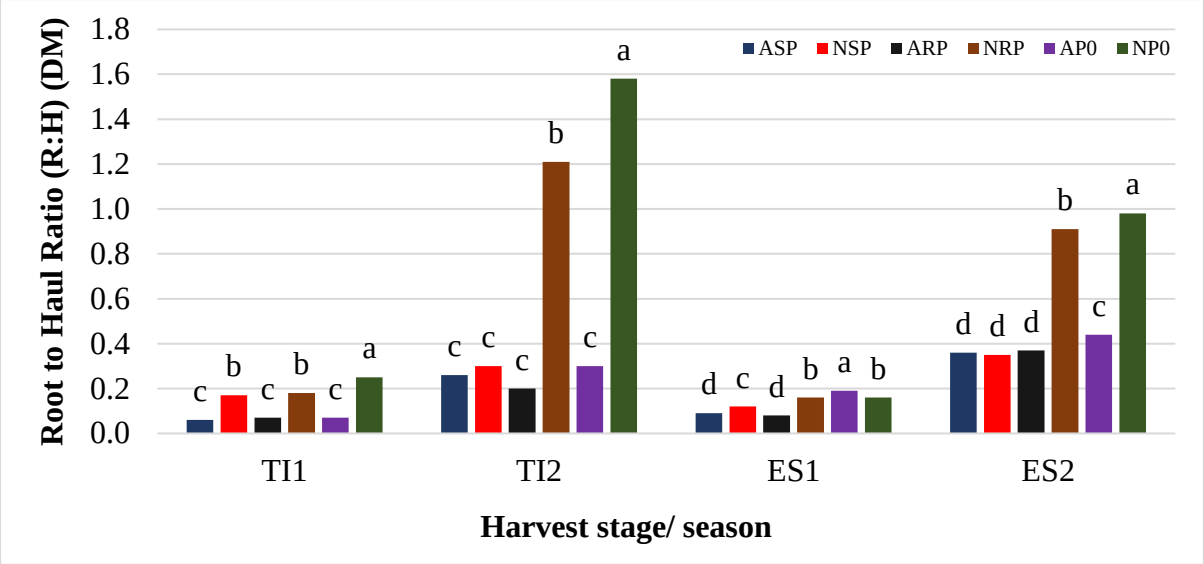
4.5.8 Root to haulm ratio as affected by nitrogen forms and phosphorus source

Two-way ANOVA analysis showed a positive interaction between P source and N form for root: haulm dry mass at TI1 (P = 0.0002), TI2 (P = <0.0001), ES1 (P = <0.0001) and ES2 (P = <0.0001) (Appendix B). P source and N form, therefore, had a significant interaction effect on potato root length at all four observation times.

In S1, all treatments produced a lower root to haulm (R:H) dry mass ratio than in S2 (Fig 4.10). At TI1, all ammonium treated plants produced a significantly lower R:H ratio than all nitrate treated plants. There were no significant differences across ammonium treatments. Nitrate P_0 had the highest R:H ratio and was significantly higher than NRP and NSP. Nitrate RP and NSP showed no significant differences.

At ES1, a similar trend to TI1 was noted, this time with a lower R:H ratio. Ammonium P_0 produced significantly higher R:H ratio than all treatments. Nitrate P_0 and NRP did not differ significantly, but produced a significantly higher ratio than NSP. Ammonium SP and ARP produced significantly lower R:H ratio than all treatments, but did not differ significantly from each other.

In the second season, NP_0 and NRP gave significantly higher R:H ratio than all treatments both at TI and ES. Nitrate P_0 had significantly higher R:H ratio than NRP at both TI and ES. At TI2, all ammonium treatments did not differ significantly. At ES2, Ammonium P_0 had significantly higher R:H ratio than ARP and both SP treatments, but was significantly lower than AP_0 and NRP.



N = nitrate, A = ammonium, SP = superphosphate, RP = rock phosphate and P_0 = zero phosphorus. TI= tuber initiation, ES = end of the season. Bars with the same letter within a column group have no significant differences. N= 4. LSD for TI1 = 0.03, TI2 = 0.10, ES1 = 0.02 and ES2 = 0.04.

Figure 4. 10. Root to haulm ratio at tuber initiation (TI) and end of the growing season (ES) in two seasons (1 or 2) as influenced by different nitrogen forms and phosphorus sources.

Plants treated with SP tended to have a lower R:H ratio, compared to RP and P_0 over the two seasons at TI and ES. For plants under low available P (RP and P_0) supply, ammonium supply tended to result in a lower R:H ratio, compared to nitrate supplied treatments. Hermans et al. (2006) also reported a high R:H ratio under P and N deficiency. The higher R:H ratio in nitrate treated plants could be due to increased allocation of assimilates to root development under P deficiency (section 3.5.7), which favoured root growth and development. The reduced HDM and RDM resulted in the lower R:H ratio for the ammonium treatments. The high R:H ratio is a positive response to P starvation, as was observed in the soil and tissue P assessments. The poor root growth resulted in lower root dry mass and subsequently, lower ratio. This was not evident in the first season, as the plants tended to have sufficient P supply. The high haulm to root ratio noted for RP and P_0 treatments are a clear indicator of P starvation (more profound at TI2 due to the extremely low available P in the soils, coupled with increased P demand at TI growth stage), which stimulated root proliferation, as was noted by Gaur et. al. (2017). Kim and Li (2016) also noted that under low P supply, lantana plants allocated a higher amount of biomass to roots compared to the shoots, thus a greater root-to-haulm ratio. This, therefore, suggests that plants supplied with RP and P_0 experienced P starvation at varying degrees, with P_0 being more affected. The findings of this study also strongly agree with those of Hu et al.

(2010) as well as Fernandes and Soratto (2012). Plants have been reported to alter their root topology, morphology, distribution and architecture, in response to limited P supply (Shen et al. 2011). These changes result in an increase in the root to haulm ratio, which enhances PUE (Machado and Furlani 2004, Schenk 2006). The changes in the roots are as a result of the plant adjusting carbohydrate partitioning between roots and haulms (Shen et al. 2011). This is achieved via adjustments in sugar signalling pathways (Karthikeyan et al. 2007, Vance 2010) and plant hormones (Neumann and Römheld 2002, Nacry et al. 2005).

4.5.9 Tuber initiation as affected by nitrogen form and phosphorus source

Two-way ANOVA analysis showed a positive interaction between P source and N form for the number of tubers initiated at season two only ($P = <0.0001$) (Appendix B). There was no significant interaction between N form and P source at TI1 ($P = 0.5236$). For the main effects, P source had a significant effect in terms of number of tubers initiated at TI1($P = <0.0001$) while N form did not have a significant effect (($P = 0.0555$).

In S1, potato plants receiving no P, initiated the fewest tubers, followed by plants receiving RP and with SP treated plants giving the highest number of tubers initiated (Fig 4.11A). Potato plants receiving ammonium or nitrate had no significant differences in the number of tubers initiated (Fig 4.11B).

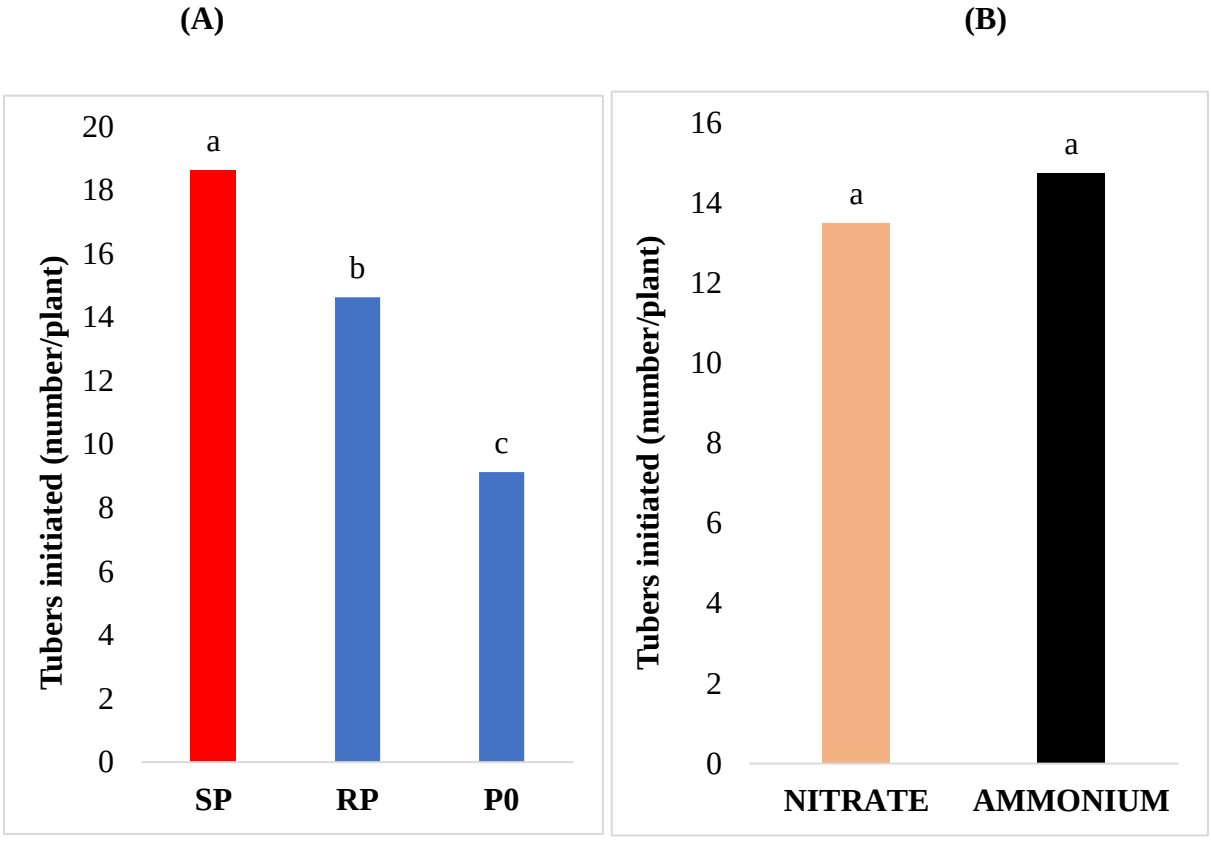


Figure 4. 11. Number of tubers initiated in the first season as affected by different phosphorus sources (A) and nitrogen forms (B).

In the second season, ASP initiated significantly higher tuber number than all other treatments, followed by NSP (Table 4.10 and Figs 4.12, 4.13). Both SP treatments gave significantly higher tuber initiation (TI) than RP and P_0 with either N form. There were no significant differences in tuber number for both RP treatments, regardless of N source, but ARP initiated more tubers. Ammonium RP initiated significantly higher tuber number than both P_0 treatments. Ammonium P_0 gave significantly higher tuber number than NP_0. The number of tubers initiated at TI2 was higher than TI1, but showed a similar trend over the two seasons.

Table 4. 10. Number of tubers initiated at tuber initiation stage (TI) in two seasons (1 or 2) as affected by varying nitrogen forms and phosphorus sources

	Tuber initiation (number per plant)	
TREATMENT	TI1	TI2
ASP	19.75a	43.50a
NSP	17.50a	36.75b
ARP	15.00a	9.75c
NRP	14.25a	8.00cd
AP_0	9.50a	6.25d
NP_0	8.75a	1.75e

N = nitrate, A = ammonium, SP = superphosphate, RP = rock phosphate and P_0 = zero phosphorus. TI1= tuber initiation season one and TI2= tuber initiation season two. Figures followed by the same letter within a column have no significant differences ($P < 0.05$). N =4, LSD: TI1 = NS (not significant) and TI2 = 2.62.

