Eesti Maaülikool
Estonian University of Life Sciences

RESPONSE OF ROOT FUNGAL COMMUNITIES TO FERTILISATION, CROP SPECIES AND CULTIVAR

VÄETAMISE, PÕLLUKULTUURI NING SORDI MÕJU TAIMEJUURTE SEENTE KOOSLUSTELE

LIINA SOONVALD

A thesis for applying for the degree of
Doctor of Philosophy in Agricultural Sciences

Väitekiri
filosoofiadoktori kraadi taotlemiseks
põllumajanduse erialal

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers, referred to by their Roman numerals in the text. All papers are reproduced with the kind permission of the publishers.

I   **Soonvald, L.**, Loit, K., Runno-Paurson, E., Astover, A., Tedersoo, L. 2019. The role of long-term mineral and organic fertilisation treatment in changing pathogen and symbiont community composition in soil. Applied Soil Ecology 141, 45–53. https://doi.org/10.1016/j.apsoil.2019.05.003

II   **Soonvald, L.**, Loit, K., Runno-Paurson, E., Astover, A., Tedersoo, L. 2020. Characterising the effect of crop species and fertilisation treatment on root fungal communities. Scientific Reports 10: 18741 https://doi.org/10.1038/s41598-020-74952-7

III   Loit, K., **Soonvald, L.**, Astover, A., Runno-Paurson, E., Öpik, M., Tedersoo, L. 2020. Temporal and cultivar-specific effects on potato root and soil fungal diversity. Agronomy-Basel 10: 1535 https://doi.org/10.3390/agronomy10101535

Table 1. Authors' contributions to each paper

Paper	Idea and study design	Sample collection, analysis of samples	Data analysis	Manuscript writing
I	**LS**, KL, ERP	**LS**, KL, AA	**LS**, KL	**LS**, KL, ERP, AA, LT
II	**LS**, KL	**LS**, KL, AA	**LS**, KL	**LS**, KL, ERP, AA, LT
III	KL, **LS**, ERP, MÖ, LT	KL, **LS**, ERP	KL, **LS**, AA, ERP, MÖ, LT	**LS**, KL, ERP, AA, LT

AA – Alar Astover; ERP – Eve Runno-Paurson; KL – Kaire Loit; **LS** – Liina Soonvald; LT – Leho Tedersoo; MÖ – Maarja Öpik

ABBREVIATIONS

AMF . . . .   Arbuscular mycorrhizal fungi
AICc . . . .   Akaike corrected value
AOF . . . .   Alternative organic fertilisation
$C_{organic}$ . . . . .   Organic carbon
DNA . . . . . .   Deoxyribonucleic acid
DistLM . . . .   Distance-based linear modelling
FYM . . . . . .   Mineral nitrogen fertilisation combined with farmyard manure amendment
GLM . . . . . .   General linear model
IOSDV . . . .   (in German: Der internationale organische Stickstoff-dauerdüngungsversuch) International Organic Nitrogen Long-term Fertilisation Experiment
K . . . .   Potassium
LMER . . . . .   Linear mixed-effect model
N . . . .   Nitrogen
N_{total} . . . . .   Total nitrogen
OTU . . . .   Operational transcriptional unit
$P_{available}$ . . . . .   Available phosphorus
PCR . . . .   Polymerase chain reaction
PERMANOVA   Permutational multivariate analysis of variance
WOM . . . . . .   Mineral nitrogen fertilisation without organic manure

1. INTRODUCTION

In her book "Silent Spring", Rachel Carson wrote the following: "Nature has introduced great variety into the landscape, but man has displayed a passion for simplifying it" (Carson, 1962, p 27). Almost 60 years after the book's publication, the observation still remains valid. For instance, the conversion of natural land for agricultural use has a long-lasting effect on ecosystems (Foley et al., 2005; Vitousek et al., 2008). The use of chemical fertilisers and pesticides has contributed to the decline of soil health and biodiversity (Carvalho, 2017; Foley et al., 2005; Tilman et al., 2001). However, agriculture is the primary food source in the world. As the human population increases steadily, intensification of agricultural production is inevitable. Thus, there is an ongoing need for further research on sustainable agriculture.

Soil is a habitat with a huge microbial diversity, and one gram of it comprises millions of microorganisms (Van Der Heijden et al., 2008). Furthermore, microorganisms establish complex genetic, biochemical, physical and metabolic interactions with plants (Trivedi et al., 2020). In addition, plant-associated microbes can play an important role in sustainable agriculture (Bender et al., 2016). There is a clear indication that certain microbes can promote plant growth, and improve nutrient uptake and pathogen resistance (Backer et al., 2018; Berendsen et al., 2012). For instance, the bacterial genus *Rhizobium* is frequently used in agriculture due to its ability to fix nitrogen in leguminous plants (Mus et al., 2016), whereas arbuscular mycorrhizal fungi (AMF) are applied to improve plant mineral nutrition and increase tolerance against abiotic stress (Begum et al., 2019). However, several microorganisms can also cause diseases in plants (Agrios, 2005). For example, the most common pathogens that have caused substantial yield losses globally in recent years have been ascomycete fungi of the genus *Zymoseptoria* and basidiomycete fungi of the genus *Puccinia* on cereals, and the oomycete *Phytophtora infestans* on potato (Figueroa et al., 2018; Kamoun et al., 2015). In addition, there are still multiple unknown microorganisms living in the soil whose function remains unclear. Studying the microbiome inside plant roots may enable us to find new beneficial and pathogenic microorganisms and contribute to our understanding on how we could benefit from microbial interactions accordingly.

