

## Temporal shifts of fungal communities in the rhizosphere and on tubers in potato fields

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### Highlights

- Fungal communities were investigated using ARISA and Illumina ITS sequencing.
- Alpha-diversity was shown to be stable within and across seasons.
- Beta-diversity varied between plant developmental stages in each season.
- The phylum *Ascomycota* dominated the potato rhizosphere at all plant growth stages in both seasons.

## **Abstract**

Soil fungal communities perform important ecological roles determining, at least in part, agricultural productivity. This study aimed at examining the fungal community dynamics in the potato rhizosphere across different development stages in two consecutive growing seasons (winter and summer). Microbial fingerprinting of rhizosphere soil samples collected at pre-planting, tuber initiation, flowering and at senescence was performed using ARISA in conjunction with Next Generation Sequencing (Illumina MiSeq). The epiphytic fungal communities on tubers at harvest were also investigated. Alpha-diversity was stable over time within and across the two seasons. In contrast, rhizospheric fungal community structure and composition were different between the two seasons and in the different plant growth stages within a given season, indicating the significance of the rhizosphere in shaping microbial communities. The phylum *Ascomycota* was dominant in the potato fungal rhizosphere, with Operational Taxonomic Units (OTUs) belonging to the genus *Peyronellaea* being the most abundant in all samples. Important fungal pathogens of potato, together with potential biological control agents and saprophytic species, were identified as indicator OTUs at different plant growth stages. These findings indicate that potato rhizosphere fungal communities are functionally diverse, which may contribute to soil health.

**Keywords:** Fungal communities; rhizosphere; potato; ARISA; sequencing

## 1. Introduction

The rhizosphere is defined as a narrow soil volume in close proximity to root surfaces, with chemical properties directly affected by root exudates (Berendsen et al. 2012; Huang et al. 2014). Plants have been shown to invest up to 40 % of fixed carbon in root exudation (Chaparro et al. 2012). Heterotrophic microbes, including bacteria, fungi, protozoa, archaea and nematodes, are attracted to these organic compounds released by the plants (Philippot et al. 2013). In addition to chemotaxis, electronic signals characterised by root surface electric potentials have been implicated in the attraction of swimming zoospores of plant pathogenic oomycetes to root surfaces (Bais et al. 2006). A cross talk between the plant roots and associated microbiome develops, which is necessary in selective microbial root colonisation (Huang et al. 2014).

The understanding of root-associated microbiomes is crucial in sustainable agriculture as they are considered indicators of soil health and fertility (Lindahl et al. 2013; Ofek-Lalzar et al. 2014). These microbiomes perform complex biological and chemical processes important in decomposition of organic matter, absorption of nutrients and either promoting or suppressing plant diseases (Berendsen et al. 2012). One of the most important current research objectives is to establish the relationship between root microbiomes of different plant species and variables determining plant fitness, as a key to plant productivity (Lakshmanan et al. 2014).

Studies on potato (*Solanum tuberosum* L.) rhizosphere microbiomes have focussed mainly on bacterial spatio-temporal dynamics between plant growth stages, genotypes and cropping systems (Lukow et al. 2000; Ferreira et al. 2008; Micallef et al. 2009; İnceoğlu et al. 2010; 2011; Gschwendtner et al. 2011; Weinert et al. 2011; Liu et al. 2014). As a consequence, despite their contribution to the productivity of agricultural soils, very little is known about

soil-borne fungi associated with potato plants. Previous studies have, however, demonstrated the influence of potato cropping systems and genotypes on the prevalence of phytopathogenic fungi in soil (Manici and Caputo 2009; Weinert et al. 2009; Sugiyama et al. 2010; Liu et al. 2014). Knowledge of the characterisation of fungal community structure in the potato rhizosphere in response to seasonal changes and plant development is limited. Such information will assist, for example, in identifying members of the rhizospheric community that are typically associated with a plant species.

Seasonal variations in precipitation and temperature may have an impact on the diversity and abundance of microbial communities (Buckeridge et al. 2013). Plant age can also shape rhizosphere communities (Micallef et al. 2009; Chaparro et al. 2014). For example, the roots of seedlings and early vegetative growth stages of the model plant *Arabidopsis thaliana* produce sugars and sugar alcohols that promote proliferation of microbial generalists (Chaparro et al. 2014). An elevated secretion of defence-related proteins was also noted at the flowering stage of many plants (De-la-Pena et al. 2010). Changes in rhizosphere bacterial communities with plant age have also been reported in some agricultural crops, including potato (Lukow et al. 2000), alfalfa (Mougel et al. 2006), soybean (Sugiyama et al. 2014), pea, wheat and sugar beet (Houlden et al. 2008).

Most research studies of the rhizosphere have used denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), phyloChip hybridization and terminal restriction fragment length polymorphism (T-RFLP) approaches (Lukow et al 2000; Ferreira et al. 2008; Manici and Caputo 2009; İnceoğlu et al. 2010; Weinert et al. 2011). While these techniques give an insight into the patterns of the numerically dominant members of the microbial communities in relation to biotic or abiotic factors, they do not provide