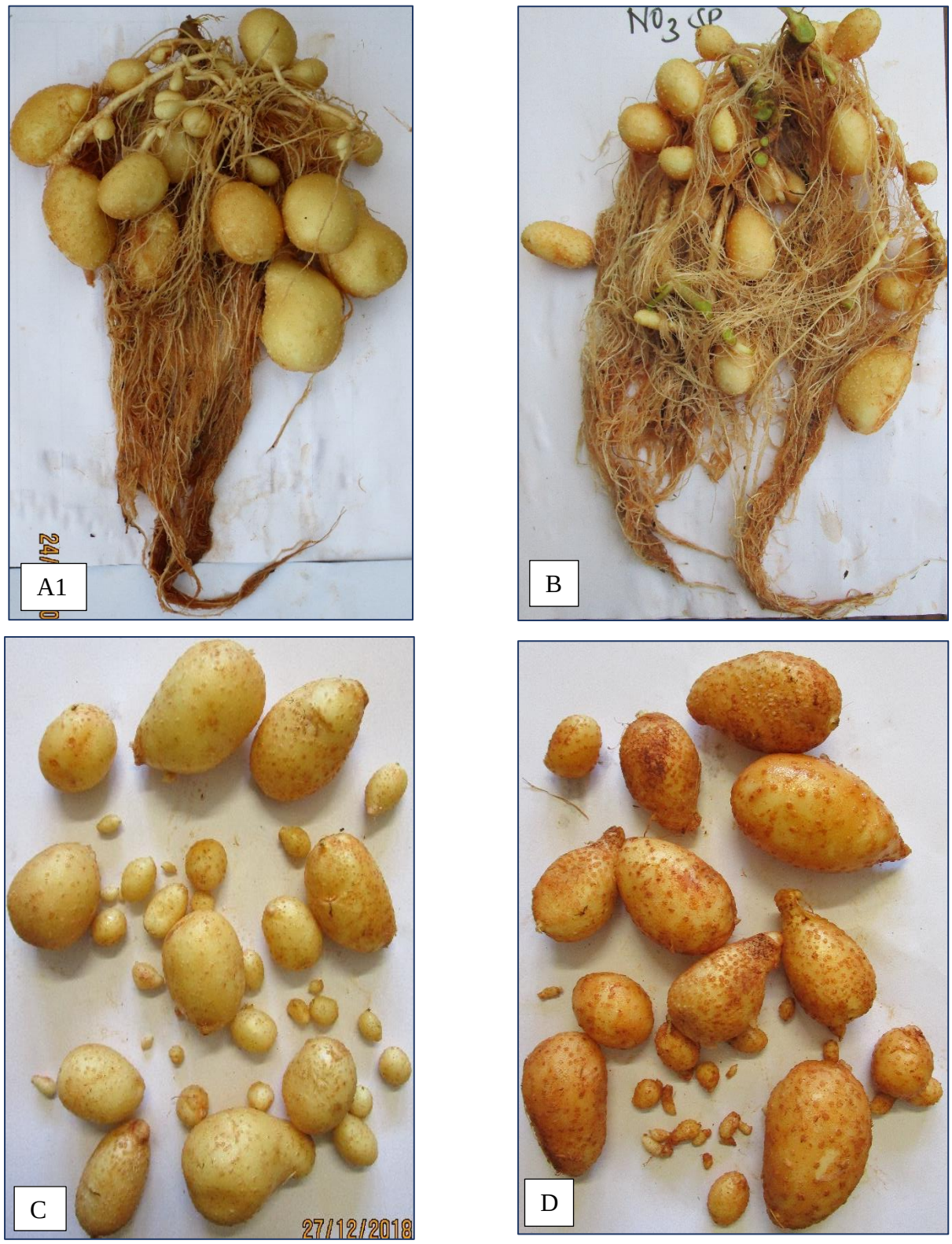


Figure 4. 12. Tuber initiation as influenced by different forms of N and different P sources. A & B= ASP, C & D = NSP.

Figure 4. 13. Tuber initiation as influenced by different forms of N and different P sources. A = ARP, B = NRP, C = AP_0, D = NP_0.

The findings of the second season (with appreciably lower soil P levels) in this current study indicated that tuber initiation in potato is affected by P addition, considering that P in soils ranged from moderate to high in the preceding season (i.e., season one). Several authors also reported a higher tuber number in P-fertilised plants, compared to unfertilised controls and) (Jenkins and Ali 2000; Sanderson et al. 2003; Rosen and Bierman 2008). This was evident in the current study regarding the difference in response between the two seasons, where season two showed obvious differences in response to P addition due to the extremely low soil P level at the start of the trial. It has previously been reported that P deficiency negatively influences TI (Tukaki and Mahler 1990; Grewal and Trehan 1993). Phosphorus fertilisation enhances the ability of plants to intercept solar radiation in P deficient medium (Jenkins and Ali 2000) increasing the plants photosynthetic capacity through enhanced haulm development.

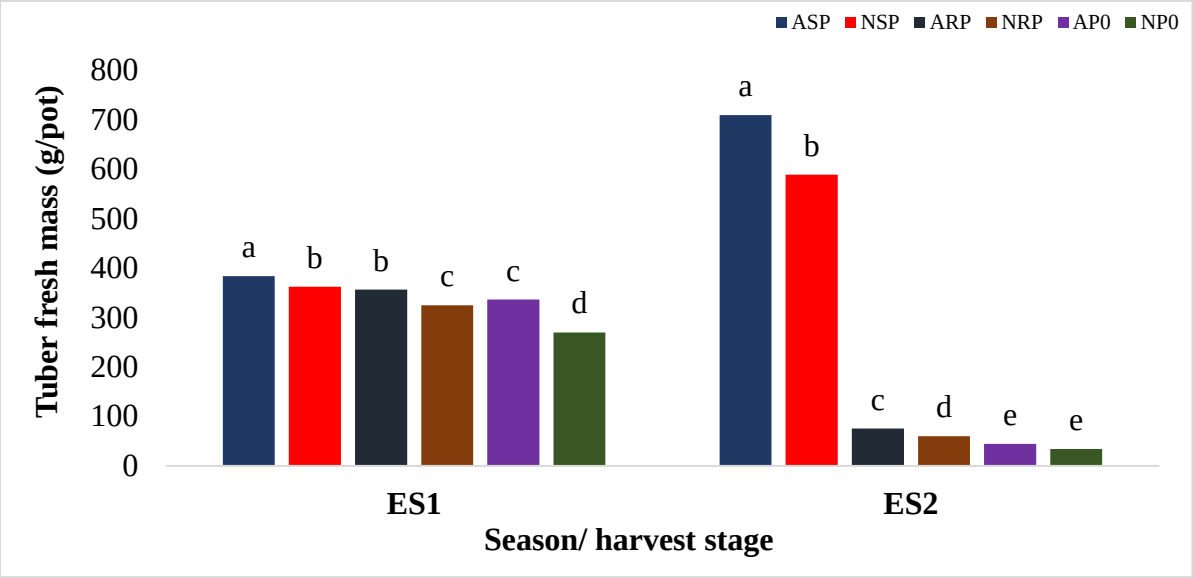
Temperature has also been reported to affect TI (Struik et al. 1989), with low temperatures inhibiting TI. This could serve to explain the variations between S1 and S2 under the varying climatic conditions. Conclusive findings on the effects of other plant nutrients on TI in potato are lacking. In spite of the effects of N on growth, there has been little evidence about its effect on both the timing and number of TI, even under different conditions (O'Brien et al. 1998). It is possible that the reduction in assimilate supply due to accentuated competition between potato stems restricted the aptitude of the respective stems to initiate tubers (Jenkins and Ali 2000).

4.5.10 Tuber fresh and dry mass yields as affected by nitrogen forms and phosphorus sources

Two-way ANOVA analysis showed a positive interaction between P source and N form for tuber fresh mass in season one ($P = 0.0009$) and season two ($P = <0.0001$) (Appendix B). Therefore, P source and N form interaction significantly influenced tubers fresh mass in both seasons.

Ammonium treatments generally produced a higher tuber fresh mass (TFM) compared to nitrate treated plants (Fig 4.14). Ammonium SP produced the highest TFM and was

significantly higher than all other treatments in both seasons. Nitrate SP gave the highest TFM of the nitrate treatments in both seasons.



N = nitrate, A = ammonium, SP = superphosphate, RP = rock phosphate and P_0 = zero phosphorus. TI= tuber initiation, ES = end of the season. Bars with the same letter within a column group have no significant differences. N =4, LSD: ES1 = 15.19 and ES2 = 12.56. CV: ES1 = 3.02 and ES2 = 3.35.

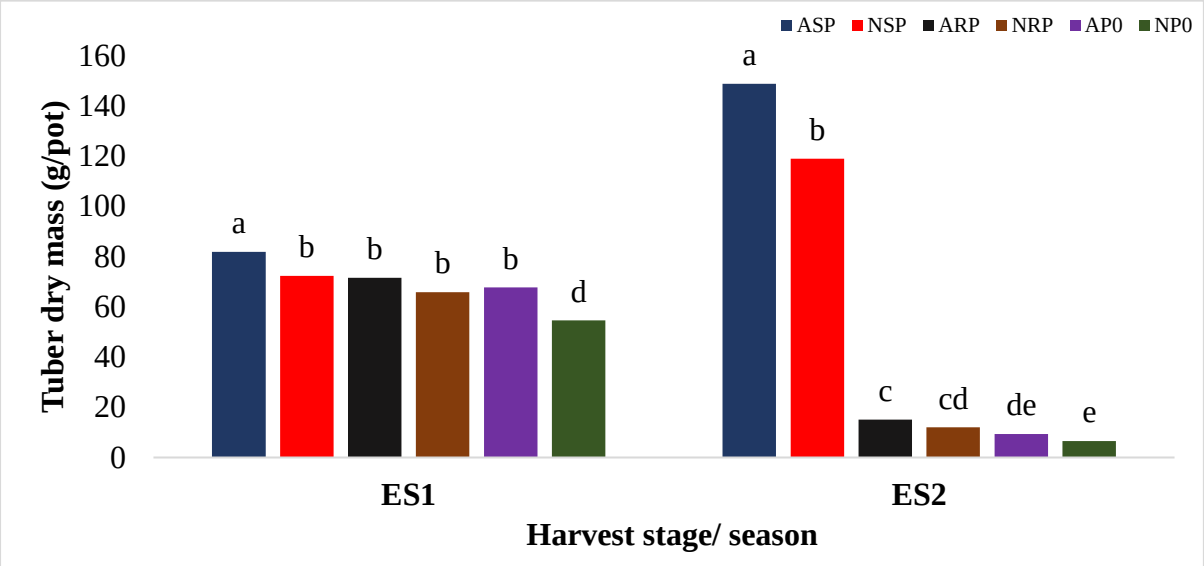
Figure 4. 14. Tuber fresh mass yield at end of the growing season (ES) in two seasons (1 or 2) as affected by different nitrogen forms and phosphorus sources.

In S1, NP_0 resulted in the lowest TFM, followed by NRP and AP_0, which did not differ significantly from each other. Similarly, ARP and NSP did not differ significantly from each other. Ammonium + SP supplied plants produced significantly higher TFM than all other treatments.

There were also clear variations in yield in the second season, with the two SP treatments having a significantly higher yield than RP and P_0 for either N form. In addition, RP treatments produced significantly higher TFM than the two P_0 treatments. Ammonium RP had significantly higher TFM than NRP. Ammonium P_0 and NP_0 treatments produced significantly lower TFM than all other treatments, but were not significantly different from each other. The yield in the second season was higher than in season one for the SP treatments, but the RP and P_0 treatments had much lower yields at ES2, probably due to the very low initial soil P content and the low P supply from RP and P_0.

Two-way ANOVA analysis showed a positive interaction between P source and N form for tuber dry mass in season one (P = 0.0368) and season two (P = < 0.0001) (Appendix B). Therefore, P source and N form interaction significantly influenced the tubers dry mass in both seasons.

The dry tuber masses followed a similar trend to the TFM, where the SP treatments produced higher TDM than RP and P_0 (Fig 4.15). Ammonium SP had significantly higher TDM than all other treatments, followed by NSP. There was a clear dominance of the SP treatments when supplied with either of the N forms. Nitrate SP showed no significant difference from ARP at ES1, but there was a significant difference at ES2, where NSP gave nearly 8 times the TDM of ARP. Nitrate RP and AP_0 gave no significant differences in either of the seasons. Nitrate P_0 accumulated a significantly lower TDM than all treatments at ES1 and ES2, but did not differ significantly from AP_0 in ES2.