Over the last few years, an increasing number of studies have examined plant–microbial interactions in crops. However, most of them have focused on bacterial communities (Backer et al., 2018; Bulgarelli et al., 2015; Edwards et al., 2015) and have given relatively less attention to fungal interactions. Moreover, there is a shortage of studies investigating root fungal communities in response to agricultural management practices (Banerjee et al., 2019). The root microbiome is less diverse than the soil microbiome, implying an importance of host-specific factors in establishing the root microbiome (Trivedi et al., 2020). Therefore, it can be speculated that within the plant roots, microbes, including fungi, utilise a different life strategy compared to soil microbes. Thus, most likely, these microbes also respond differently to various disturbances, such as agricultural management practices.

Most studies on fungal communities in agricultural soils have been conducted in East Asia, whereas information about Europe and North America in this context is poorly represented in the literature. Furthermore, we have contradictory knowledge of how agricultural management practices affect fungal communities. For example, measurements conducted in 12 long-term experiments revealed that the fertilisation treatment on fungal communities depends on site and soil conditions, as well as on crop species (Hannula et al., 2021). The current thesis examines the effect of soil properties and fertilisation treatment on root fungal communities. As host-plant identity and its interaction with fungal communities may provide valuable insights into plant performance, this thesis also reports the effects of crop species and cultivar on root fungal communities. Furthermore, in addition to the overall mycobiome, we study the response of two fungal guilds: pathogens and mutualists (arbuscular mycorrhizal fungi). This allows a more in-depth understanding of the processes regulating the assembly and functionality of the plant-associated mycobiome. The knowledge obtained in this thesis could provide a valuable contribution as part of a novel approach to integrated disease management and soil quality assessment to enhance agricultural productivity with minimal harm to ecosystems.

2. LITERATURE OVERVIEW

Soil plays a fundamental role in ecosystem processes such as nutrient cycling, organic matter decomposition, water regulation, and control of pests and diseases (Smith et al., 2015). However, due to inappropriate agricultural management practices, about one third of global soils are degraded, and some of the best agricultural lands are threatened by erosion, loss of nutrients and salinisation (FAO, 2018). Impoverished soil has a reduced capacity to support biodiversity and the growth of crops.

During the last century, most research on soil focused on its physical and chemical properties rather than its biology (Sherwood and Uphoff, 2000). However, in recent years, the emphasis has started shifting to soil microbial organisms due to their significant contribution to ecosystem services such as increasing soil nutrient availability, defending against plant diseases and improving plant health (Bender et al., 2016; Mendes et al., 2013; Philippot et al., 2013). Some research does even not consider plants as independent entities, as the microbes inside and outside the plants are related by direct or indirect mechanisms to primary functions such as plant growth and health (Vandenkoornhuyse et al., 2015). Moreover, molecular methods for studying microorganisms have become cheaper and more accessible (Llaca, 2012). In particular, high-throughput sequencing has enabled a large-scale analysis of microbial communities (Zhou et al., 2015).

2.1. The importance of soil fungi

Fungi play a key role in soil ecology (Agrios, 2005; De Boer et al., 2005) by decomposing organic matter, mediating nutrient cycling and stabilising soil aggregates (reviewed by Bardgett and Van Der Putten, 2014 and Bender et al., 2016). Moreover, fungi include plant mutualistic and plant pathogenic taxa besides saprotrophs (Agrios, 2005; Smith and Read, 2008). Fungal abundance and diversity are strongly affected by climatic, edaphic and biotic conditions (Tedersoo et al., 2014) (Figure 1). Further research is required to study soil and root fungal communities in agricultural systems. In agroecosystems, where anthropogenic factors are important, different agricultural management practices can place selection pressure on fungi (Verbruggen and Kiers, 2010). It is also important to study the effects of plant species and their cultivars on fungal communities, as species-specific genetic factors can determine the rhizosphere as well as the root microbiome (Bulgarelli et al., 2013).
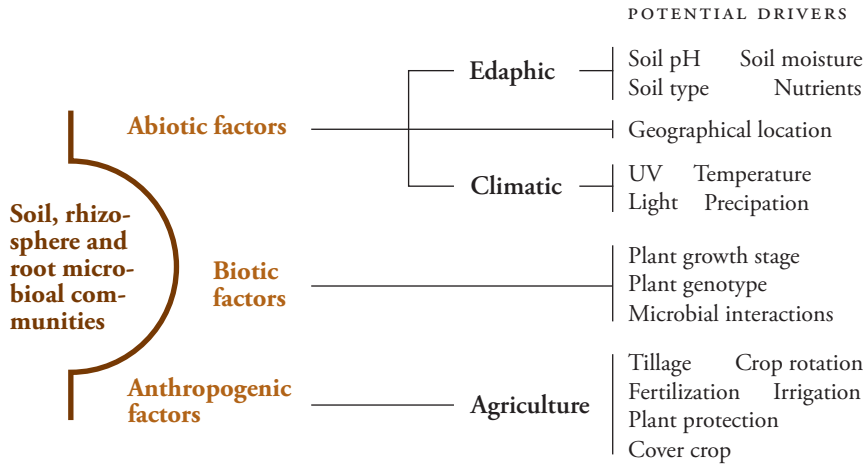
Figure 1. Potential drivers of root, rhizosphere and soil fungal communities.