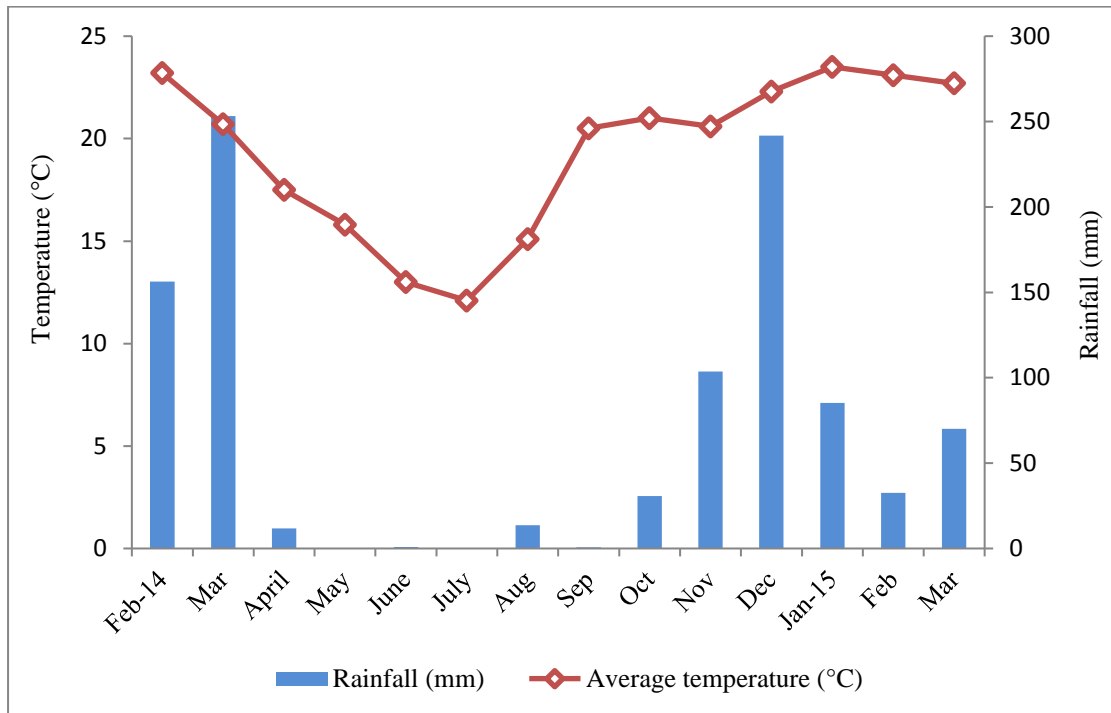
comprehensive information on which microbes are present (Chaparro et al. 2014). Sequencing approaches are better suited to obtain in depth taxonomic information (Schmitt et al. 2012; Mizrahi-Man et al. 2013). The current study used automated ribosomal intergenic spacer analysis (ARISA) in combination with the sequencing of the ITS region using the Illumina MiSeq platform to examine inter- and intra-seasonal variation of fungal communities associated with the potato rhizosphere. Specifically, two fundamental questions were asked. (i) How do fungal diversity and composition change as potato plants grow? (ii) Are these patterns consistent across consecutive seasons?

## **2. Materials and Methods**

### **2.1. Trial layout and sample collection**

Two field trials were prepared at the University of Pretoria experimental farm (25°44'49"S, 28°15'40"E; 66 % sand, 27 % clay and 7 % silt) in Pretoria, South Africa. The first trial was planted in March and the second trial in October 2014, representing winter and summer crops, respectively. The rainfall and temperature data for the study period are presented in Fig. 1. A trial area of approximately 125 m<sup>2</sup> (13 x 9.6 m) was divided into three blocks with three plots in each block. Plots of 4.05 m<sup>2</sup> were separated by 1 m wide paths. The Up-to-Date potato variety was cultivated as per standard agricultural practices. Destructive rhizosphere soil sampling (from one plot per block and four plants per plot per sampling time) was done at tuber initiation, flowering and senescence in both seasons following the procedure by Chaparro et al. (2014) with minor modifications. Briefly, the whole plant system was carefully uprooted together with soil adhering to tubers and intact root systems. The samples were placed in cooler boxes and immediately transported to the laboratory. Excess soil was gently shaken off and approximately 10 g of soil loosely attached to the root system of each plant was collected. Control soil samples were collected pre-plant from the trial area and between plant rows at

each sampling time point using a soil auger to an approximate depth of 20 cm. The soil samples were homogenised and kept at - 80 °C for DNA extraction. Potato peel samples from three tubers per plant at harvest were also kept at - 80 °C.



**Figure 1:** Rainfall and temperature data recorded at the University of Pretoria experimental farm from February, 2014 to February, 2015

## 2.2. DNA extraction from rhizosphere and peel samples

Soil DNA extraction was performed from 0.5 g of each soil sample using PowerSoil<sup>®</sup> DNA Isolation Kit (Mo Bio, Carlsbad, CA, USA) according to the manufacturer's instructions. DNeasy<sup>®</sup> Plant Mini DNA extraction Kit (Qiagen, Hilden, Germany) was used to isolate DNA from tuber peel samples following the manufacturer's recommendations. DNA was quantified using a NanoDrop-2000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA) and checked for quality by agarose gel electrophoresis. The DNA samples were kept at -20 °C for future use.

### 2.3. Automated Ribosomal Intergenic Spacer Analysis (ARISA)

The fluorescently labelled fITS9 (FAM) (5'-GAACGCAGCGAAATGCGA-3') (Ihrmark et al. 2012) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990) primers were used in the PCR reactions to produce short amplicons spanning the fungal ITS 2 region. PCR amplification was conducted according to the protocol by Ihrmark et al. (2012) with minor modifications. Briefly, 50 µl reaction mixes were comprised of 0.25 ng µl<sup>-1</sup> genomic DNA template, 200 µM of each dNTP, 2.75 mM MgCl<sub>2</sub>, 200 nM primers, 1.28 mg ml<sup>-1</sup> Bovine Serum Albumin (BSA), 2 U DreamTaq DNA polymerase (Thermo Scientific) and 1x DreamTaq buffer (Thermo Scientific). These were amplified in triplicate for each sample in a thermal cycler (Bio-Rad, Hercules, CA, USA) using the following thermal PCR profile: initial denaturation at 94 °C for 5 mins; 30 cycles of 94 °C for 30s, 55 °C for 30s and 72 °C for 30s; with final elongation at 72 °C for 7 mins. Replicate amplicons were mixed prior to gel electrophoresis and clean up using BioSpin PCR Purification Kit (Bioflux, Tokyo, Japan) following the manufacturer's instructions.