N = nitrate, A = ammonium, SP = superphosphate, RP = rock phosphate and P_0 = zero phosphorus. ES1 = season ES2= season two. Bars with the same letter within a column group have no significant differences. N =4, LSD: ES1 = 3.87 and ES2 = 3.14. CV: ES1 = 3.78 and ES2 = 4.07.

Figure 4. 15. Tuber dry mass yield at the end of the growing season (ES) in two seasons (1 or 2) as affected by different nitrogen forms and phosphorus sources.

The findings in this trial highlight the magnitude of the importance of sufficient P supply for potato if any substantial yields are to be realised. Gaur et al. (2017) similarly reported increased

dry mass production in plants supplied with optimal P, compared to the treatments under limited P. This suggest that RP and P_0 probably supplied insufficient P for optimal tuber production. Rosen and Bierman (2008) similarly reported a linear relationship between the P level and the total plant yield. Zewide et al (2012) also noted that higher P supply produced the highest yield, compared to a limited P supply for a trial in Southern Ethiopia. This agrees with the findings in the present trial in both seasons, where RP and P_0 recorded a significantly lower number of tubers initiated, hence reduced yield. The greater yield in plants supplied with SP could be attributed to the general increase in plant biomass, which led to an increase in above-ground organs, thus increased photosynthesis capacity (Jenkins and Mahmood 2003, Fleisher et al. 2013, Soratto and Fernandes 2016). Potato tuber yield is positively influenced by P fertilisation, mostly on soils with limited P supply. However, positive yield response has also been reported in soils with relatively high P content upon P addition (Sanderson et al. 2003, Rosen and Bierman 2008).

The higher yield for ammonium treated plants could be due to the increased P dissolution and uptake, as was confirmed by the plant available P analysis as well as leaf P content. Bundy et al. (1986) in a 5-year trial reported higher yield in potato supplied with ammonium as the sole N source, as compared to nitrate. Other researchers also reported higher potato tuber yields under ammonium supply when compared to nitrate (Gou et al. 2011; Jiao et al. (2012). The findings in these past studies and in this current trial suggest that ammonium supply might enhance potato tuber yields.

These effects of N form on tuber yield have been attributed to possible involvement of ammonium in the regulation of the completion of stolon elongation and kickstarting stolon tip swelling, resulting in tuber formation (Qiqige et al 2017). Ammonium as the sole N source has also been suggested to result in increased chlorophyll content, which eventually causes higher dry matter accumulation and better tuber bulking, ultimately resulting in higher yields (Qiqige et al 2017).

4.6 Concluding remarks/Synopsis

The findings of this study indicate that ammonium combined with SP produced the highest yield, which was more pronounced than nitrate combined with either P source. Rock phosphate as a P source proved to be inefficient in supplying adequate P for optimal potato growth, but had a positive effect on potato yield, compared to the zero P treatment. Even under optimal N supply, P deficiency significantly impacts crop growth, suggesting a co-limitation between these elements, as was reported by Jeschke et al. (1996) and De Groot et al. (2001). This has been attributed to poor N redistribution in the plant, especially to the organs low in N concentration, presumably the roots (Rufty et al. 1993, Gniazdowska and Rychter 1999) or limited energy availability to sustain N uptake (Rufty et al. 1993) under these P deficient conditions.

As expected, nitrate resulted in reduced P dissolution and availability in the soil and uptake by potato plants due to increased soil alkalinity, compared to ammonium. This was due to reduced soil acidity as a result of the uptake of H^+ and the extrusion of OH^-.

Superphosphate as P source resulted in increased P dissolution and availability in the soil and uptake by plants when supplied with either N form. Superphosphate, as expected, had higher P dissolution, availability in the soil and uptake by plants, compared to both RP and P_0. The P source thus has a direct influence on the P concentration in the plant and in the soil. Phosphorus limitation is a big obstacle that needs to be overcome if target yields are to be attained. Rock phosphate, unfortunately, was proven not to be a viable alternative in supplying P for optimum potato production.

The soil and plant analysis indicated that ammonium supply resulted in reduced soil pH hence enhanced P availability and uptake by potato combined with all the three P sources. Therefore, we accept the hypothesis that would result in enhanced P dissolution and availability in the soil and uptake by potato due to increased soil acidification, regardless of the P source and the soil type. The lower P concentration in soil and plant tissue and higher pH in nitrate treatments leads us to accept the second hypothesis that nitrate would result in reduced P dissolution and availability in the soil and uptake by potato due to increased soil alkalinity. The high P content in leaf tissue and the high plant-available P in the soil in SP treatments leads us to accept the

hypothesis that SP as P source would result in increased P dissolution and availability in the soil and uptake by potato under either N form. The higher P content in plants grown on soil supplied with ARP leads us to accept the hypothesis that rock phosphate would enhance P dissolution, availability in the soil and uptake by potato plants when applied together with ammonium, compared to nitrate. The higher P content in soil and plant tissues of ARP treatment leads us to accept the hypothesis that the treatment without P application would result in increased P dissolution, availability in the soil and uptake by potato plants when supplied with ammonium, compared to nitrate.

It is very critical to analyse and know both the P and N requirements for optimal potato growth as well as maximum yield. Further research is also needed to evaluate the effect of varying levels of the different N forms and P sources on the productivity of the potato crop to quantify an optimal application rate. This is because there are some benefits in RP application, compared to the non-application of any P. Secondly, considering the changes currently being experienced in the environment as well as the improved potato genotypes from improved breeding techniques Further research on the effect of P-N interaction on their effectiveness in the uptake and utilisation of plant nutrients. This is due to the apparent indication that prevailing weather conditions (Appendix C/D) may have affected nutrient availability and uptake. More so, depending on the N form supplied to the plants, which eventually affects the concentration of other elements and eventually plant growth as a whole.

Upon the availability of funding, further in-depth analysis can be conducted for the elements that were not monitored in this current trial to provide an overview of the effect of the P-N interactions on the concentration of the other plant nutrients in the soil and in plants, and what contributions they may have to potato yield.

CHAPTER 5

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Potato (*Solanum tuberosum* L.) is regarded as a very important food crop in Africa due to its high dietary energy, potassium, protein, vitamin C and dietary fibre. Potato has a shorter production cycle and produces more food calories per hectare, using less water compared to most cereals. This makes potato an important hunger-breaker crop. In terms of production, South Africa ranks thirds in Africa, behind Algeria and Egypt. However, potato production is very expensive with average production costs of about R150 000 ha^{-1} where 20% of the costs are fertilisers.

In addition to the high production costs, most potato cultivars require a consistent supply of plant nutrients with any deficiencies resulting in poor yields. Of these fertilisers, phosphorus is one of the key elements required for potato growth and development right from the young seedling stage to tuber initiation and tuber bulking. The potato crop has a very high demand for this critical element as compared to crops such as cereals. Most potato cultivars are known to have a shallow root system which hampers P uptake.

To address this challenge, farmers respond by applying phosphatic fertilisers. After application, a marginal 20% is available for the utilisation by plants within a few days with the amount available dropping to 4% within just 10 days. This is due to P fixation in the soil by iron and aluminium in acidic soils and calcium as well as magnesium in alkaline soils.

The sustainability of this heavy P fertilisation in potato fields is greatly threatened by the finite nature of the RP which is used as raw material for P fertiliser production. Therefore, these resources must be wisely used by ensuring application only when necessary and in the required amounts. This is in order to prolong the service period of the existing reserves as well as countering any possible environmental degradation accompanying their misuse.

In addition to the threat of the depleting RP, the processing of this material into P fertilisers is expensive, has very high energy demands and contributes to greenhouse gas emissions through the production on fluorine. The heavy fertilisation also results in the accumulation of cadmium

(a carcinogen) in the soil and in plants posing a health risk to humans, animals and aquatic life. The run off P bound in soil particles results in eutrophication of water bodies. The declining RP reserves (expected to hit a low by 2200, at current mining rates) means that low concentration ores will be adopted, further increasing the costs of production. With the global population continuously increasing, more pressure will be on the crop production systems to supply the increasing food demands under conditions of declining RP reserves.

Sustainable agricultural practices aim to increase food production without or at least with minimal harm to the environment, or even with a positive contribution to the environment. Producing crops sustainably calls for the avoidance of practices that are harmful to the environment and human health. Sustainable fertiliser use is among one of the most important facets that need to be addressed if crop production is to be sustainable.

It is evident that any measures available that can be adopted to improve the efficiency of P fertilisers are of great importance. Past studies, widely done on tree species, grasslands and majorly in cereal crops indicate that the N form applied has a direct effect on the soil pH and eventually, P availability in the soil and its subsequent uptake by plants. Limited studies exist on these P – N interaction in relation to tuber crops, particularly for potato. There has also been growing investigations on the use of ground raw RP as a direct application to supply P needs. However, these findings have varied greatly depending on the soil type, climatic conditions, plant species and even within cultivars of the same species. There is, therefore, a need to adopt soil, region, species and even cultivar specific studies to shed more light on the P – N interaction.

In this current study, a column study was set to investigate the P – N interaction on two different soil types, sandy and clay soil, in a laboratory without a test crop. A second study was set up to investigate the P – N interaction using South Africa's most produced cultivar, Mondial as the test crop in soils varying in P concentration across two growing seasons (winter and summer). The two trials involved the investigation of the effect of ammonium and nitrate as N forms, and superphosphate and rock phosphate as P sources on P dissolution, availability and uptake by potato.

The objective of the column study was to assess the effect of the P-N interaction on P dissolution and availability through alteration of the soil pH followed by induced leaching events. Furthermore, the effect of this interaction on the availability of soil cations (K, Ca and Mg) was assessed. Ammonium application tended to enhance dissolution and availability of P, both in the soil and in the leachate, more so than nitrate in both soil types. This was largely due to ammonium application resulting in lower soil pH as compared to nitrate, with the pH difference ranging between 0.54 to 1.05 pH units. The low pH, therefore, favoured P dissolution and availability in ammonium treatments. The sandy soil tended to have an alkaline pH, regardless of the N form and P source. However, ammonium treated soils did tend to have a lower pH compared to nitrate. These findings agreed with the first hypothesis of this trial that ammonium would favour P dissolution and its eventual availability compared to nitrate.

The P source also had a substantial effect on P availability in the soil and leachate. Superphosphate treated soils gave the highest available P concentration in the soil and leachate, compared to RP and P_0. This scenario was also noted when the three P sources were combined with either N form in both the clay and sandy soils. Ammonium + SP treated soils gave the lowest leachate and soil pH and subsequently gave the highest leachate P content compared to nitrate + SP and both RP and P_0 treatments combined with either of the N forms. This was an indication of a positive P – N interaction. There was a negligible amount of P detected in the leachate from the sandy soil on most of the watering events when ammonium and nitrate were combined with either RP and P_0 suggesting a minimal effect of the P – N interaction. Generally, the leachate P content on in the clay soil did tend to be higher compared to the sandy soil.

Seemingly, the soil and leachate K content was not influenced by the P-N interaction at certain stages. The P source tended to have minimal to no effect on soil potassium content at three of the assessed stages. In contrast, the N form had a significant effect on the leachate and soil K content on all occasions indicating that N form rather than P source influenced K concentration. Soil and leachate Ca content tended to follow a similar trend as K where at three of the assed stages, P source had minimal to no effect on Ca concentration in the leachate. However, N form significantly influenced Ca concentration in leachate on all assessed events. Superphosphate tended to have an influence on the Mg content especially in the clay soil leachate at W1 and end of the trial while RP and P_0 showed no effect. Nitrogen form significantly influenced the soil and leachate Mg concentration. There appeared to be no specific trend in K, Ca and Mg

content in the soil and leachate as some watering events and end of season analysis showed significant and non-significant interaction. Despite this, the N form seemingly having a greater effect even where the interaction was not significant.

These findings indicated that even in the absence of a crop growing in different soils, soil chemical reactions significantly influence the pH and eventually P availability, especially nitrogen form applied to the soil, depending on the soil type.

In the second trial, potato response to different N forms and P sources was assessed. Potato is a vegetable crop with very high demand for nutrients, including P, partly due to its root architecture as well as the complex chemistry of P in the soil. The aim of the trial was to evaluate the interaction between different P sources and nitrogen forms and how this affects P dissolution and its eventual availability in the soil for uptake by plants. The possibility of RP (raw material for chemical phosphatic fertilisers) as a substitute for chemical phosphatic fertilisers, such as super phosphate, was also assessed.