2.2. The effect of soil properties on fungal communities

A range of soil characteristics can cause variation in soil and root fungal community structure. Soil nitrogen can shift the fungal communities directly (changing nutrient availability) and indirectly (changing plant community composition) (Egerton-Warburton et al., 2007; Valliere et al., 2017). Nitrogen was the strongest predictor among soil properties in determining the soil fungal community composition in a long-term field experiment (Hartmann et al., 2015); however, it explained only 6.4% of the total variation. Furthermore, soil fungal diversity has been shown to decrease with increasing nitrogen inputs, but with the fungal richness remaining unchanged (Zhou et al., 2016). Previous studies have also shown the effects of soil nitrogen and phosphorus on plant-beneficial AMF communities. High soil phosphorus content is shown to reduce AMF root colonisation, whereas colonisation increases under moderate soil phosphorus levels (Liu et al., 2016). Similarly, nitrogen and phosphorus additions enhance AMF biomass in nitrogen- and phosphorus-limited sites (Treseder and Allen, 2002). A large-scale study on an agricultural field in Switzerland demonstrated changes in root AMF community composition in response to soil pH changes (Jansa et al., 2014). Rousk et al. (2010) observed that certain taxonomic orders changed their abundance in response to changes in soil pH; however, these differences were

more pronounced for bacteria. Nearly all the above-mentioned studies have been conducted on different soil types, but soil type and land-use history can be important drivers determining fungal community structure (Schlatter et al., 2020; Simonin et al., 2020). For example, soil type has been reported as a significant driver in determining fungal rhizosphere richness and phylogenetic diversity in wheat fields (Simonin et al., 2020). Fungal community composition also responds to soil moisture, as rhizosphere fungal diversity has been shown to increase with decreasing soil water content (Azarbad et al., 2018, 2021).

2.3. The effect of fertilisation on fungal communities

Generally, there are three types of fertilisers: synthetic, organic and combined. All these fertilisation treatments can significantly influence belowground fungal communities (Francioli et al., 2016; Hartman et al., 2018; Hartmann et al., 2015; Wang et al., 2017; Zhou et al., 2016). Lupatini et al. (2017) suggested that mineral fertilisation reduces microbial competition and promotes the dominance of a small number of microorganisms. This assumption is supported by Banerjee et al. (2019), who revealed a reduction in fungal network complexity and a decrease in keystone taxa after mineral fertilisation. On the contrary, both manure- and plant-based organic fertilisation may increase fungal biomass and diversity (García-Gil et al., 2000; Heijboer et al., 2016; Sun et al., 2016). García-Gil et al. (2000) suggested that the organic matter can act as an additional fungal substrate, promoting fungal diversity. Xiang et al. (2020) confirmed this by demonstrating an increase in less abundant fungal phyla after manure application.

After manure amendment, a decline in pathogen infection rate is presumed, as plant pathogens must compete with the increased amount of resident microorganisms (Garbeva et al., 2004). An experiment conducted by Bonanomi et al. (2017) described increased fungistasis in response to organic matter amendment, which can suppress plant diseases by increasing fungal nutrient deficiency and reducing pathogen residence time in the soil (Garbeva et al., 2011).

2.4. The effect of plant species on fungal communities

Biotic factors may affect soil and root fungal communities. The plant species identity is one such determinant. Studies by Burns et al. (2015), Sweeney et al. (2020) and Leff et al. (2018) demonstrated that plant species

harbour distinct rhizosphere fungal communities. Similar results have also been shown in root fungal communities (Schroeder *et al.,* 2019). Furthermore, Bergelson et al. (2019) studied replicated *Arabidopsis thaliana* accessions and showed that genetic differences in host plants had a substantial effect in shaping root fungal communities. The underlying reasons for changes in fungal communities in response to plant species may be related to plant root exudates. Root exudates are defined by the host genotype and contain several compounds, such as primary (sugars, amino and organic acids) and secondary (terpenes, phenols, alkaloids) metabolites (Vives-Peris et al., 2020). By being a food source for microorganisms or by being involved in signalling and defence mechanisms (Chaparro et al., 2012), root exudates can attract various fungi (Sasse et al. 2018; Vives-Peris et al., 2020). Microorganisms use plant-released root border cells and root cap mucilage for nutrients, and these all differ between plant species (Badri and Vivanco, 2009; Hawes et al., 2000; Hu et al., 2018, Sasse et al. 2018).

The differences in root and rhizosphere fungal communities may also rely on root traits. The plasticity of root traits can create ecological niches for diverse bacterial and fungal species and affect their functions (Saleem et al., 2018). Knowing the root mycobiome and its relation to crop species may also improve our understanding of how different root traits influence fungal interactions and structure their environment. Sweeney et al. (2020) examined 21 grassland species and concluded the importance of root diameter, root nitrogen content and specific root length on rhizosphere fungal composition. Furthermore, their results indicated that rhizosphere fungal communities are determined by interspecific trait variation compared to intraspecific trait plasticity. Francioli et al. (2020) demonstrated that saprotrophic root fungal community composition was driven by root lignin content and C:N ratio, whereas Eissenstat et al. (2015) showed the importance of root thickness in determining AM colonisation. In general, there is a lack of knowledge concerning the study of the effect of crop species on root and rhizosphere fungal communities. Several of the studies mentioned above have been conducted on grassland or forest species (Barberán et al., 2015; Bergelson et al., 2019; Burns et al., 2015; Francioli et al., 2020; Leff et al., 2018; Schroeder et al., 2019; Sweeney et al., 2020).

2.5. The effect of crop cultivar on fungal communities

Not only plant species but also crop cultivars can influence root-associated microbial communities. Modern plant breeding is usually conducted with increased external inputs (Pérez-Jaramillo et al., 2018, 2016). Therefore, in this context, plant–microbe interactions that benefit plants may become redundant since nutrients become freely available in the soil (Porter and Sachs, 2020). For example, An et al. (2010) compared the variation of AMF colonisation among maize cultivars and demonstrated the importance of cultivar origin and release date on AMF colonisation. These results imply that crop breeding history can be an influential determinant in plant–fungal interactions.

Agricultural crop breeding targets only a few genetic markers, meaning that cultivars are genetically similar, whereas in natural ecosystems, the host population comprises genetically diverse individuals (Möller and Stuckenbrock, 2017). This, in turn, can impact pathogenic interactions. A diverse host population can decrease disease prevalence through antagonistic host–pathogen coevolution (Möller and Stuckenbrock, 2017). This, however, requires continually evolving genetic variation in both partners (Möller and Stuckenbrock, 2017).