Restriction enzyme digestion was carried out overnight at 37 °C in 20 µl reaction mix containing 200 ng purified PCR products, 2 µl reaction buffer and 1 µl *Msp*1 (Thermo Scientific). Four micro litre aliquots of purified digests were mixed with GeneScan™ 600 LIZ® size standard (Applied Biosystems, Carlsbad, USA) and denatured at 95 °C for 5 mins. An automated Applied Biosystems 3500xl DNA Genetic Analyzer (Applied Biosystems™, Carlsbad, USA) was used to electrophoretically separate restriction fragments in relation to the lane standard. The instrument used the signal intensity of fluorescently labelled terminal fragments for each sample to produce ARISA electropherograms. GeneMapper® Software v. 4.1 was used to analyse fragment peak patterns across samples and present them in binary format allowing for easy downstream analysis.

#### **2.4. ITS sequencing of rhizosphere and peel samples**

DNA samples were pooled to end up with three rhizosphere samples for each sampling time point (tuber initiation, flowering and senescence) and three potato peel samples per season (n = 24). PCR reactions were performed with primers ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990; Gardes and Bruns, 1993) with a unique barcode on the forward primer. HotStar Plus PCR Master Mix Kit (Qiagen, USA) was used in PCR amplification reactions. Amplicons were run on a 2 % agarose gel followed by pooling of multiple samples in equal proportions depending on molecular weight and DNA concentrations. Calibrated Ampure XP beads were used to purify pooled samples and DNA libraries were prepared using the Illumina TruSeq DNA library preparation protocol. Sequencing was done at Mr DNA ([www.mrdnalab.com](http://www.mrdnalab.com), Shallowater, TX, USA) on the MiSeq platform (Illumina) according to the manufacturer's guidelines to produce 2 x 250 paired end reads. The raw read data from Illumina Miseq was deposited in GenBank Sequence Read Archive (SRA) under accession number SAMN07427124.

#### **2.5. Data analysis**

ARISA fragments generated by Peak Scanner software v 1.0 (Applied Biosystems) were filtered and binned by the method developed by Abdo et al. (2006). A standard deviation of 3 was used to remove baseline noise and 1 bp distance was used for binning adjacent fragments into the same operational taxonomic unit (OTU). Bray-Curtis similarity matrices were generated using Primer 6 v 6.1.11 (Clarke 1993). and the matrices were subjected to non-metric multidimensional scaling (NMDS) ordination analyses for seasonal and growth stage communities. The Kruskal-Wallis test was performed in R (<http://www.r-project.org/>; R Core Team) to determine differences in OTU richness between sampling time points. A Student's t-



test was performed to compare diversity between seasons using R software. Beta diversity within seasons was determined by permutational analysis of variance (PERMANOVA) (Anderson et al. 2003) using the *adonis* function in the *vegan* package (Oksanen et al. 2016) for R.

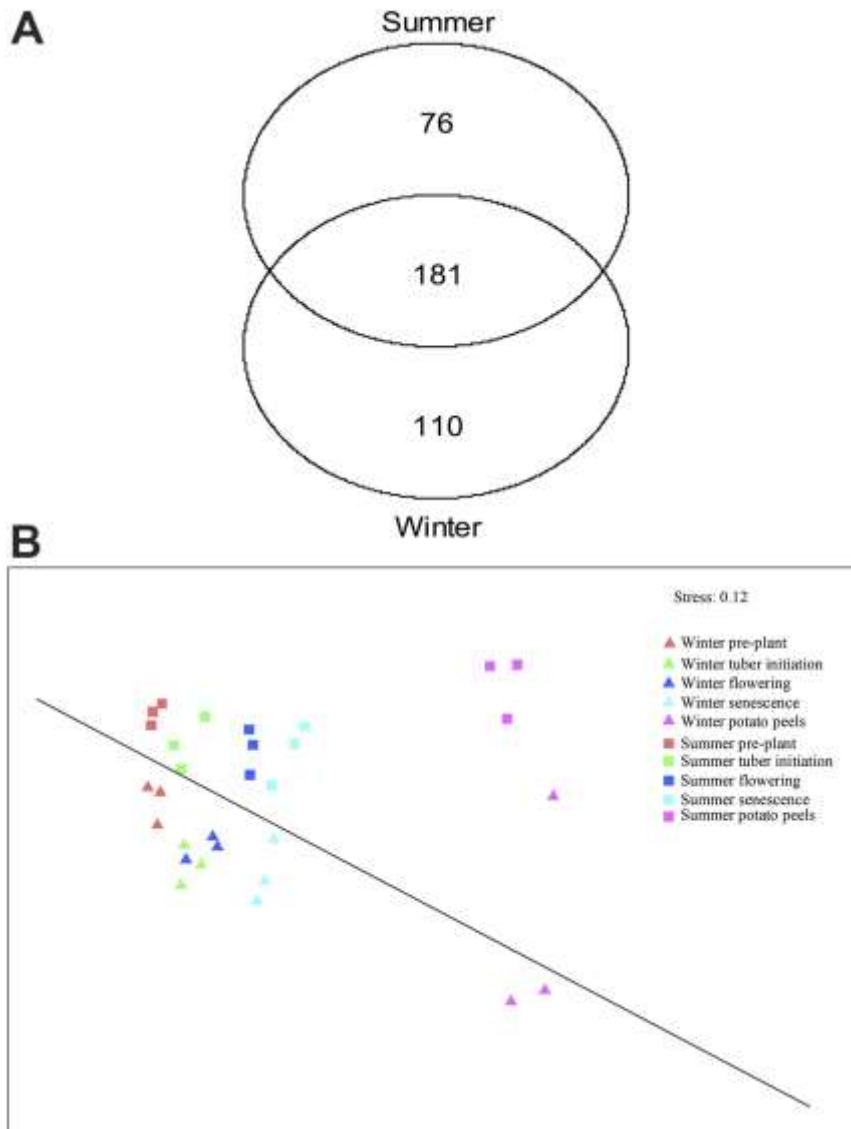
The ITS gene sequencing data were analysed using MOTHUR v1.27.0 (Schloss et al. 2009). Only the forward reads were used for further analysis, as the quality of the forward reads was far superior to that of the reverse reads. Sequences were trimmed using the *trim.seqs* command and sequences shorter than 200 bp and longer than 272 bp were removed. Reads with ambiguities, homopolymers (>8 bp) or poor quality (average score <25) were also removed. Unique sequences were generated using the *unique.seqs* command and Uchime v4.2.40 was implemented for chimera identification. The *cluster* command with default parameters was used to assign sequences to OTUs at 97 % similarity. Taxonomic affiliation was performed using the UNITE v6 database (Kõljag et al. 2013; <http://unite.ut.ee>) with mothur at a cut-off of 80 %. Refined taxonomy assignment of indicator OTUs was performed using BLASTn against the NCBI database. Venn diagrams were generated using the *gplots* package (Warnes et al. 2009) in R. The *labdsv* package (Roberts 2013) in R was used to identify indicator OTUs for the sampling time points. Indicator OTUs are defined as OTUs that are more relatively abundant and frequent (Dufrêne and Legendre 1997) at a specific sampling time point.