Significant interactions were recorded and similar to the column trial ammonium proved to be a superior N form to apply in potato production in soils with limited P supply. This is because ammonium treatments gave the highest P concentration in column leachate and the soil at the end of the column study as well as in plant tissue and in the soils at the end of the pot trial. Furthermore, ammonium fertilisation further contributed to the increased number of tubers initiated and eventual tuber yield, compared to nitrate.

The findings of the pot trial were in agreement with those of the column study, namely that the P source used had a direct effect on the P content in the soil. The crop response to the application of RP and P0, regardless of the N form used, resulted in lower yield and tuber initiation, compared to SP treatments combined with ammonium or nitrate. Phosphorus limitation is a big hurdle, which must be overcome if sufficient nutrients for the target yields are to be supplied. Rock phosphate as a P source proved to be inefficient in supplying adequate P for optimal potato growth even when used together with ammonium, but it had a positive effect on crop yield, compared to when no P was applied.

Significant P – N interaction was noted on all the assessed plant growth parameters in S1 with the exception of three instances where the HDM at TI in S1 and root length at the end of the first

season showed no significant interaction between P and N. These two stages however showed that the main factors (P source and N form) significantly influenced the assessed parameters. The number of tubers initiated in the first season also showed no significant interaction between P and N. However, the P source had a significant effect on the number of tubers initiated. All assessed plant growth parameters showed a significant P – N interaction at all stages in the second season.

P – N interaction altered the soil pH, plant available P and leaf P concentration at all stages in both seasons. Ammonium + SP tended to have the lowest pH, the highest plant available P and eventually the highest leaf P content compared to all other treatments. This finding suggested that ammonium combined with SP induced noticeable effects in soil pH, which in turn affected P availability in the soil and subsequently, the plant tissue P content. Ammonium + SP also tended to produce the highest number of tubers initiated and the eventual yield. This finding also indicates that ammonium combined with SP results in higher potato tuber yield.

Crops respond to P deficiency by firstly altering their root architecture through allocating greater biomass to the roots, and secondly, by exudation of organic acids and enzymes into the rhizosphere and finally, by forming symbiotic relationships with mycorrhizae. Plant root exudates and enzyme activities were not assessed in this study. However, the plants showed increased root growth and reduced haulm to root ratio, as well as purple colouring under the leaves (P deficiency symptom) in the P0 and RP treatments with both forms of N especially in the second season. These observations indicated positive response of the crop to the deficient conditions, though not sufficient to maintain growth and yield.

The findings of this current pot and column study indicated that ammonium combined with SP produces the highest tuber yield, significantly higher than nitrate combined with either P source. Even under optimal N supply, P deficiency significantly impacts crop growth, suggesting a co-limitation between these elements, as was reported by Jeschke et al. (1996) and De Groot et al. (2001). This has been attributed to N redistribution in the plant to the organs

low in N concentration, presumably the roots (Rufty et al. 1993, Gniazdowska and Rychter 1999) or limited energy availability to sustain N uptake (Rufty et al. 1993) under such P deficiency conditions.

It is, therefore, very critical to analyse the soil to assess the soil P status and know both the P and N requirements for optimal potato growth and maximum yield. Further research is much needed to evaluate the effect of varying levels of the different N forms and P sources on the productivity of the potato crop in order to quantify an optimal application rate. This is because there exist some benefits in RP application compared to not applying any P. Secondly, considering the changes currently being experienced in the environment, as well as the release of improved potato genotypes from improved breeding techniques, further research is required to assess their effectiveness in nutrient use efficiency.

When sufficient funds are available, further in-depth work should be considered to carry out analysis for the elements that were not monitored, specifically in the pot trial. This will offer an overview concentration of the rest of the elements and what contributions they might have to improving tuber yield. It is of great importance to also assess plant root exudates and enzymatic activities as well as the symbiotic relationships developed between microorganisms and potato roots to assess their effect on P availability in the soil. The role of N form on the effectiveness of these exudates also needs to be elaborated on further.

It is of great importance to note that other factors, such as environmental conditions, also tended to have an effect on general crop growth, where the cold weather (Appendix C) in the first season tended to result in minimal treatment effect on crop growth. This was presumably due to the varying effect of temperature on ammonium and nitrate nutrition in plants. Growth did tend to be less affected by low temperature under ammonium supply as ammonium uptake tended to be favoured by low temperature due to reduced nitrification in the soil. Higher temperature (Appendix D) did tend to favour biomass accumulation under nitrate nutrition. Caution should, therefore, be taken on what fertiliser combination need to be applied if the optimal yield is to be attained in different growing seasons.

It was evident in the findings of this study that potato production cannot be optimised without chemical P fertiliser application. Considering that RP is a finite resource, any management

practices that can improve P availability in the soil and its use efficiency need to be tried and adopted if possible. Further research on how P fertilisers can be sustainably used, considering the costs and their environmental impacts, is of great importance.

Limitations of the study

The column study was conducted in a laboratory environment, while the second study (pot trial) was conducted in a glasshouse where the temperature was regulated. The column study was conducted at room temperature, while for the pot trial, cooling was during the hot season using mechanical means, which limited the temperature to a maximum of 29°C, while no heating was done during the cold season. Therefore, the trials were conducted in varying climatic conditions, which could have definitely affected the crop performance and the nutrient composition in the soil. In addition, the controlled environment does not always reflect the actual field conditions, which vary widely during the growing season and from one season to another. Therefore, this study gives an insight into what kind of a scenario to expect under various N-P combinations, but might not necessarily reflect what the scenario could be under field conditions because there was probably some masking of the dynamics that can be expected in the field.

Time constraints, as well as funding limitations, restricted the carrying out of the experiment in the field and as a consequence, confirmatory tests, as well as conclusive recommendations, could not be given as to what may happen to both the crop and nutrient availability under field conditions.

It may have been of great interest to conduct an in-depth analysis of both the nutritional status of the crop and soil, including all the elements that affect crop growth and development. However, the analysis was not conducted due to the limitation of the project funding.

The target pH for the sandy soil was not achieved, because there was a drastic rise in pH after treatment with $Ca(OH)_2$, which raised the pH beyond the target value. Therefore, the findings reported in the two soils during the column study could have been influenced by the variations in pH.

Appropriate measures to take into account in future work

First, it is of great importance to also conduct field studies to assess the actual effect of the P-N interaction under field conditions and how the growth of potato as well as nutrient availability are affected. Thereafter, recommendations can be made to farmers on the best P source and N form combination for potato production.

With the soil being very dynamic under actual field conditions, it would be of great importance to conduct complete plant and soil analysis. Therefore, upon the availability of funding, the analysis should be conducted for all micro and macro elements in the plant and in soil to evaluate how the P-N interactions affect not only P, but all other elements in both soil and plants.

It would be important to critically evaluate the amount of lime to be applied in sandy soils, and probably also the incubation period of the soils. It would also be of great importance to assess the pH of the soils over a period of time to ensure that the required pH is actually achieved.

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APPENDICES

Appendix A: Column study leachate and soil analysis ANOVA tables

TABLE 1a. ANOVA table for effect of P source and N form on clay soil leachate pH after first watering event

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	2.77	0.55	122.75	<.0001
Error	18	0.08	0.00		
Corrected total	23	2.85			

R-square	Coefficient of variation	Root mean square error	Mean
0.97	1.33	0.07	5.05

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	0.02	0.01	2.02	0.1613
Nitrogen form	1	2.71	2.71	602.08	<.0001
P source*nitrogen form	2	0.03	0.02	3.81	0.0418

TABLE 1b. ANOVA table for effect of P source and N form on sandy soil after first watering event pH

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	0.49	0.10	147.57	<.0001
Error	18	0.01	0.00		
Corrected total	23	0.51			

R-square	Coefficient of variation	Root mean square error	Mean
0.98	0.21	0.03	12.30

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	0.00	0.00	0.17	0.8415
Nitrogen form	1	0.49	0.49	736.53	<.0001
P source*nitrogen form	2	0.00	0.00	0.47	0.6306

TABLE 2a. ANOVA table for effect of P source and N form on clay soil after second watering event pH

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	2.40	0.48	215.22	<.0001
Error	18	0.04	0.00		
Corrected total	23	2.44			

R-square	Coefficient of variation	Root mean square error	Mean
0.98	0.96	0.05	4.91

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	0.09	0.04	19.89	<.0001
Nitrogen form	1	2.29	2.29	1027.60	<.0001
P source*nitrogen form	2	0.02	0.01	4.36	0.0285

TABLE 2b. ANOVA table for effect of P source and N form on sandy soil after second watering event pH

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	5.45	1.09	255.72	<.0001
Error	18	0.08	0.00		
Corrected total	23	5.53			

R-square	Coefficient of variation	Root mean square error	Mean
0.99	0.56	0.07	11.68

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	1.71	0.46	200.72	<.0001
Nitrogen form	1	3.20	3.20	749.88	<.0001
P source*nitrogen form	2	0.54	0.27	63.65	<.0001

TABLE 3a ANOVA table for effect of P source and N form on clay soil after third watering event pH

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	2.28	0.46	454.38	<.0001
Error	18	0.02	0.00		
Corrected total	23	2.29			

R-square	Coefficient of variation	Root mean square error	Mean
0.99	0.65	0.03	4.86

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	0.06	0.03	29.83	<.0001
Nitrogen form	1	2.19	2.19	2187.07	<.0001
P source*nitrogen form	2	0.03	0.01	12.60	0.0004

TABLE 3b. ANOVA table for effect of P source and N form on sandy soil after third watering event pH

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	18.31	3.66	857.61	<.0001
Error	18	0.08	0.00		
Corrected total	23	18.39			

R-square	Coefficient of variation	Root mean square error	Mean
1.00	0.61	0.07	10.79

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	5.81	2.90	679.65	<.0001
Nitrogen form	1	11.58	11.58	2711.11	<.0001
P source*nitrogen form	2	0.93	0.46	108.83	<.0001

TABLE 4a. ANOVA table for effect of P source and N form on clay soil after fourth watering event pH

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	2.58	0.52	438.91	<.0001
Error	18	0.02	0.00		
Corrected total	23	2.60			

R-square	Coefficient of variation	Root mean square error	Mean
0.99	0.71	0.03	4.81

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	0.11	0.06	48.77	<.0001
Nitrogen form	1	2.41	2.41	2056.05	<.0001
P source*nitrogen form	2	0.05	0.02	20.47	<.0001

TABLE 4b. ANOVA table for effect of P source and N form on sandy soil after fourth watering event pH

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	11.90	2.38	1400.28	<.0001
Error	18	0.03	0.00		
Corrected total	23	11.93			

R-square	Coefficient of variation	Root mean square error	Mean
1.00	0.46	0.04	8.94

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	2.25	1.13	663.14	<.0001
Nitrogen form	1	9.25	9.25	5441.42	<.0001
P source*nitrogen form	2	0.40	0.20	116.86	<.0001

TABLE 5a. ANOVA table for effect of P source and N form on clay soil at the end of trial pH

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	2.75	0.55	609.13	<.0001
Error	18	0.02	0.00		
Corrected total	23	2.77			

R-square	Coefficient of variation	Root mean square error	Mean
0.99	0.63	0.03	4.78

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	0.10	0.05	54.51	<.0001
Nitrogen form	1	2.61	2.61	2895.06	<.0001
P source*nitrogen form	2	0.04	0.02	20.78	<.0001

TABLE 5b. ANOVA table for effect of P source and N form on sandy soil at the end of trial pH

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	4.46	0.89	1190.41	<.0001
Error	18	0.01	0.00		
Corrected total	23	4.48			

R-square	Coefficient of variation	Root mean square error	Mean
1.00	0.40	0.03	6.82

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	0.08	0.04	54.96	<.0001
Nitrogen form	1	4.30	4.30	5734.76	<.0001
P source*nitrogen form	2	0.08	0.04	53.69	<.0001

TABLE 6a. ANOVA table for effect of P source and N form on plant available P in clay soil leachate after first watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	19137.90	3827.58	2093.35	<.0001
Error	18	32.91	1.83		
Corrected total	23	19170.81			

R-square	Coefficient of variation	Root mean square error	Mean
1.00	6.80	1.35	19.88

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	17925.11	8962.56	4901.72	<.0001
Nitrogen form	1	459.11	159.11	251.09	<.0001
P source*nitrogen form	2	753.67	376.84	206.10	<.0001

TABLE 6b. ANOVA table for effect of P source and N form on plant available P in sandy soil leachate after first watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	0.06	0.01	118.82	<.0001
Error	18	0.00	0.00		
Corrected total	23	0.06			

R-square	Coefficient of variation	Root mean square error	Mean
0.97	24.41	0.01	0.04

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	0.01	0.00	47.84	<.0001
Nitrogen form	1	0.04	0.04	402.78	<.0001
P source*nitrogen form	2	0.01	0.00	47.84	<.0001

TABLE 7a. ANOVA table for effect of P source and N form on plant available P in clay soil leachate after second watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	101.44	20.29	748.50	<.0001
Error	18	0.49	0.03		
Corrected total	23	101.93			

R-square	Coefficient of variation	Root mean square error	Mean
1.00	10.55	0.15	1.56

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	100.79	50.40	1859.26	<.0001
Nitrogen form	1	0.47	0.47	17.35	0.0006
P source*nitrogen form	2	0.18	0.09	3.32	0.0592

No detectable P in the leachate from the sandy soil after the second leaching event.