3. AIMS AND HYPOTHESES OF THE STUDY

This research provides valuable information on how different fungal guilds inhabit the roots of common crops and explains their response to different agricultural management practices. This knowledge can contribute to the development of sustainable agriculture. In the first paper, the study included both fungi and the oomycetes. The oomycetes are fungus-like microorganisms, which are known to include a large number of plant pathogens. By using high-throughput sequencing, we expand our knowledge on both plant pathogens and mutualists. This expertise is important for both scientists and farmers. Knowing local fungal communities allows us to exploit them to our advantage and offers a promising strategy in improving soil quality and plant productivity. Modern crop production needs to devise new solutions that take advantage of the benefits of microbiota instead of disrupting it. For example, beneficial fungi may be used in practice in protecting host plants against various environmental stress. However, without a fundamental understanding of how fungi respond to different agricultural practices on a local scale, it is not possible to develop these microbiome-based, sustainable agricultural practices. Furthermore, information about fungal community structure can provide an indication of the soil quality of the field. For example, species richness can indicate healthy soil, as diverse fungal species play an essential role in soil ecological processes related to plant growth and stress resistance, whereas the pathogen community structure can indicate potential threats for the field site.

The main aims of this thesis are as follows:
1) To determine the effect of soil properties on root fungal communities (Studies **I**, **II**).
2) To characterise root fungal richness and community composition under different fertilisation treatments (Studies **I**, **II**).
3) To compare the root fungal community diversity and community composition across different crop species (Study **II**).
4) To describe the root fungal community richness and community composition across 21 potato cultivars (Study **III**).

The hypotheses of the study were:
1) Soil properties have a significant impact on root fungal community composition.

2) Manure amendment and alternative organic fertilisation treatment increase overall fungal and AMF richness but decrease pathogen richness.
3) All fertilisation treatments have distinct fungal community composition.
4) Root fungal richness and diversity (both overall fungal richness, as well as AMF and pathogen richness) are different between wheat, barley and potato.
5) The root fungal richness and community composition differ between the studied potato cultivars.

4. MATERIAL AND METHODS

4.1. Experimental sites

The studies in this thesis addressed the effect of fertilisation treatment and crop species on root fungal communities were conducted on the IOSDV (International Organic Nitrogen Long-term Fertilisation Experiment) experimental site in Tartu, Estonia (58°22'30.0"N, 26°39'48.0"E, **I**, **II**). The soil at the experimental site is *Fragic Glossic Retisol* associated with *Stagnic Luvisol* (IUSS WG WRB 2015), with a sandy loam texture. The IOSDV experimental site (**I**, **II**) was arranged in a split-block design, with three replicates (Figure 2A). The crop species grown in rotation were potato (cultivar 'Manitou'), spring wheat (cultivar 'Vinjett') and spring barley (cultivar 'Anni'). The fertilisation treatments included mineral nitrogen fertilisation (WOM), mineral nitrogen fertilisation combined with farmyard manure 40 t ha^{-1} (FYM) and alternative organic fertilisation (AOF). Five nitrogen fertiliser application rates were used: 0, 40, 80, 120 and 160 kg ha^{-1}. Five alternative organic fertiliser application rates were also used: N0, PS (pulp sludge compost used in 2008–2011),

Figure 2. Experimental design of the IOSDV (A) and Reola (B) field sites.

C-200, C-275, and C-350. The latter three alternative fertiliser rates are waste compost separated from food and green waste, as well as category III animal by-products corresponding to three application rates with N totals of 200, 275 and 350 kg ha^{-1}, respectively.

The study assessing cultivar-specific effects on potato root fungal communities was conducted in Reola, Estonia (58°17'02.0"N, 26°43'19.6"E, **III**). The Reola experimental site (**III**) was arranged in a randomised block design with three replicate plots per cultivar (Figure 2B). The dominant soil here is *Stagnic Luvisol* (IUSS WG WRB 2015), with a sandy loam texture. The 21 commercial potato cultivars studied were 'Antonia', 'Arielle', 'Bellefleur', 'Catania', 'Concordia', 'Champion', 'Erika', 'Esmee', 'Excellency', 'Fontane', 'Glorietta', 'Karlena', 'Laudine', 'Madeleine', 'Manitou', 'Mariska', 'Merlot', 'Ranomi', 'Rosagold', 'Solist' and 'Viviana'. Root samples were collected in 2014 during the plant flowering stage (BBCH60) (**I**, **II**, **III**) and at the plant senescence stage (BBCH95) (**III**).

For these analyses, the entire root systems of three randomly chosen plants were collected from the 10–15 cm topsoil layer using a clean shovel. The samples for experiment **I** were kept at –20 °C for further analyses. The samples for experiments **II** and **III** were dried at 70 °C for 48 h and stored dry at room temperature until molecular analysis.

4.2. Molecular analysis

DNA was extracted from 0.075 g of roots per plot using the PowerSoil DNA Isolation Kit (MoBio Laboratories Inc, Carlsbad, CA, USA) with minor modifications (**I**, **II**, **III**). In 2014, PCR was performed using three forward primers – ITS1ngs, ITS1Fngs (Oja et al., 2015), ITS1Oo (Riit et al., 2016) – and the reverse primer ITS4ngs (Tedersoo et al., 2014) (**I**). The samples from studies **II** and **III** were analysed using the ITS3-Mix1-5 (Tedersoo et al., 2014) and ITS3Oo (Riit et al., 2016) forward primers and the degenerate reverse primer ITS4ngs (Tedersoo et al., 2014). The detailed PCR protocols and sequencing preparations are described in studies **I**, **II** and **III**. The samples were sequenced either on a PacBio RSII instrument using P6-C4 chemistry at the University of Oslo (**I**) or on an Illumina MiSeq system (2×300 bp) at the Estonian Genome Centre (**II**, **III**).