### **3. Results**

#### **3.1. Inter-seasonal fungal microbial diversity**

ARISA profiling showed a total of 367 OTUs across seasons, of which 181 were common to both seasons, 110 were distinct to winter and 76 to summer (Fig. 2a). There were no differences in richness (alpha-diversity) between winter (107.2 (mean)  $\pm$  9.3 (sd)) and summer (92.7  $\pm$

10.1) (Student's t-test,  $P > 0.05$ ). Similar results were obtained with the analysis performed using ITS sequencing data (winter:  $113.8 \pm 39.6$ , summer:  $95.2 \pm 28.8$ ) (Student's t-test,  $P > 0.05$ ). A non-metric multidimensional scaling (NMDS) analysis revealed differences in fungal community composition for both rhizosphere and peel samples in both seasons (Fig. 2b), which was corroborated using PERMANOVA analysis (Table 1,  $P < 0.001$ ).



**Figure 2:** (a) Venn diagram generated from ITS sequencing data illustrating communities that are shared between seasons (b) Non-metric multidimensional (NMDS) scaling ordination plot showing seasonal variation of fungal communities from ARISA profiles. Three samples per sampling point were presented on the non-metric multidimensional (NMDS) ordination plot, each representing an average of four samples. ▲ represents winter communities, ■ represents summer communities. .

**Table 1:** Beta diversity analysis of soil fungal communities at different plant growth stages using PERMANOVA based on Bray-Curtis similarity matrices derived from ARISA profiles.

Treatment combination	Season	
	Winter	Summer
Overall	19.534 * (0.001) **	15.387 (0.001)
Tuber initiation vs. flowering	4.047 (0.001)	5.618 (0.001)
Tuber initiation vs. senescence	13.307 (0.001)	14.261 (0.001)
Tuber initiation vs. potato peels	28.256 (0.001)	29.800 (0.001)
Flowering vs. senescence	10.695 (0.001)	4.529 (0.001)
Flowering vs. potato peels	29.237 (0.001)	18.570 (0.001)
Senescence vs. potato peels	20.530 (0.001)	21.424 (0.001)