TABLE 8a. ANOVA table for effect of P source and N form on plant available P in clay soil leachate after third watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	29.94	5.99	301.19	<.0001
Error	18	0.36	0.02		
Corrected total	23	30.30			

R-square	Coefficient of variation	Root mean square error	Mean
0.99	17.72	0.14	0.80

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	29.64	14.82	745.43	<.0001
Nitrogen form	1	0.08	0.08	3.99	0.0611
P source*nitrogen form	2	0.22	0.11	5.54	0.0133

No detectable P in the leachate from the sandy soil after the third leaching event

TABLE 9a. ANOVA table for effect of P source and N form on plant available P in clay soil leachate after fourth watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	18.65	3.73	335.53	<.0001
Error	18	0.20	0.01		
Corrected total	23	18.85			

R-square	Coefficient of variation	Root mean square error	Mean
0.99	18.21	0.11	0.58

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	16.11	8.05	723.99	<.0001
Nitrogen form	1	0.85	0.85	75.56	<.0001
P source*nitrogen form	2	1.70	.85	75.56	<.0001

No detectable P in the leachate from the sandy soil after the fourth leaching event

TABLE 10a. ANOVA table for effect of P source and N form on plant available P in clay soil at the end of the trial

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	4804.64	960.93	672.40	<.0001
Error	18	25.73	1.43		
Corrected total	23	4830.36			

R-square	Coefficient of variation	Root mean square error	Mean
0.99	6.90	1.20	17.32

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	4729.86	2364.93	1654.84	<.0001
Nitrogen form	1	38.71	38.71	27.09	<.0001
P source*nitrogen form	2	36.07	18.04	16.62	0.0004

TABLE 10b. ANOVA table for effect of P source and N form on plant available P in sandy soil at the end of the trial

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	22688.31	4537.66	844.51	<.0001
Error	18	96.72	5.37		
Corrected total	23	22785.03			

R-square	Coefficient of variation	Root mean square error	Mean
1.00	10.14	2.32	22.86

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	21445.45	10722.73	1995.61	<.0001
Nitrogen form	1	441.18	441.18	82.11	<.0001
P source*nitrogen form	2	801.68	400.84	74.60	<.0001

TABLE 11a. ANOVA table for effect of P source and N form on K concentration in clay soil leachate after first watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	25053.07	5010.61	16.64	<.0001
Error	18	5418.60	301.03		
Corrected total	23	30471.67			

R-square	Coefficient of variation	Root mean square error	Mean
0.82	7.49	17.35	231.77

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	3514.43	1707.72	5.67	0.0123
Nitrogen form	1	18345.78	18345.78	60.94	<.0001
P source*nitrogen form	2	3291.86	1645.93	5.47	0.0140

TABLE 11b. ANOVA table for effect of P source and N form on K concentration in sany soil leachate after first watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	1402380.50	280476.10	131.28	<.0001
Error	18	38457.50	2136.53		
Corrected total	23	1440838.00			

R-square	Coefficient of variation	Root mean square error	Mean
0.97	3.00	46.22	1542.67

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	20569.94	10284.97	4.81	0.0212
Nitrogen form	1	1338121.38	1338120.38	626.31	<.0001
P source*nitrogen form	2	43690.19	21845.09	10.22	0.0011

TABLE 12a. ANOVA table for effect of P source and N form on K concentration in clay soil leachate after second watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	11925.10	2385.02	89.56	<.0001
Error	18	479.33	26.63		
Corrected total	23	12404.43			

R-square	Coefficient of variation	Root mean square error	Mean
0.96	5.02	5.16	102.84

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	163.66	81.83	3.07	0.0711
Nitrogen form	1	11654.75	116454.75	437.66	<.0001
P source*nitrogen form	2	106.68	53.34	2.00	0.1639

TABLE 12b. ANOVA table for effect of P source and N form on K concentration in sandy soil leachate after second watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	28601.24	5720.25	194.23	<.0001
Error	18	530.13	29.45		
Corrected total	23	29131.36			

R-square	Coefficient of variation	Root mean square error	Mean
0.98	3.96	5.43	137.08

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	20942.65	10471.32	355.55	<.0001
Nitrogen form	1	4983.84	4983.84	169.22	<.0001
P source*nitrogen form	2	2674.75	1337.37	45.41	<.0001

TABLE 13a. ANOVA table for effect of P source and N form on K concentration in clay soil leachate after third watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	8066.23	1613.25	94.95	<.0001
Error	18	305.82	16.99		
Corrected total	23	8372.05			

R-square	Coefficient of variation	Root mean square error	Mean
0.96	5.62	4.12	73.36

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	265.29	132.65	7.81	0.0036
Nitrogen form	1	7772.76	7772.76	457.49	<.0001
P source*nitrogen form	2	28.18	14.09	0.83	0.4524

TABLE 13b. ANOVA table for effect of P source and N form on K concentration in sandy soil leachate after third watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	6858.92	1371.78	107.28	<.0001
Error	18	230.17	12.79		
Corrected total	23	7089.09			

R-square	Coefficient of variation	Root mean square error	Mean
0.97	6.61	3.58	54.07

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	776.75	388.38	30.37	<.0001
Nitrogen form	1	4906.33	4906.33	383.69	<.0001
P source*nitrogen form	2	1175.84	587.92	45.98	<.0001

TABLE 14a. ANOVA table for effect of P source and N form on K concentration in clay soil leachate after fourth watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	7355.19	1471.04	395.99	<.0001
Error	18	66.87	3.71		
Corrected total	23	7422.06			

R-square	Coefficient of variation	Root mean square error	Mean
0.99	3.00	1.93	64.32

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	362.96	181.48	48.85	<.0001
Nitrogen form	1	6948.25	6948.25	1870.41	<.0001
P source*nitrogen form	2	43.98	21.99	5.92	0.0106

TABLE 14b. ANOVA table for effect of P source and N form on K concentration in sandy soil leachate after fourth watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	556.58	111.32	10.56	<.0001
Error	18	189.67	10.54		
Corrected total	23	746.25			

R-square	Coefficient of variation	Root mean square error	Mean
0.75	10.37	3.25	31.30

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	91.06	45.53	4.32	0.0293
Nitrogen form	1	350.45	350.45	33.26	<.0001
P source*nitrogen form	2	115.07	57.54	5.46	0.0140

TABLE 15a. ANOVA table for effect of P source and N form on K concentration in clay soil at the end of the trial

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	5646.15	1129.23	90.11	<.0001
Error	18	225.57	12.53		
Corrected total	23	5871.72			

R-square	Coefficient of variation	Root mean square error	Mean
0.96	1.86	3.54	189.85

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	1.89	0.95	0.08	0.9276
Nitrogen form	1	5590.65	5590.65	446.12	<.0001
P source*nitrogen form	2	53.60	26.80	2.14	0.1468

TABLE 15b. ANOVA table for effect of P source and N form on K concentration in sandy soil at the end of the trial

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	30.21	6.04	17.35	<.0001
Error	18	6.27	0.35		
Corrected total	23	36.47			

R-square	Coefficient of variation	Root mean square error	Mean
0.83	9.53	0.59	6.19

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	0.59	0.29	0.84	0.4471
Nitrogen form	1	25.17	25.17	72.29	<.0001
P source*nitrogen form	2	4.44	2.22	6.38	0.0080

TABLE 16a. ANOVA table for effect of P source and N form on Ca concentration in clay soil leachate after first watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	419579.63	83915.93	100.03	<.0001
Error	18	15100.38	838.91		
Corrected total	23	434680.00			

R-square	Coefficient of variation	Root mean square error	Mean
0.96	2.81	28.96	1031.71

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	359329	179664.59	214.16	<.0001
Nitrogen form	1	13490.04	13490.04	16.08	0.008
P source*nitrogen form	2	46760.40	23380.20	27.87	<.0001

TABLE 16b. ANOVA table for effect of P source and N form on Ca concentration in sandy soil leachate after first watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	862335.25	172467.05	228.43	<.0001
Error	18	13590.38	755.02		
Corrected total	23	875925.63			

R-square	Coefficient of variation	Root mean square error	Mean
0.98	2.88	27.48	953.09

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	2089.19	1044.59	1.38	0.2761
Nitrogen form	1	858060.17	858060.17	1136.47	<.0001
P source*nitrogen form	2	2185.90	1092.95	1.45	0.2612

TABLE 17a. ANOVA table for effect of P source and N form on Ca concentration in clay soil leachate after second watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	347734.10	69546.82	73.40	<.0001
Error	18	17055.76	947.54		
Corrected total	23	364789.86			

R-square	Coefficient of variation	Root mean square error	Mean
0.95	11.07	30.78	278.16

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	14142.01	7071.01	7.46	0.0044
Nitrogen form	1	333157.26	333157.26	351.60	<.0001
P source*nitrogen form	2	434.83	217.41	0.23	0.7973

TABLE 17b. ANOVA table for effect of P source and N form on Ca concentration in sandy soil leachate after second watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	257072.64	51414.53	623.57	<.0001
Error	18	1484.13	82.45		
Corrected total	23	258556.77			

R-square	Coefficient of variation	Root mean square error	Mean
0.99	1.77	9.08	511.72

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	42946.83	21473.41	260.44	<.0001
Nitrogen form	1	212101.60	212101.60	2572.44	<.0001
P source*nitrogen form	2	2024.22	1012.11	12.28	0.0004

TABLE 18a. ANOVA table for effect of P source and N form on Ca concentration in clay soil leachate after third watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	338709.60	67741.92	496.39	<.0001
Error	18	2456.46	136.47		
Corrected total	23	341166.05			

R-square	Coefficient of variation	Root mean square error	Mean
0.99	5.40	11.68	216.05

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	47064.63	23532.32	172.44	<.0001
Nitrogen form	1	286394.51	286394.51	2098.59	<.0001
P source*nitrogen form	2	5250.45	2625.23	19.24	<.0001

TABLE 18b. ANOVA table for effect of P source and N form on Ca concentration in sandy soil leachate after third watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	112603.65	22520.73	95.86	<.0001
Error	18	4228.73	234.93		
Corrected total	23	116832.38			

R-square	Coefficient of variation	Root mean square error	Mean
0.96	6.96	15.33	220.29

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	3694.62	1847.31	7.86	0.0035
Nitrogen form	1	61568.11	61568.11	262.07	<.0001
P source*nitrogen form	2	47340.91	23670.46	100.76	<.0001

TABLE 19a. ANOVA table for effect of P source and N form on Ca concentration in clay soil leachate after fourth watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	342295.83	68459.17	259.06	<.0001
Error	18	4756.68	264.26		
Corrected total	23	347052.51			

R-square	Coefficient of variation	Root mean square error	Mean
0.99	8.39	16.26	193.74