4.3. Soil chemical analysis

For soil chemical analyses, eight subsamples were collected at a depth of 20 cm from each plot before fertiliser application (**I**, **II**) or during planting (**III**). In spring, all samples were air-dried, sieved to < 2 mm and pooled to obtain composite samples for each plot. Soil chemical analyses were performed to assess the concentrations of total nitrogen (N_{total}), organic carbon ($C_{organic}$), plant-available phosphorus ($P_{available}$), soil potassium (K) and soil pH. The N_{total} was measured using the Kjeldahl method (Reeuwijk, 2002) (**I**, **II**) or the dry combustion method on a varioMAX CNS elemental analyser (ELEMENTAR, Langenselbold, Germany) (**III**). $C_{organic}$ was measured using the Tjurin method (Nikitin, 1999) (**I**, **II**) or the dry combustion method on a varioMAX CNS elemental analyser (**III**). The ammonium lactate method (Egnér et al., 1960) was used to determine the $P_{available}$ and K (**I**, **II**, **III**). Soil pH was measured in 1 M KCl solution (**I**, **II**, **III**).

4.4. Bioinformatics

Bioinformatic analyses were performed using the PipeCraft analysis platform (Anslan et al., 2017) (**I**, **II**). At first, either circular consensus sequences from PacBio RSII data were created using default settings (**I**), or paired-end reads were quality-trimmed and assembled using vsearch v1.1.11 (Rognes et al., 2016) (**II**). In experiment **III**, reads were quality-filtered and assigned to samples using MOTHUR v1.34.4 (Schloss et al., 2009) and after that assembled using PANDAseq Assembler (Masella et al., 2012). This was followed by quality filtering and demultiplexing (Schloss et al., 2009) (**I**, **II**, **III**). In experiment **I**, putative chimeric sequences were removed using UCHIME de novo filtering (Edgar et al., 2011) and reference-based filtering against UNITE reference dataset v7.0 (Abarenkov et al., 2010), whereas in experiment **II**, chimeras were removed using de novo and reference-based (UNITE v7.2) methods as implemented in vsearch (Rognes et al., 2016). In experiment **III**, potential chimeric sequences were removed using USEARCH v7.0.1090 (Edgar, 2010). Flanking rRNA gene regions were removed using ITSx v1.011 (**I**) or ITSx v1.0.9 (**II**, **III**) to extract the full-length ITS (**I**) or ITS2 region (**II**, **III**) (Bengtsson-Palme et al., 2013). The high-quality sequences were clustered into operational taxonomic units (OTUs) at a 98% sequence similarity threshold using the usearch algorithm (Edgar, 2010) (**I**) or at a 97% sequence similarity threshold with CD-Hit v4.6 (Fu et al., 2012)

(**II**, **III**). Singleton OTUs were removed from further analyses (**I**, **II**, **III**). For taxonomic assignment, a representative sequence from each OTU was selected for the BLASTn search against the UNITE (**I**, **II**, **III**) and GenBank (**I**) databases. We conservatively considered e-values < e^{-50} of BLASTn search results and query coverage that was > 50% reliable to assign OTUs taxonomically. The OTU taxonomy was assigned based on the consensus taxonomy assignment if at least eight out of ten BLAST hits agreed on the same taxonomy level (**I**, **II**). In experiment **III**, OTUs with 75.0%, 80.0%, 85.0%, 90.0%, 95.0%, and 97.0% sequence similarity thresholds were considered to represent the phylum, class, order, family, genus and species levels, respectively (Tedersoo et al., 2014).

4.5. Functional assignment

OTUs were classified using FUNGuild (Nguyen et al., 2016) (**II**, **III**) and were based on their species-level identification according to the United States Department of Agriculture, Agricultural Research Service (https://nt.ars-grin.gov/fungaldatabases/) (**I**, **II**, **III**). Oomycetes assigned to the genus level (except *Lagenidium* spp.) (Lévesque, 2011), as well as the fungal genera *Alternaria*, *Fusarium* and *Phoma*, were all considered pathogenic (Aoki et al., 2014; Aveskamp et al., 2008; Thomma, 2003) (**I**). All OTUs belonging to the phylum Glomeromycota were considered as arbuscular mycorrhiza (**I**, **II**, **III**).

4.6. Data analysis

We used two ecological measures – species richness (**I**, **II**, **III**) and the Simpson index of diversity (**II**, **III**) – to study the α-diversity of root-inhabiting fungi. To account for the variation in sequencing depth, species richness was calculated from a linear regression of obtained OTUs and the square root of the number of obtained sequences (Bálint et al., 2016; Tedersoo et al., 2014). In the case of a significant regression, standardised residuals were used as a proxy for taxonomic richness; otherwise, the analyses were carried out with the number of obtained OTUs. The Simpson diversity index was calculated on standardised OTU-by-sample and transformed matrices using Primer7+ software (Clarke and Gorley, 2015). Linear mixed-effects models (LMERs) (**II**) and general linear models (GLMs) (**I**, **III**) were used to test the effect of studied variables on root fungal communities. The tests were followed by Tukey HSD post hoc tests (α = 0.05). For this thesis, additional LMER analyses were carried

out. Firstly, the effects of fertilisation treatment and fertiliser application rate on overall root fungal richness were assessed (**I**). Secondly, the effects of cultivar and sampling time on overall root fungal richness were examined (**III**). All these tests were carried out using Statistica 12.0 (Palo Alto, CA, USA) or R (R Development Team; https://www.R-project.org).

The fungal community composition was analysed using PERMANOVA+ (Anderson et al., 2008) (**II**, **III**). Additionally, PERMANOVA analysis was carried out on the overall root fungal community presented in study **I**. Before calculating the Bray–Curtis similarity index, samples were standardised (by samples), which was followed by square-root transformation. DistLM (McArdle and Anderson, 2001) was performed to estimate the proportion of variance explained in fungal community composition by soil variables (**II**). For this thesis, DistLM was also conducted for the study **I** data matrices. In brief, the environmental variables examined were pH, $C_{organic}$, N_{total}, K, and $P_{available}$. The latter four were log-transformed before DistLM analysis. Models were generated using the BEST procedure, which examines all possible combinations of predictor variables. Before DistLM analysis, overall and pathogen-only fungal data matrices were standardised and square-root transformed, and followed by Bray–Curtis similarity index calculation. DistLM analysis for AMF was calculated on a modified Gower log10 resemblance matrix. The final models were chosen using the corrected Akaike Information Criterion (AICc). P values were calculated with 9999 permutations.