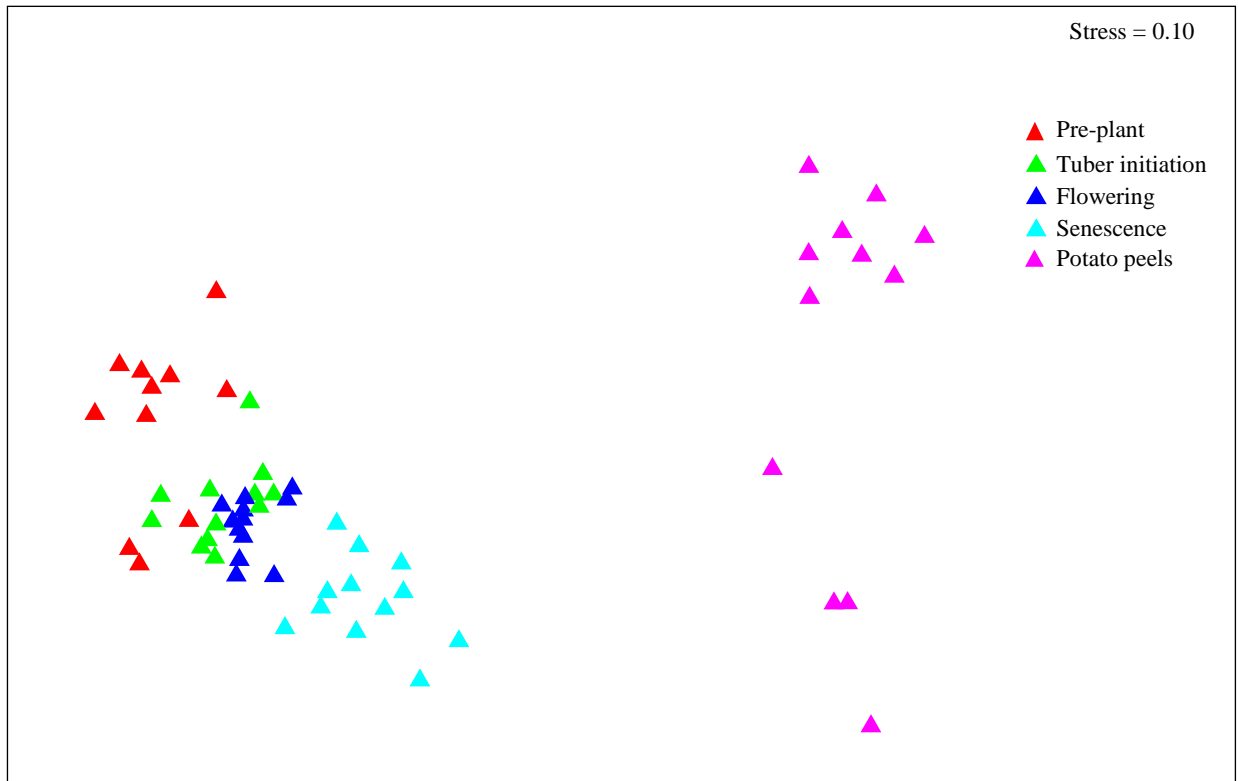
\*F ratio

\*\*P value

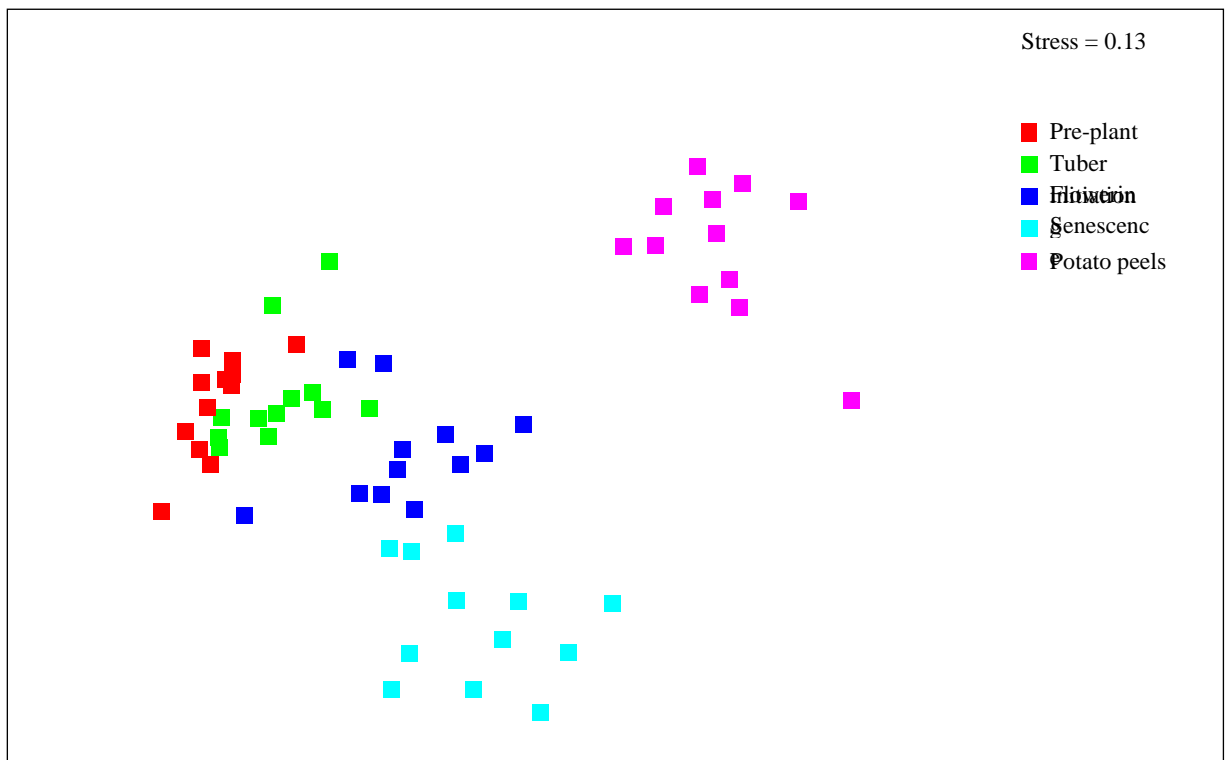
### 3.2. Within-seasonal fungal microbial diversity

Fungal community structure and composition differed between sampling time points in winter (PERMANOVA,  $P < 0.001$ ) (Table 1). A developmental trend was noticed within the communities (Fig. 3a, b). Rhizosphere communities gradually diverged from pre-plant communities and substantial changes occurred between pre-plant and peel communities. The patterns were similar in the summer season (PERMANOVA,  $P < 0.001$ ). Separation between bulk soil and rhizosphere communities at each sampling stage was also significant in both seasons (PERMANOVA,  $P < 0.001$ ), but it was more apparent in winter than in summer (Supplementary Fig. 1).