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	30280.96	15140.48	57.29	<.0001
Nitrogen form	1	310175.88	310175.88	1173.75	<.0001
P source*nitrogen form	2	1838.99	919.50	3.48	0.0528

TABLE 19b. ANOVA table for effect of P source and N form on Ca concentration in sandy soil leachate after fourth watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	122735.07	24547.01	307.27	<.0001
Error	18	1437.98	79.89		
Corrected total	23	124173.06			

R-square	Coefficient of variation	Root mean square error	Mean
0.99	7.34	8.94	121.83

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	2647.33	1323.67	16.57	<.0001
Nitrogen form	1	111526.12	111526.12	1396.04	<.0001
P source*nitrogen form	2	8561.62	4280.81	53.59	<.0001

TABLE 20a. ANOVA table for effect of P source and N form on Ca concentration in clay soil at the end of the trial

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	40918.29	8183.66	169.29	<.0001
Error	18	870.14	48.34		
Corrected total	23	41788.43			

R-square	Coefficient of variation	Root mean square error	Mean
0.98	2.52	6.95	275.50

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	24682.54	12341.27	255.29	<.0001
Nitrogen form	1	1049.40	1049.40	21.71	0.0002
P source*nitrogen form	2	15186.35	7593.17	157.04	<.0001

TABLE 20b. ANOVA table for effect of P source and N form on Ca concentration in sandy soil at the end of the trial

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	21660.28	4332.06	148.02	<.0001
Error	18	526.81	29.27		
Corrected total	23	22187.09			

R-square	Coefficient of variation	Root mean square error	Mean
0.98	2.50	5.41	216.60

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	18228.64	9114.32	311.42	<.0001
Nitrogen form	1	2954.82	2954.82	100.96	<.0001
P source*nitrogen form	2	476.83	238.41	8.15	0.0030

TABLE 21a. ANOVA table for effect of P source and N form on Mg concentration in clay soil leachate after first watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	2968.62	593.72	41.73	<.0001
Error	18	256.11	14.23		
Corrected total	23	3224.73			

R-square	Coefficient of variation	Root mean square error	Mean
0.92	6.17	3.77	61.16

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	1165.64	582.82	40.96	<.0001
Nitrogen form	1	1791.24	1791.24	125.89	<.0001
P source*nitrogen form	2	11.74	5.87	041	0.6681

No detectable Mg in the leachate from the sandy soil after the first leaching event

TABLE 22a. ANOVA table for effect of P source and N form on Mg concentration in clay soil leachate second first watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	9441.46	1888.29	298.48	<.0001
Error	18	113.87	6.33		
Corrected total	23	9555.34			

R-square	Coefficient of variation	Root mean square error	Mean
0.99	5.70	2.52	44.11

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	669.61	334.81	52.92	<.0001
Nitrogen form	1	8663.24	8663.24	1369.40	<.0001
P source*nitrogen form	2	108.61	54.31	8.58	0.0024

TABLE 22b. ANOVA table for effect of P source and N form on Mg concentration in sandy soil leachate after second watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	0.78	0.16	121.07	<.0001
Error	18	0.02	0.00		
Corrected total	23	0.80			

R-square	Coefficient of variation	Root mean square error	Mean
0.97	44.52	0.04	0.08

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	0.31	0.16	121.07	<.0001
Nitrogen form	1	0.16	0.16	121.07	<.0001
P source*nitrogen form	2	0.31	0.16	121.07	<.0001

TABLE 23a ANOVA table for effect of P source and N form on Mg concentration in clay soil leachate after third watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	4739.17	947.83	197.83	<.0001
Error	18	86.24	4.79		
Corrected total	23	4825.41			

R-square	Coefficient of variation	Root mean square error	Mean
0.98	7.34	2.19	29.84

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	464.58	232.29	48.48	<.0001
Nitrogen form	1	4212.97	4212.97	879.31	<.0001
P source*nitrogen form	2	61.61	30.81	6.43	0.0078

TABLE 23b. ANOVA table for effect of P source and N form on Mg concentration in sandy soil leachate after third watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	43.86	8.77	489.67	<.0001
Error	18	0.32	0.02		
Corrected total	23	44.18			

R-square	Coefficient of variation	Root mean square error	Mean
0.99	22.14	0.12	0.60

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	17.55	8.77	489.67	<.0001
Nitrogen form	1	8.77	8.77	489.67	<.0001
P source*nitrogen form	2	17.55	8.77	489.67	<.0001

TABLE 24a. ANOVA table for effect of P source and N form on Mg concentration in clay soil leachate after fourth watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	5539.66	1107.93	255.03	<.0001
Error	18	78.20	4.34		
Corrected total	23	5617.86			

R-square	Coefficient of variation	Root mean square error	Mean
0.99	7.43	2.08	28.07

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	405.64	202.82	46.69	<.0001
Nitrogen form	1	5064.29	5064.29	1165.71	<.0001
P source*nitrogen form	2	69.73	34.87	8.03	0.0032

TABLE 24b. ANOVA table for effect of P source and N form on Mg concentration in sandy soil leachate after fourth watering

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	511.97	102.39	114.95	<.0001
Error	18	16.03	0.89		
Corrected total	23	528.00			

R-square	Coefficient of variation	Root mean square error	Mean
0.97	36.02	0.94	2.62

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	309.54	154.77	173.75	<.0001
Nitrogen form	1	71.83	71.83	80.64	<.0001
P source*nitrogen form	2	130.60	65.30	73.31	<.0001

TABLE 25a. ANOVA table for effect of P source and N form on Mg concentration in clay soil at the end of the trial

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	233.68	46.74	11.44	<.0001
Error	18	73.51	4.08		
Corrected total	23	307.19			

R-square	Coefficient of variation	Root mean square error	Mean
0.76	4.58	2.02	44.17

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	69.52	34.76	8.51	0.0025
Nitrogen form	1	152.66	152.66	37.38	<.0001
P source*nitrogen form	2	11.49	5.75	1.41	0.2705

TABLE 25b. ANOVA table for effect of P source and N form on Mg concentration in sandy soil at the end of the trial

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	174.93	34.99	46.41	<.0001
Error	18	13.57	0.75		
Corrected total	23	188.50			

R-square	Coefficient of variation	Root mean square error	Mean
0.93	6.00	0.87	11.47

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	166.44	83.44	110.41	<.0001
Nitrogen form	1	1.23	1.23	1.63	0.2180
P source*nitrogen form	2	7.26	3.63	4.81	0.0211

Appendix B: Season one and two tuber initiation and end of season ANOVA tables

TABLE 1a. ANOVA table for tuber initiation season one soil pH

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	1.91	0.38	152.16	<.0001
Error	18	0.05	0.003		
Corrected total	23	1.96			

R-square	Coefficient of variation	Root mean square error	Mean
0.98	0.94	0.05	5.31

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	0.03	0.02	6.48	0.0076
Nitrogen form	1	1.77	1.77	702.82	<.0001
P source*nitrogen form	2	0.11	0.07	22.51	<.0001

TABLE 1b. ANOVA table for end of season one soil pH

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	9.01	1.80	982.89	<.0001
Error	18	0.03	0.00		
Corrected total	23	9.04			

R-square	Coefficient of variation	Root mean square error	Mean
1.00	0.83	0.04	5.14

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	0.13	0.06	34.98	<.0001
Nitrogen form	1	8.26	8.26	4505.60	<.0001
P source*nitrogen form	2	0.62	0.31	169.45	<.0001

TABLE 2a. ANOVA table for tuber initiation season two soil pH

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	1.87	0.37	36.24	<.0001
Error	18	0.19	0.01		
Corrected total	23	2.06			

R-square	Coefficient of variation	Root mean square error	Mean
0.91	2.32	0.10	4.38

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	0.05	0.02	2.24	0.1348
Nitrogen form	1	1.65	1.65	160.00	<.0001
P source*nitrogen form	2	0.17	0.09	8.35	0.0027

TABLE 2b. ANOVA table for end of season two soil pH

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	13.55	2.71	309.26	<.0001
Error	18	0.16	0.01		
Corrected total	23	13.70			

R-square	Coefficient of variation	Root mean square error	Mean
1.00	1.94	0.01	4.82

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	0.25	0.12	14.24	0.0002
Nitrogen form	1	12.85	12.85	1466.49	<.0001
P source*nitrogen form	2	0.45	0.22	25.67	<.0001

TABLE 3a. ANOVA table for tuber initiation season one plant available P

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	2339.23	467.85	739.95	<.0001
Error	18	11.38	0.63		
Corrected total	23	2350.61			

R-square	Coefficient of variation	Root mean square error	Mean
1.00	2.75	0.80	28.87

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	2047.73	1023.87	1619.36	<.0001
Nitrogen form	1	159.19	159.19	251.77	<.0001
P source*nitrogen form	2	132.31	66.16	104.63	<.0001

TABLE 3b. ANOVA table for end of season one plant available P

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	1881.44	376.29	217.18	<.0001
Error	18	31.19	1.73		
Corrected total	23	1912.63			

R-square	Coefficient of variation	Root mean square error	Mean
0.98	5.01	1.32	26.27

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	1708.50	854.25	493.04	<.0001
Nitrogen form	1	93.58	93.58	54.01	<.0001
P source*nitrogen form	2	79.36	39.68	22.90	<.0001

TABLE 4a. ANOVA table for tuber initiation season two plant available P

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	8944.34	1788.87	1470.82	<.0001
Error	18	21.89	1.22		
Corrected total	23	8966.23			

R-square	Coefficient of variation	Root mean square error	Mean
1.00	4.60	1.10	23.98

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	8519.53	4259.77	3502.41	<.0001
Nitrogen form	1	269.81	269.81	221.84	<.0001
P source*nitrogen form	2	155.00	77.50	63.72	<.0001

TABLE 4b. ANOVA table for season two plant available P

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	5743.83	1148.77	1094.96	<.0001
Error	18	18.88	1.05		
Corrected total	23	5762.72			

R-square	Coefficient of variation	Root mean square error	Mean
1.00	5.34	1.02	19.17

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	5472.16	2736.08	2607.94	<.0001
Nitrogen form	1	144.16	144.16	137.41	<.0001
P source*nitrogen form	2	127.51	63.76	60.77	<.0001

TABLE 5a. ANOVA table for tuber initiation season one leaf P concentration

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	30529713.71	6105942.74	1414.24	<.0001
Error	18	77714.25	4317.46		
Corrected total	23	30607427.96			

R-square	Coefficient of variation	Root mean square error	Mean
1.10	1.10	65.71	5959.46

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	27232693.58	13616346.79	3153.79	<.0001
Nitrogen form	1	28.9713.38	28.9713.38	581.29	<.0001
P source*nitrogen form	2	787306.75	393653.37	91.18	<.0001

TABLE 5b. ANOVA table for end of season one leaf P concentration

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	10559102.21	2111820.44	180.01	<.0001
Error	18	2111783.83	11731.88		
Corrected total	23	10770276.04			

R-square	Coefficient of variation	Root mean square error	Mean
0.98	6.21	108.31	1743.74

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	10140665.20	5070332.60	432.18	<.0001
Nitrogen form	1	307315.40	307315.40	26.19	<.0001
P source*nitrogen form	2	111121.60	55560.80	4.74	0.0223

TABLE 6a. ANOVA table for tuber initiation season two leaf P concentration

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	138004402.70	27600880.5	1337.95	<.0001
Error	18	371326.30	20629.20		
Corrected total	23	138375729.00			

R-square	Coefficient of variation	Root mean square error	Mean
1.00	4.06	143.63	3537.29

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	127130420.3	63565210.2	3081.32	<.0001
Nitrogen form	1	6471932.0	6471932.0	313.73	<.0001
P source*nitrogen form	2	4402050.3	2201025.2	106.69	<.0001

TABLE 6b. ANOVA table for end of season two leaf P concentration

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	16289737.33	3257947.47	307.00	<.0001
Error	18	191020.00	10612.22		
Corrected total	23	16480757.33			

R-square	Coefficient of variation	Root mean square error	Mean
0.99	5.87	103.12	1755.67