5. RESULTS

5.1. Relationship between root fungal communities and soil properties

DistLM analysis was performed in order to model the relationship of root fungal community composition with environmental variables. The best-fitting model for the overall fungal community composition in study **I** included only soil pH, accounting for 9.6% of the variation. In study **II**, soil pH and $C_{organic}$ were the two variables that explained most of the variation (11.3%) in overall root fungal community composition. The results for root pathogen community composition showed that either N_{total} (**I**) or both N_{total} and soil pH (**II**) best explained this, accounting for 6.2% and 11.0% of the variation, respectively. Among the soil properties, soil pH (10.3%) was the strongest environmental predictor in explaining the variation in AMF community composition in study **I**, whereas in study **II**, only $P_{available}$ (3.7%) was included in the model.

5.2. The effect of fertilisation treatment on root fungal communities

Fertilisation treatment, fertiliser application rate and their interaction had no significant effect on the combined overall root fungal and oomycete richness in potato roots (**I**). Furthermore, fertilisation treatment, as well as fertiliser application rate, did not change the overall root fungal community richness or diversity in study **II**. Changes occurred in species richness when fungal guilds were studied separately. In 2014, pathogen richness was significantly lower in WOM treatment compared to both FYM and AOF treatments (**I**). Similarly, WOM plots harboured decreased pathogen richness compared to FYM plots two years later (**II**). AMF richness was higher under WOM treatment in both studies.

According to PERMANOVA, both fertilisation treatment and fertiliser application rate did not determine the combined overall root fungal and oomycete community composition (**I**). Fertilisation treatment had a marginal effect on root fungal community composition in study **II.** However, fertilisation treatment changed the root pathogen community composition (**I**). In study **II,** fertilisation treatment had a weak effect on both root pathogen and AMF community composition.

5.3. Response of root fungal communities to crop species

Crop species had a significant impact on root fungal richness and diversity (**II**). Of the three crop species, wheat roots harboured the highest fungal richness and diversity, whereas the diversity indices were the lowest in potato roots (**II**). The effect of crop species prevailed over the effect of fertilisation treatment, fertiliser application rate and their interaction (**II**).

Similarly, pathogen richness and diversity were significantly different among the three crop species (**II**); values were the highest in wheat roots and lowest in potato roots. There was no significant difference in the AMF species richness among the studied crops (**II**). However, AMF diversity was significantly different among the crop species (**II**). Potato roots harboured the lowest diversity compared to both wheat and barley. Furthermore, among all crops, AMF richness was highest in potato roots grown in WOM plots, whereas the lowest values were observed in potato roots grown in FYM plots (**II**). In PERMANOVA analysis, crop species explained 36.2% of the variation in root fungal community composition (**II**). Crop species was the main factor in determining the differences in pathogen (40.7%) and AMF (4.7%) community composition (**II**).

5.4. Response of root fungal communities to cultivar

Root fungal richness was significantly affected by cultivar, sampling time and their interaction (**III**). In general, root fungal richness was higher during the flowering stage. The highest fungal richness was observed during the flowering stage in cultivar 'Merlot' roots, whereas the lowest richness was observed in the roots of 'Laudine'. Furthermore, potato cultivar was the main variable determining fungal community composition. Cultivar explained 8.2% and sampling time 5.3% of variation in fungal community composition (**III**).

6. DISCUSSION

6.1. Root fungal communities are affected by soil properties

Soil pH was the main factor determining the overall root fungal community composition, as well as AMF (**I**) and pathogen (**II**) composition, respectively. In general, fungi are considered more tolerant than bacteria to acidic conditions and have a wider pH optimum (Rousk et al., 2010). Fungi are known for their plasticity and, thus, for altering their growth and dispersal in response to changing environmental variables (Slepecky and Stramer, 2009). Moreover, pH can regulate genes that encode essential fungal compounds such as enzymes, permeases, antibiotics or toxins (Peñalva and Arst, 2004). Some of these compounds are related to fungal pathogenicity (Prusky and Yakoby, 2003), which in turn may explain the effect of pH on pathogen community composition. Among soil properties, N_{total} also significantly accounts for the variation in pathogen community composition (**I**, **II**). This may be associated with nitrogen acting as a limiting resource for pathogens (Sun et al., 2020). A sufficient amount of nitrogen in the environment enables pathogens to acquire nitrogen more easily and causes them to thrive. Furthermore, nitrogen is an essential nutrient for plant growth. When plants are suffering from nitrogen deficiency, they are more susceptible to pathogens. Conversely, it may also be that nitrogen availability can elicit plant defence mechanisms (Sun et al., 2020) and consequently affect pathogen communities.

6.2. Root fungal community composition is weakly affected by fertilisation

Fertilisation treatment did not change root fungal richness, diversity or community composition (**I**, **II**). Interestingly, even different fertiliser application rates did not alter root fungal community structure. It may be that the local fungal communities are adapted to the site conditions. This assumption is supported by a study conducted on a neighbouring field (Esmaeilzadeh-Salestani et al., 2021); also, the results of that study revealed a relatively stable soil fungal community structure in response to fertilisation treatment, with the effect of crop rotation prevailing over the effect of other management practices. Similarly, other experiments conducted on field sites with contrasting site conditions have highlighted the importance of site characteristics and the interaction effects of man-

agement and region in determining the response of root fungal structure to chemical and organic fertilisation (Kracmarova et al., 2020; Sternhagen et al., 2020).