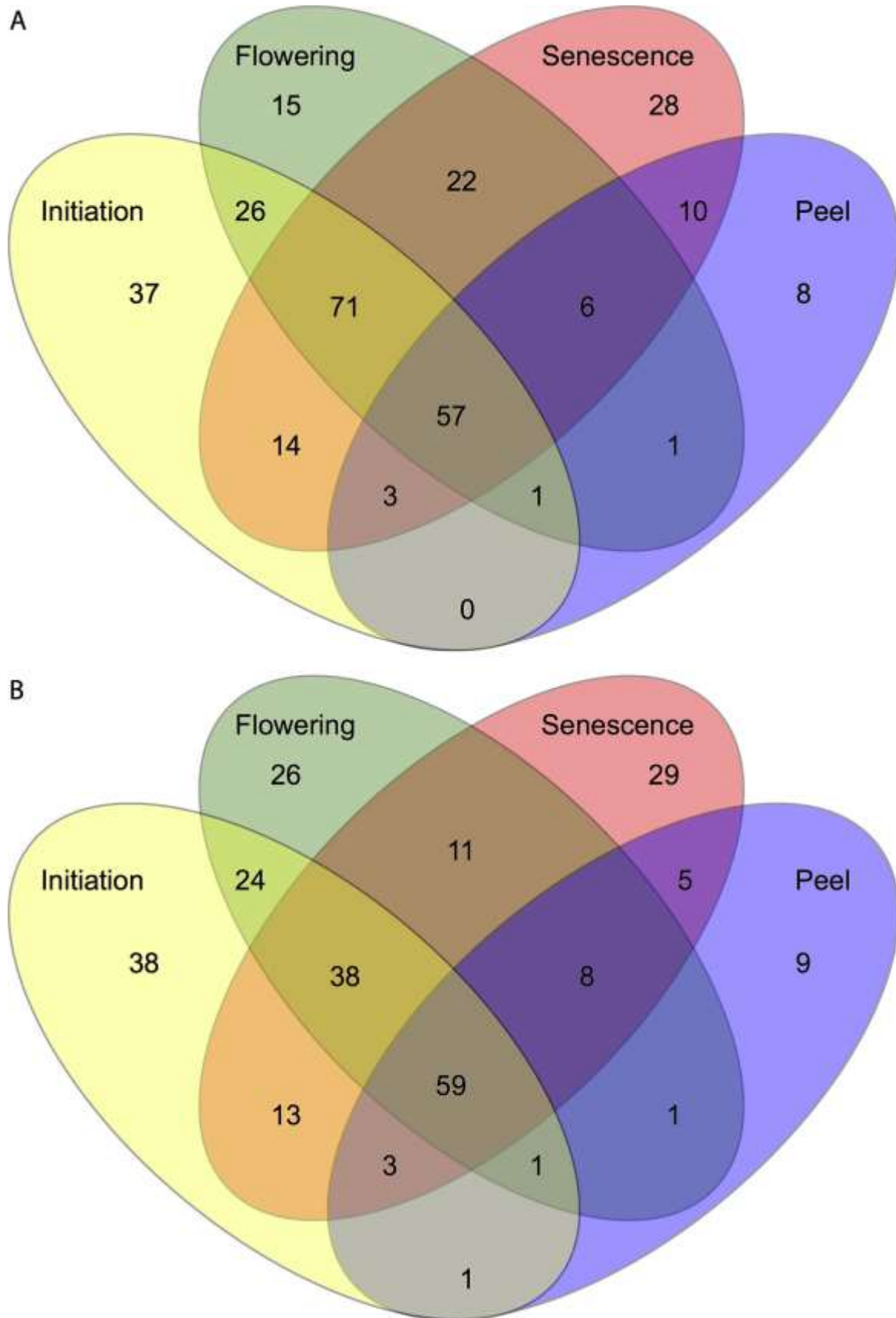
**a**



**b**



**Figure 3:** Non-metric multidimensional scaling (NMDS) ordination plot generated from ARISA derived profiles for fungal communities at different sampling points in (a) winter (b) summer.



**Figure 4:** Venn diagram from ITS gene sequencing showing rhizospheric fungal communities shared between different stages of plant growth in (a) winter and (b) summer.

Venn diagrams constructed from MiSeq-derived ITS fragments of pooled rhizospheric and peel samples indicated that 57 and 59 OTUs were shared amongst all samples in winter and summer, respectively (Fig. 4a, b). The highest number of OTUs was shared between senescence and peel samples in both seasons (76 and 75 OTUs in winter and summer, respectively). The lowest number of OTUs (61 in winter and 64 in summer) was common between tuber initiation and peels. Overall, the tuber initiation stage in both seasons had the highest number of distinct OTUs whilst peel samples scored the fewest distinct OTUs (Fig. 4a, b). There was a general decline in total number of OTUs in both seasons in the order of tuber initiation > flowering > senescence > peels (Fig. 4a, b), although the differences were not statistically significant (Kruskal-Wallis test,  $P > 0.05$ ).