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	14380258.08	7190129.04	677.53	<.0001
Nitrogen form	1	1045002.67	1045002.67	98.47	<.0001
P source*nitrogen form	2	864476.58	432238.29	40.73	<.0001

TABLE 7a. ANOVA table for tuber initiation season one haulm length

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	881.76	176.35	139.09	<.0001
Error	18	22.82	1.27		
Corrected total	23	904.59			

R-square	Coefficient of variation	Root mean square error	Mean
0.97	3.31	1.23	34.01

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	486.93	243.47	192.02	<.0001
Nitrogen form	1	22.82	381.60	300.97	<.0001
P source*nitrogen form	2	13.23	6.62	5.22	<0.0163

TABLE 7b. ANOVA table for end of season one haulm length

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	1707.19	341.44	307.91	<.0001
Error	18	19.96	1.11		
Corrected total	23	1727.15			

R-square	Coefficient of variation	Root mean square error	Mean
0.9	2.64	1.05	39.93

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	1195.80	597.90	539.16	<.0001
Nitrogen form	1	454.14	454.14	409.55	<.0001
P source*nitrogen form	2	57.25	28.63	25.81	<.0001

TABLE 8a. ANOVA table for tuber initiation season two haulm length

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	9012.31	1802.46	746.41	<.0001
Error	18	42.28	2.35		
Corrected total	23	9054.59			

R-square	Coefficient of variation	Root mean square error	Mean
1.00	2.33	1.53	65.91

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	7653.39	3826.70	1629.25	<.0001
Nitrogen form	1	1339.52	1339.52	570.31	<.0001
P source*nitrogen form	2	19.40	9.70	4.13	0.0334

TABLE 8b. ANOVA table for end of season two haulm length

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	7961.15	1592.23	213.09	<.0001
Error	18	134.50	7.47		
Corrected total	23	8095.65			

R-square	Coefficient of variation	Root mean square error	Mean
0.98	3.42	2.73	79.98

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	6362.79	3181.40	425.78	<.0001
Nitrogen form	1	1526.42	1526.42	204.29	<.0001
P source*nitrogen form	2	71.94	35.97	4.81	0.0211

TABLE 9a. ANOVA table for TUBER INITIATION season one haulm dry mass

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	34.71	6.94	45.03	<.0001
Error	18	2.77	0.15		
Corrected total	23	37.49			

R-square	Coefficient of variation	Root mean square error	Mean
0.93	7.10	0.39	5.53

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	27.35	13.67	88.69	<.0001
Nitrogen form	1	6.73	6.73	43.66	<.0001
P source*nitrogen form	2	0.63	0.32	2.06	<.1571

TABLE 9b. ANOVA table for end of season one haulm dry mass

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	129.68	25.94	191.29	<.0001
Error	18	2.44	0.14		
Corrected total	23	132.12			

R-square	Coefficient of variation	Root mean square error	Mean
0.98	4.70	0.37	7.84

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	81.53	40.76	300.64	<.0001
Nitrogen form	1	38.25	38.25	282.13	<.0001
P source*nitrogen form	2	9.90	4.95	36.50	<.0001

TABLE 10a. ANOVA table for tuber initiation season two haulm dry mass

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	4038.28	807.66	994.84	<.0001
Error	18	14.61	0.81		
Corrected total	23	4052.90			

R-square	Coefficient of variation	Root mean square error	Mean
1.00	6.03	0.90	14.95

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	3802.86	1901.43	2342.11	<.0001
Nitrogen form	1	21.19	21.19	26.10	<.0001
P source*nitrogen form	2	214.24	107.12	131.95	<.0001

TABLE 10b. ANOVA table for season two haulm dry mass

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	3563.74	712.75	3000.52	<.0001
Error	18	4.28	0.24		
Corrected total	23	3568.01			

R-square	Coefficient of variation	Root mean square error	Mean
1.00	2.70	0.49	18.10

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	3304.63	1562.32	6955.91	<.0001
Nitrogen form	1	0.19	0.19	0.80	0.3819
P source*nitrogen form	2	258.91	129.46	544.99	<.0001

TABLE 11a. ANOVA table for tuber initiation season one root length

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	1188.69	237.74	122.11	<.0001
Error	18	35.05	1.95		
Corrected total	23	1223.73			

R-square	Coefficient of variation	Root mean square error	Mean
0.97	5.35	1.40	26.08

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	231.42	115.71	59.43	<.0001
Nitrogen form	1	930.02	930.02	477.68	<.0001
P source*nitrogen form	2	27.25	13.63	7.00	<.0056

TABLE 12b. ANOVA table for end of season one root length

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	891.07	178.21	65.42	<.0001
Error	18	49.03	2.72		
Corrected total	23	940.10			

R-square	Coefficient of variation	Root mean square error	Mean
0.95	5.28	1.65	31.25

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	228.91	114..45	42.02	<.0001
Nitrogen form	1	656.26	656.26	240.92	<.0001
P source*nitrogen form	2	5.90	2.95	1.08	<.3596

TABLE 13a. ANOVA table for tuber initiation season two root length

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	1941.78	388.36	405.95	<.0001
Error	18	17.22	0.96		
Corrected total	23	1959.00			

R-square	Coefficient of variation	Root mean square error	Mean
0.99	3.01	0.98	32.44

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	1228.75	614.38	642.20	<.0001
Nitrogen form	1	552.96	552.96	578.01	<.0001
P source*nitrogen form	2	160.07	80.03	83.66	<.0001

TABLE 13b. ANOVA table for end of season two root length

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	1906.95	381.39	159.17	<.0001
Error	18	43.13	2.40		
Corrected total	23	1950.08			

R-square	Coefficient of variation	Root mean square error	Mean
0.98	4.01	1.55	38.59

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	1334.11	667.06	278.39	<.0001
Nitrogen form	1	530.16	530.16	221.26	<.0001
P source*nitrogen form	2	42.68	21.34	8.91	0.0020

TABLE 14a. ANOVA table for tuber initiation season one root dry mass

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	1.97	0.39	180.74	<.0001
Error	18	0.04	0.002		
Corrected total	23	2.01			

R-square	Coefficient of variation	Root mean square error	Mean
0.98	7.12	0.05	0.66

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	1.46	0.73	334.75	<.0001
Nitrogen form	1	0.32	0.32	145.37	<.0001
P source*nitrogen form	2	0.19	0.10	11.40	<.0001

TABLE 14b. ANOVA table for end of season one root dry mass

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	1.52	0.30	12.39	<.0001
Error	18	0.13	0.01		
Corrected total	23	1.65			

R-square	Coefficient of variation	Root mean square error	Mean
0.92	8.63	0.08	0.98

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	0.94	0.47	65.17	<.0001
Nitrogen form	1	0.45	0.45	63.12	<.0001
P source*nitrogen form	2	0.13	0.07	9.25	<.0017

TABLE 15a. ANOVA table for tuner initiation season two root dry mass

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	238.32	47.66	1564.40	<.0001
Error	18	0.55	0.03		
Corrected total	23	238.87			

R-square	Coefficient of variation	Root mean square error	Mean
1.00	3.38	0.17	5.17

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	216.53	108.27	3553.46	<.0001
Nitrogen form	1	2.81	2.81	92.18	<.0001
P source*nitrogen form	2	18.98	9.49	311.46	<.0001

TABLE 15b. ANOVA table for season two root dry mass

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	250.70	50.14	898.72	<.0001
Error	18	1.00	0.06		
Corrected total	23	251.70			

R-square	Coefficient of variation	Root mean square error	Mean
1.00	2.93	0.24	8.06

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	230.82	115.41	2068.62	<.0001
Nitrogen form	1	1.85	1.85	33.23	<.0001
P source*nitrogen form	2	18.03	9.01	161.57	<.0001

TABLE 16a. ANOVA table for tuber initiation season one root to haulm dry mass ratio

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	0.12	0.02	81.22	<.0001
Error	18	0.01	0.00		
Corrected total	23	0.13			

R-square	Coefficient of variation	Root mean square error	Mean
0.96	13.53	0.02	0.13

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	0.01	0.01	18.83	<.0001
Nitrogen form	1	0.10	0.10	340.42	<.0001
P source*nitrogen form	2	0.01	0.00	14.00	0.0002

TABLE 16b. ANOVA table for end of season one root to haulm dry mass ratio

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	0.04	0.01	44.55	<.0001
Error	18	0.00	0.00		
Corrected total	23	0.04			

R-square	Coefficient of variation	Root mean square error	Mean
0.93	9.88	0.01	0.13

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	0.02	0.01	61.66	<.0001
Nitrogen form	1	0.00	0.00	27.00	<.0001
P source*nitrogen form	2	0.01	0.01	36.22	<.0001

TABLE 17a. ANOVA table for tuber initiation season two root to haulm dry mass ratio

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	7.06	1.41	293.36	<.0001
Error	18	0.09	0.00		
Corrected total	23	7.14			

R-square	Coefficient of variation	Root mean square error	Mean
0.99	10.81	0.07	0.64

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	1.75	0.88	182.87	<.0001
Nitrogen form	1	3.60	3.60	749.05	<.0001
P source*nitrogen form	2	1.69	0.85	176.01	<.0001

TABLE 17b. ANOVA table for the end of season two root to haulm dry mass ratio

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	1.74	0.35	511.99	<.0001
Error	18	0.01	0.00		
Corrected total	23	1.75			

R-square	Coefficient of variation	Root mean square error	Mean
0.99	4.60	0.03	0.57

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	0.57	0.29	418.79	<.0001
Nitrogen form	1	0.76	0.76	1121.53	<.0001
P source*nitrogen form	2	0.41	0.20	300.42	<.0001

TABLE 18. ANOVA table for season one tuber initiation

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	376.38	75.28	33.66	<.0001
Error	18	40.25	2.24		
Corrected total	23	416.63			

R-square	Coefficient of variation	Root mean square error	Mean
0.90	10.59	1.50	14.13

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	364.00	182.00	81.39	<.0001
Nitrogen form	1	9.38	9.38	4.19	<.0001
P source*nitrogen form	2	3.00	1.50	0.67	0.5236

TABLE 19. ANOVA table for season two tuber initiation

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	7182.85	1436.57	817.07	<.0001
Error	18	31.65	1.76		
Corrected total	23	7214.50			

R-square	Coefficient of variation	Root mean square error	Mean
1.00	7.23	1.33	18.35

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	6901.85	3450.93	1962.77	<.0001
Nitrogen form	1	194.37	194.37	110.55	<.0001
P source*nitrogen form	2	86.62	43.31	24.63	<.0001

TABLE 20a. ANOVA table for end of season one tuber fresh mass

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	31267.71	6235.54	59.83	<.0001
Error	18	1881.25	104.51		
Corrected total	23	33148.96			

R-square	Coefficient of variation	Root mean square error	Mean
0.94	3.02	10.22	338.96

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	19633.33	9816.67	39.93	<.0001
Nitrogen form	1	9401.04	9401.04	89.95	<.0001
P source*nitrogen form	2	2233.33	1116.67	10.68	0.0009

TABLE 20b. ANOVA table for season one tuber dry mass

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	1613.27	322.65	47.47	<.0001
Error	18	122.34	6.80		
Corrected total	23	1735.61			

R-square	Coefficient of variation	Root mean square error	Mean
0.63	3.78	2.61	68.96

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	1017.55	508.77	74.86	<.0001
Nitrogen form	1	541.50	541.50	79.67	<.0001
P source*nitrogen form	2	54.22	27.11	3.99	0.0368

TABLE 21a. ANOVA table for end of season two tuber fresh mass

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	1924080.23	384816.05	5384.08	<.0001
Error	18	1286.51	71.47		
Corrected total	23	1925366.75			

R-square	Coefficient of variation	Root mean square error	Mean
1.00	3.35	8.45	252.15

Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	1894386.21	947193.11	13252.50	<.0001
Nitrogen form	1	14202.88	14202.88	198.72	<.0001
P source*nitrogen form	2	15491.14	7745.57	108.37	<.0001