Secondly, it may be that root fungal communities are determined to a greater extent by host genotype. In study **II**, the effect of crop species prevailed over the effect of fertilisation treatment in determining fungal diversity and community composition. Fungi colonising plant roots may be more sheltered from the outer environment and, thus, be less responsive to environmental changes. Therefore, it can be speculated that the assembly of fungi inhabiting roots is shaped by the host plant rather than by the environment (Trivedi et al., 2020).

However, fertilisation treatment changed the diversity of fungal guilds; pathogen richness and diversity were higher in FYM plots (**II**). Organic compounds may generate a more eutrophic environment and increase pathogenic fungal genera capable of acting as saprotrophs. In agreement with this, soil analyses showed higher nutrient levels in FYM and AOF plots. The majority of pathogenic sequences obtained in study **I** were assigned to the oomycete genus *Pythium*. With a broad habitat preference, *Pythium* species can change the nutrient acquisition strategy throughout the life cycle (Agrios, 2005). However, higher pathogen richness and diversity do not imply that the plant diseases cause more damage. In general, higher competition for nutrients and habitat may reduce pathogen dominance and lower disease incidence (Abdullah et al., 2017; Vannier et al., 2019). Fertilisation treatment also influenced AMF richness. On the contrary to the second hypothesis, AMF richness and diversity were the lowest in potato roots grown in FYM plots. It may be that in plots with higher soil nutrient levels, plants can acquire nutrients by themselves, and therefore AMF are no longer nutritionally beneficial (Johnson and Gibson, 2021). This is supported by the fact that AMF richness was highest in WOM plots, where nutrient levels were the lowest.

6.3. Root fungal communities differ between potato, wheat and barley

In line with the fourth and fifth hypotheses, the fungal richness and community composition were significantly different among crop species (**II**). The most contrasting community composition was in potato roots, particularly in comparison with wheat (**II**). Fitzpatrick et al. (2018)

proposed that plants may have developed traits responsible for shaping the root microbiome and these traits, in turn, are related to plant phylogenetic history. Closely related species (i.e. cereals) are expected to have more similar root traits (Valverede-Barrantes et al., 2017) and this may underlie the differences in root mycobiome. For example, species belonging to the family *Poaceae* (including wheat and barley) adhere soil physically to plant roots, which is also known as the rhizosheath (Brown et al., 2017; Pang et al., 2017). Consisting mainly of root hairs and mucilage, this may promote higher fungal diversity on the root surface. The contrasting community structure may also be related to differences in plant physiology. For instance, plants release species-specific root exudates. Differing in their quantity and quality, root exudates can promote or inhibit the colonisation of different microbial species (Badri and Vivanco, 2009; Haichar et al., 2008; Knights et al., 2021 and the references therein).

Another explanation may be the differences in root architecture. Potato roots are shallow, penetrate soil poorly (Joshi et al., 2016; Stalham et al., 2007) and exhibit a low capacity to extract minerals and water from the soil (Opena and Porter, 1999). Conversely, the root system in cereals is complex, with many types of branching and greater surface area (Rich and Watt, 2013; Smith and de Smet, 2012). Comparative studies by Yamaguchi et al. (1990) and Yamaguchi (2002) demonstrated that compared to potato, wheat roots have a smaller root diameter, higher root density and higher total root length. Therefore, the more complex root system of cereals may provide more adhesion and niche preferences for fungi. On the other hand, the coarser root morphology of potatoes may increase the presence of AMF taxa that facilitate nutrient uptake from the larger soil surface area (Comas et al., 2014). This is supported by the findings of study **II**, showing the highest AMF richness and diversity in potato roots grown in WOM plots, where the nutrient levels were the lowest.

6.4. Root fungal communities differ between potato cultivars

Comparing 21 potato cultivars, fungal richness was highest in the roots of the cultivar 'Merlot' (**III**). According to the information leaflet provided by Norika (Norika GmbH), the cultivar 'Merlot' has a medium-late maturity. In contrast, the lowest root fungal richness was observed in the roots of early and medium-early cultivars such as 'Laudine', 'Solist' and 'Concordia'. Studying the root characteristics of ten potato cultivars,

Iwama (2008) discovered that the roots of late cultivars generally have a longer total root length per unit area, with differences occurring at the flowering stage compared to early cultivars. In the present study also, the differences in fungal richness were most pronounced in the flowering stage. It is possible that the greater root length of late-maturing cultivars may have provided more habitat for fungi. Similar to study **II**, the differences in root fungal communities between potato cultivars may also rely on root exudates, but with less pronounced differences. In support of this assumption, a study conducted with 19 *Arabidopsis thaliana* accessions demonstrated a variation in their root exudates, including secondary metabolites such as flavonoids and salicylic acids (Monchgesang et al., 2016). Flavonoids and salicylic acids are often related to plant defence traits (Liu et al., 2020 and the references therein), which in turn are generally influenced by plant breeding. These results imply that the root exudates are genetically determined.

7. CONCLUSIONS

Based on the results of this thesis, the following conclusions can be drawn:
1) Soil pH is the primary environmental variable in determining root fungal community composition.
2) Fertilisation treatment does not affect overall root fungal community structure. However, fertilisation affects pathogen and AMF community structure. The more eutrophic environment after manure amendment may reduce beneficial AMF interactions and increase the diversity of pathogens with saprotrophic capabilities.
3) Root fungal community structure differs between crop species. The fungal communities in cereals are more similar to each other than compared to potato. These results suggest that the root microbiome is selectively recruited and may be related to host-plant genetic traits. For confirmation of the genetic effect, and to test if phylogenetic distance among crop species predicts the similarity of root fungal communities, future studies should include more than three closely related crop species, as well as phylogenetically distant species.
4) Potato cultivars differ in root fungal richness and community composition, indicating the importance of host genotype in structuring the root mycobiome.