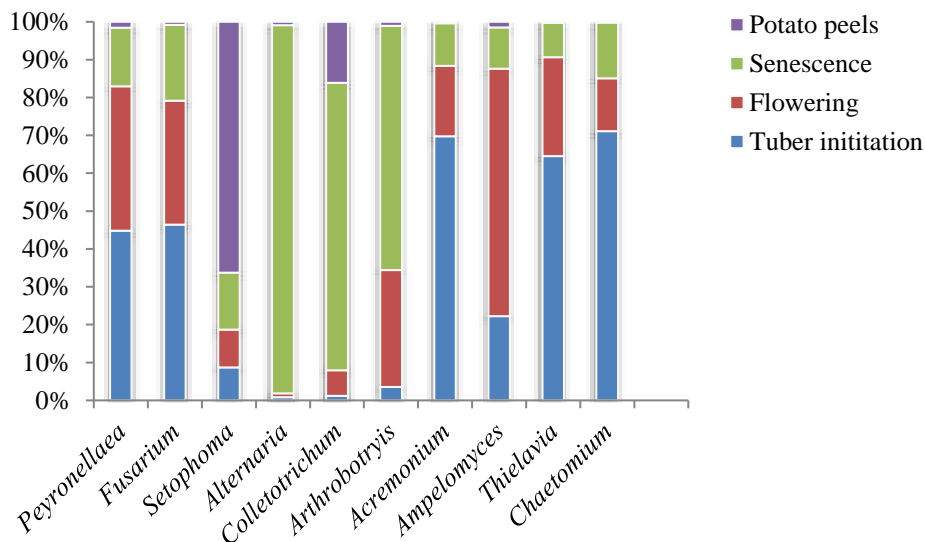
### **3.3. Fungal community composition as revealed by ITS sequencing**

A total of 602 943 unique ITS sequences were recorded after quality control and these were classified into 375 OTUs. The most dominant phylum was *Ascomycota* (63 % of total reads) followed by *Basidiomycota* (15 %), *Zygomycota* (4 %) and *Chytridiomycota* (2 %). Unclassified OTUs constituted 16 % of the total. The genus *Peyronellaea* (*Ascomycota*) was the most abundant in both seasons (Supplementary Fig. 4). Thirty-three indicator OTUs were identified (Supplementary Table 1), of which 29 belonged to the *Ascomycota*, 2 to the *Basidiomycota* and 2 to the *Zygomycota*. An overall assessment showed that five (*Peyronellaea glomerata*, *Fusarium equiseti*, *Acremonium persicinum*, *Thielavia terricola* and *Chaetomium globosum*) of the ten most abundant species detected were indicator species at tuber initiation and their numbers declined successively with plant age (Table 2). *Alternaria*, *Colletotrichum* and *Arthrobotryx* species were more abundant and predominantly found at senescence. An OTU belonging to the genus *Setophoma* was the only indicator OTU of peel samples (Fig. 5).

**Table 2:** Relative abundance of the ten most dominant indicator OTUs at each sampling time point in winter and summer

OTU	Taxonomy	Winter (%)				Summer (%)			
		TI	FL	SS	PP	TI	FL	SS	PP
2	<i>Peyronellaea glomerata</i>	44.5	40.0	13.3	2.1	45.2	36.1	17.8	1.0
5	<i>Fusarium equiseti</i>	37.7	39.6	21.8	0.8	53.4	27.7	18.1	0.8
6	<i>Setophoma terrestris</i>	15.4	15.1	23.6	46.1	2.2	5.2	7.2	85.4
7	<i>Alternaria solani</i>	0.5	0.6	91.2	7.8	21.3	18.8	25.0	35.0
13	<i>Colletotrichum coccodes</i>	1.1	2.7	77.9	18.4	37.1	21.7	67.4	7.3
27	<i>Arthrobotryis oligospora</i>	2.0	38.2	59.6	0.2	4.9	24.2	69.0	1.8
32	<i>Acremonium persicinum</i>	36.4	9.1	45.5	9.1	70.8	18.8	10.3	0.1
33	<i>Ampelomyces</i> sp.	6.6	84.2	7.4	1.9	38.0	46.4	14.6	1.1
34	<i>Thielavia terricola</i>	56.6	27.9	15.5	0	66.2	25.8	7.7	0.3
43	<i>Chaetomium globosum</i>	61.8	17.7	20.6	0	74.3	12.7	12.2	0.3

Abbreviations: TI - Tuber initiation; FL - Flowering; SS - Senescence; PP - Potato peels.  
 Sampling time points in each season are presented in rows for each fungal species.



**Figure 5:** Mean relative abundance for two seasons of the ten most dominant indicator OTUs at each sampling time point

#### 4. Discussion

The objective of the current study was to determine rhizosphere fungal community dynamics at different stages of potato plant growth across two seasons in arable land in subtropical conditions. The microbial community data revealed that fungal richness was stable over time

within and across the two seasons, similar to findings in previous studies performed on crops other than potato (Santos-Gonzalez et al. 2007; Pereira e Silva et al. 2012; Barnard et al. 2013). The lack of variation in fungal diversity between seasons may indicate resistance of fungal mycelia to desiccation (Barnard et al. 2013). Fungal diversity between seasons in most arable soils is also influenced by agronomic practices particularly monocropping and tillage. Previous studies have indicated a reduction in fungal diversity in plots under monocropping due to reduced organic matter content (Manici and Caputo 2009). Fields under monoculture resulted in a build up of fungal pathogens compared to rotation plots (Ding et al. 2018). This could probably explain our findings because trials were established on plots where the previous crop was also potato. Conventional tillage, on the other hand, was shown to reduce fungal diversity in soil due to disruption of soil structure and fungal mycelia (Wang et al. 2017).