TABLE 21b. ANOVA table for end of season two tuber dry mass

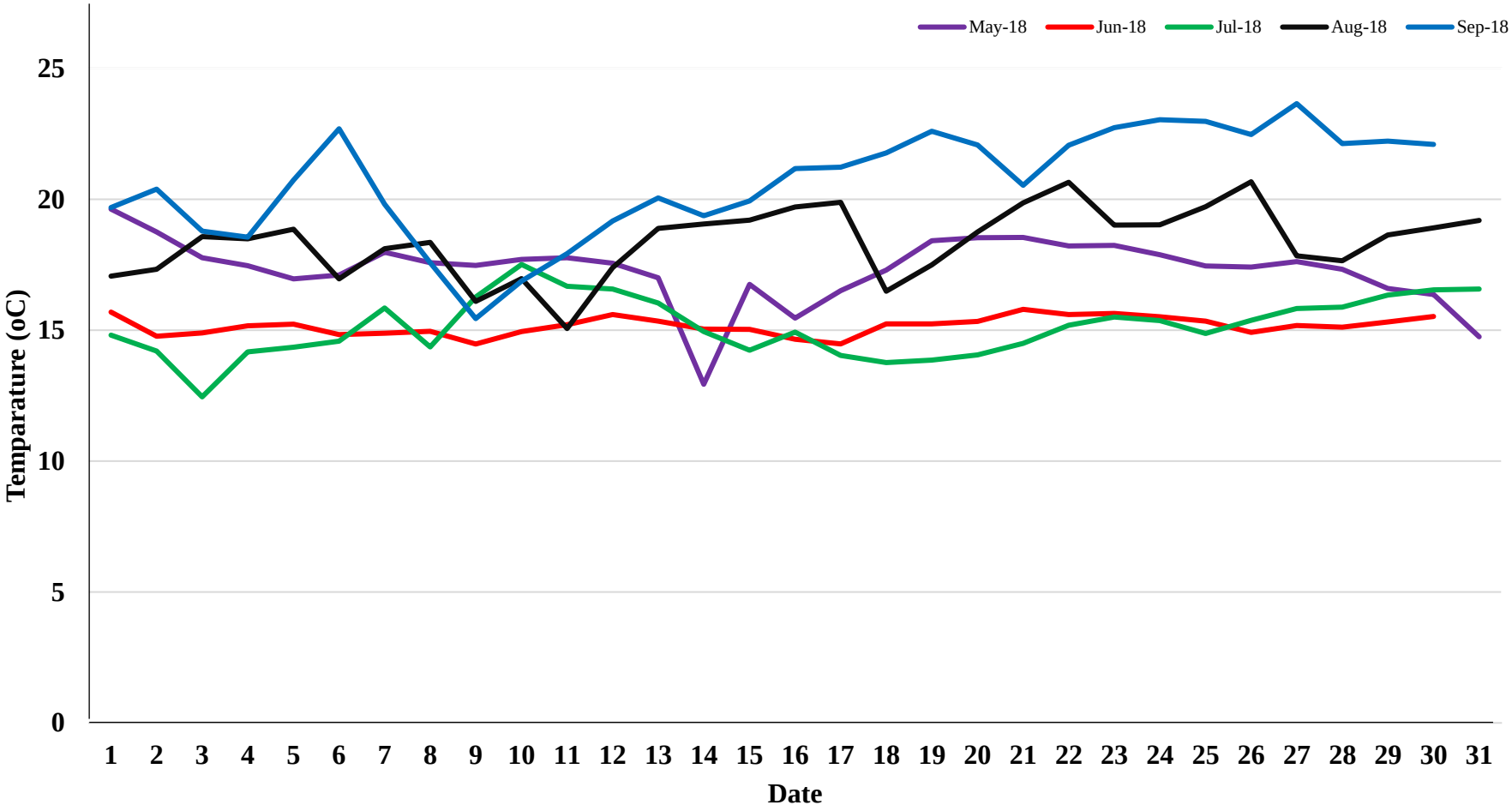
Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	5	82664.73	16532.95	3707.41	<.0001
Error	18	80.27	4.46		
Corrected total	23	82745.00			

R-square	Coefficient of variation	Root mean square error	Mean
1.00	4.07	2.11	51.84

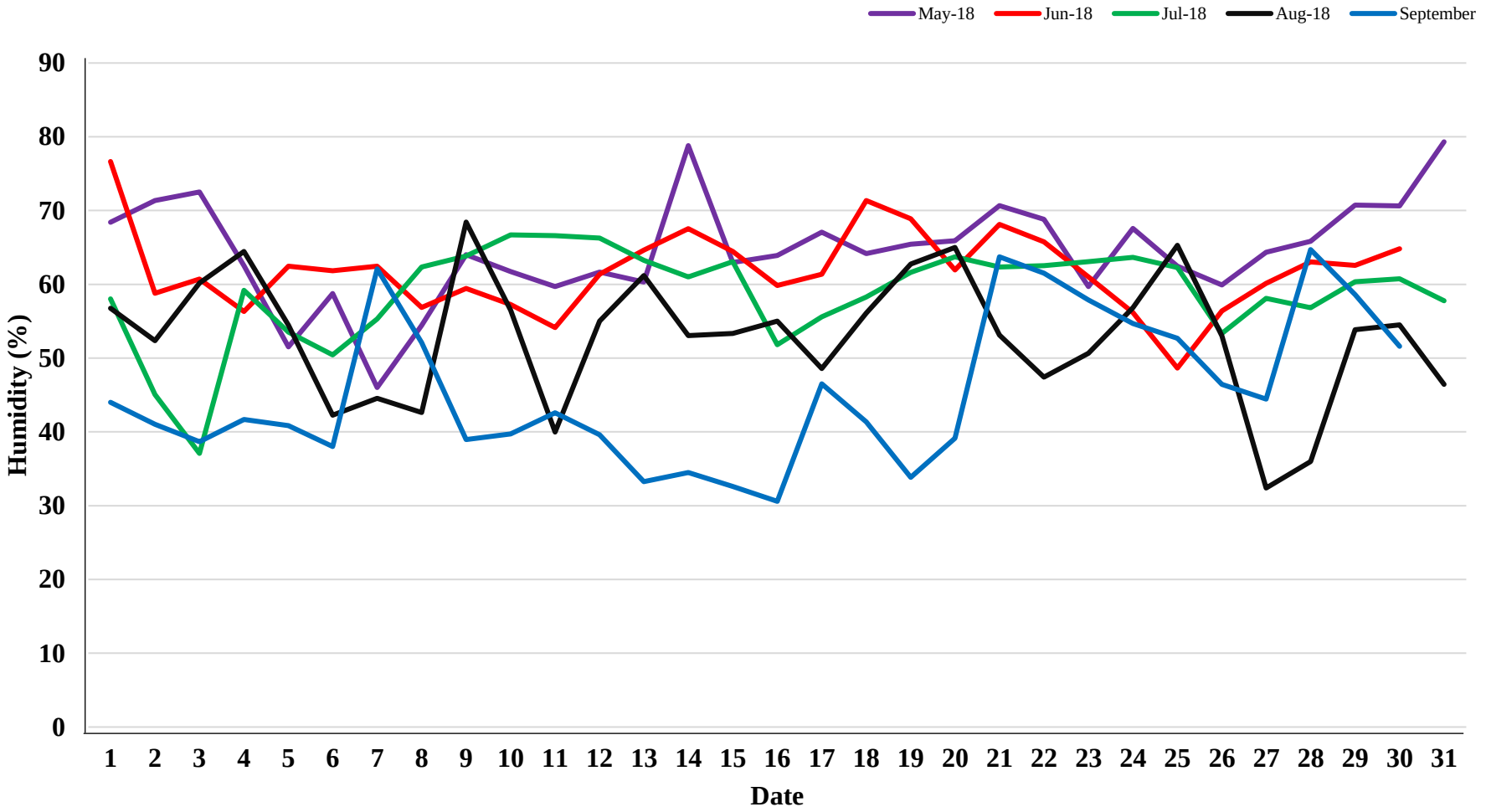
Source	DF	Type I SS	Mean Square	F Value	Pr > F
P source	2	80860.96	40430.48	9066.29	<.0001
Nitrogen form	1	828.73	828.73	185.84	<.0001
P source*nitrogen form	2	975.04	487.52	109.32	<.0001

Appendix C: Season one weather conditions

Daily temperature average over the growing period (logged on an hourly basis)

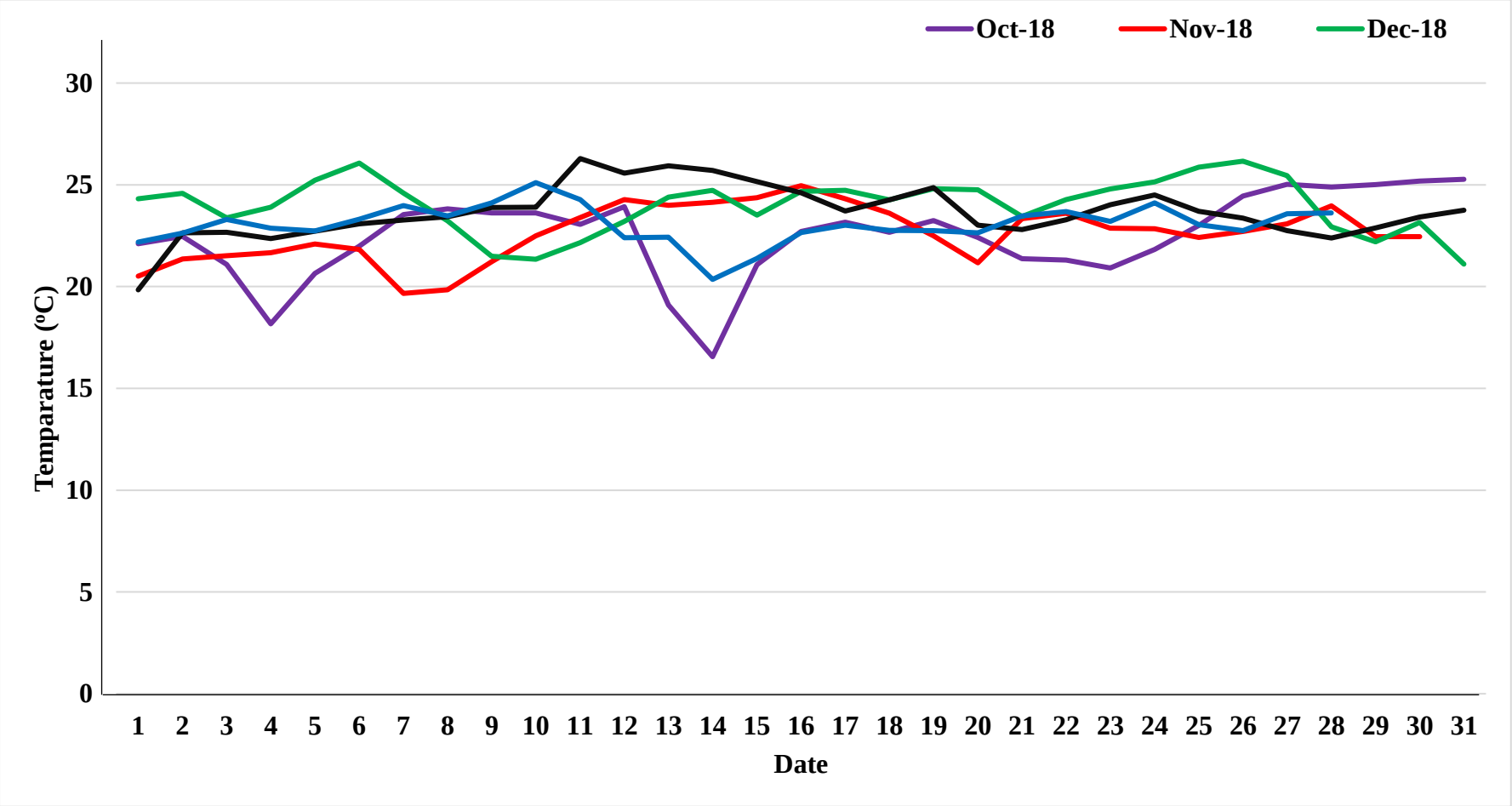


Daily humidity average over the growing period (logged on an hourly basis)



Appendix D: Season two weather conditions

Daily temperature average over the growing period (logged on an hourly basis)



Daily humidity average over the growing period (logged on an hourly basis)