This study revealed that the rhizosphere fungal communities were different from pre-plant communities and that potato plants recruited specific fungi at different plant growth stages (Fig 3a, b). Similar findings have been reported for other plant species such as *Medicago truncatula* (Mougel et al. 2006) and soybean (Wang et al. 2009). This scenario can be explained by changes in plant physiology and root architecture as the plant develops. Plant development results in shifts in composition and amount of root exudates with subsequent influence on the root microbiome (Ryan and Delhaize 2001; Ferreira et al. 2008; Berg and Smalla 2009; Gschwendtner et al. 2011). However, we noted that in winter bulk soil fungal communities remained relatively constant (Supplementary Fig. 1), whereas in summer these communities evolved concomitantly with rhizosphere communities. Despite these differences, there was an overlap between fungal communities in winter and summer (Fig 2b) and amongst all sampling points in the two seasons (Fig 4a, b), illustrating a progressive transformation of communities between plant growth stages (Chaparro et al. 2014). The differentiation process is gradual, with some communities persisting in the rhizosphere of a plant species throughout the plant growth



phases. The OTUs that consistently associate with a given plant species are considered to represent the core microbiome of that plant species (Lundberg et al. 2012) and may have co-evolved with the plant. It has been postulated that the core species are critical for plant health and productivity (Cúcio et al. 2016).

Our results confirmed previous findings that reported prevalence of *Ascomycota* and *Basidiomycota* in potato fields (Manici and Caputo 2009). *Ascomycota* are significant in arable land as they effectively decompose holocellulose. For instance, members of the Chaetomiaceae and the Nectriaceae are known for their ability to decompose cellulolytic substrates (Lumley et al. 2000; Berlemont et al. 2014). The Tremelomycetes class belonging to the phylum *Basidiomycota* was dominant in the plant senescence stages in this study. This phylum prefers organic polymers high in lignin content, typical of forest ecosystems (Moll et al. 2016). The *Zygomycota* observed at tuber initiation in this study included *Rhizopus* spp. and *Mortierella* spp., which are possibly generalists that survive on the readily accessible soluble root exudates of the seedlings (Chaparro et al. 2014; Yadav et al. 2015), although some form mycorrhizal associations with plants. *Peyronellaea*, a section of the genus *Phoma* (Aveskamp et al. 2009), was the most abundant OTU across all sampling time points and seasons. Together with *Acremonium*, *Chaetomium*, *Fusarium* and *Thielavia*, this genus was an indicator organism of the tuber initiation stage and the relative abundances gradually declined with plant age. This may explain the overall drop in OTU richness from tuber initiation to the surfaces of potato tubers.

Several of these OTUs represent fungal pathogens of agricultural crops. For example, *Peyronellaea glomerata*, initially isolated and characterised from potato tubers by Wollenweber and Hochapfel in 1936 (Chohan and Chand 1980), has been reported to cause diseases on potato tubers and leaves and is pathogenic on several plants (Kranz 1962; Chohan and Chand 1980; Ligoixigakis et al. 2013; Yang et al 2016). Other notable pathogens of potato

identified in this study were *Fusarium* species responsible for causing dry rot, *Alternaria solani* (early blight), *Colletotrichum coccodes* (black dot) and *Rhizoctonia solani* (stem canker, black scurf, tuber malformation, cracking and elephant hide) (Fiers et al. 2012). Most of the cognate symptoms of these organisms, except black dot, were observed on harvested tubers (data not shown) and the pathogens were detected in peel samples.

Most fungal species have saprophytic lifestyles in soil and are important in decomposition of agricultural residues in arable soils, thereby promoting nutrient cycling (Manici and Caputo 2009). In this study, fungal saprophytes such as *Arthrotrrys*, *Acremonium*, *Thielavia* and *Chaetomium* were identified amongst the top ten indicator OTUs. The prevalence of *Acremonium*, *Thielavia* and *Chaetomium* at tuber initiation may have coincided with decomposition of mother tubers, reported to attract saprophytic fungi (Ferreira et al. 2008). Conversely, the feeding preference of saprophytes may change with some becoming predaceous when prey is available (Yang et al. 2011). *Arthrotrrys oligospora*, an indicator OTU found at plant senescence, is one example of a saprophyte that can also be nematophagous, using adhesive trapping devices (Yang et al. 2011). The nematode-induced symptoms noticed on potato tubers at plant harvest (data not shown) may suggest that this fungus was feeding on nematodes. The observation supports claims that plant roots recruit beneficial microbes later in plant growth to combat plant pests (Bonkowski et al. 2009).

*Chaetomium globosum* and *Ampelomyces* spp. are known fungal biocontrol agents and were mostly found in rhizosphere samples. *Chaetomium globosum* is reportedly antagonistic to several soil-borne fungal pathogens, including *Pythium ultimum* (Pietro et al. 1992), *Phytophthora nicotianae* (Hung et al. 2015) and *Rhizoctonia solani* (Sriraj et al. 2014). *Rhizoctonia* spp. were detected in our samples (see above), but in low abundance, and we suggest that its population was possibly limited by fungal biocontrol agents. *Ampelomyces quisqualis* is a biocontrol agent of powdery mildew-causing fungi (Kiss et al. 2004).

## **5. Conclusion**

In conclusion, our study has revealed significant temporal heterogeneities in fungal community composition associated with the potato rhizosphere. The rhizosphere fungal communities were different from pre-plant communities and also varied between plant growth stages. Furthermore, the reproducibility of these patterns was confirmed over two seasons, suggesting that plant phenology contributed to the shaping of rhizosphere microbial communities. The study has also confirmed the dominance of *Ascomycota* in the potato rhizosphere, with an OTU classified as *Peyronellaea* being the most prevalent indicator OTU. The ten most abundant OTUs, found in almost equal proportions, were classified as plant pathogens, saprophytes and biological control agents. We suggest that this observation is indicative of an important ecological principle; that the dominant taxa recruited to the host plant rhizosphere all play important functional roles, both potentially positive and negative, but that none of the dominant taxa are 'ecological passengers'.

## **6. Acknowledgements**

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