This thesis improves our knowledge of how agricultural practices can shape root fungal community structure at a local scale. This information may help to identify sustainable farming methods; for example, employing the microbiome with persistent beneficial effects on crop plants and protecting them against biotic and abiotic stress. Future research should convert the knowledge gained from high-throughput sequencing data in order to find economically and ecologically important agricultural fungi and better determine their role in agriculture. Furthermore, to maximise their potential, these studies should include both below- and aboveground parts of the plant. This would allow the complexity of fungal communities in agriculture to be accounted for and via this, a comprehensive perspective of the crop mycobiome could be obtained.

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SUMMARY IN ESTONIAN

VÄETAMISE, PÕLLUKULTUURI NING SORDI MÕJU TAIMEJUURTE SEENTE KOOSLUSTELE

Tänapäeva põllumajandus on keskendunud peamiselt tootlikkusele ning selle mõju keskkonnale on üldiselt negatiivne. Kõrge intensiivsusega põllumajanduses kasutatavad monokultuurid, sünteetilised väetised ja pestitsiidid kahjustavad nii mulla tervist ja veekogude kvaliteeti kui ka vähendavad bioloogilist mitmekesisust. Samas on põllumajandus inimkonna peamiseks toiduallikas ning rahvaarvu kasvamisega kaasneva ressursinõudluse suurenemisega on looduslike alade muutmine põllumaaks paratamatu. Seetõttu on oluline arendada jätkusuutlikke põllumajandusstrateegiaid, mille mõju keskkonnale oleks võimalikult väike ning mis soosiks nii bioloogilist mitmekesisust kui ka sotsiaalselt õiglast majandamist.

Muld on elupaigaks tohutule hulgale mikroorganismidele nagu näiteks viirused, seened, bakterid ja algloomad. Paljud neist mikroorganismidest on pidevas vastastikmõjus taimedega, soodustades nende kasvu, parandades toitainete omastamist ning muutes neid patogeenidele vähem vastuvõtlikuks. Samuti on paljud mikroorganismid patogeensed, põhjustades mitmesuguseid taimehaiguseid. Seetõttu on oluline uurida mikroorganisme ja nende kooseksisteerimist taimedega. Kuigi viimastel aastatel on tõusnud uuringute arv, mis on keskendunud mikroorganismide ja põllukultuuride vastastikmõjule, on enamik neist keskendunud bakterikooslustele, jättes tähelepanuta seened. Seentel on aga mullaökoloogias võtmeroll, nad lagundavad orgaanilisi ühendeid, osalevad toitaineringluses ja stabiliseerivad mullaosakesi. Lisaks on vähe uuritud ka juuri asustavaid seeni. Võib oletada, et võrreldes mullas olevate seentega, on taimejuures elavatel seentel teistsugune elustrateegia ning seetõttu reageerivad nad teisiti keskkonnas toimuvatele muutustele. Keskkonnamuutusi põhjustavad tegurid võivad omakorda olla biootilised, abiootilised ja antropogeensed. Antropogeensete muutuste alla kuuluvad sealhulgas erinevad põllumajandusviisid, näiteks väetamine, kündmine, viljavaheldus ja sordi valik. Kõik need võivad mõjutada mikroobide, sealhulgas seenekooslusi.

Käesoleva doktoritöö esimeseks eesmärgiks oli uurida, kuidas mõjutavad juure seenekooslusi mineraalne väetis, orgaanika lisamine mineraalsele väetisele ning alternatiivne orgaaniline väetis, mis koosneb kompostist ja toot-

misjääkidest. Doktoritöö teiseks eesmärgiks oli uurida, kuidas mõjutab juure seenekooslusi kasvatatav põllukultuur ja põllukultuuri sort. Eelpool nimetatud tegurite mõju hinnati nii üldisele seenekooslusele kui ka kahele funktsionaalsele rühmale, milleks olid patogeenid ja mutualistid. Viimaste hulka arvestati arbuskulaar-mükorriised seened, mis moodustavad taime-dega sümbioosi ning seeläbi parandavad nende toitainete omastamist.

Väetusviiside ja põllukultuuride võrdluskatse läbi IOSDV katsepõllul. IOSDV pikaajaline külvikorrakatse rajati aastal 1989 eesmärgiga uurida pikaajalise väetamise mõju mullastikule. Käesoleva doktoritöö tarbeks koguti proovid aastal 2014 ja 2016. Esimesel katsevõtul korjati juure-proovid ainult kartulilt. 2016. aastal koguti juureproovid kõikidelt katses olnud taimedelt (nisu, oder ja kartul). Kõik proovid koguti taimede õit-semise faasis. Sordivõrdluskatse viidi läbi Reola katsepõllul 2014. aastal. Juureproovid koguti nii õitsemise kui ka vananemise faasis. Kõikide proo-vide analüüsimiseks kasutati mass-sekveneerimist.

Doktoritöö peamised hüpoteesid olid järgnevad:
a) mulla keemilised omadused mõjutavad looduslikke seenekooslusi taimejuurtes;
b) seente liigirikkus ja liigiline koosseis erineb väetusviiside vahel. Orgaa-nilised väetised suurendavad taimejuurtes seente üldist ja mutualis-tide liigirikkust, ent vähendavad patogeenide liigirikkust;
c) seente (üldine seenekooslus, mutualistid, patogeenid) liigirikkus, mitmekesisus ja liigiline koosseis erineb nisu, kartuli ja odra juurtes. Kõrgeim liigirikkus ja mitmekesisus esineb teraviljajuurtes;
d) juuri asustavate seente liigirikkus ning liigiline koosseis erineb kartu-lisortide vahel.

Doktoritöö tulemused näitasid, et mulla keemilistest omadustest oli pH peamine seenekoosluste mõjutaja. Üldiselt on varasemad uuringud näi-danud, et võrreldes bakteritega peetakse seeni happeliste tingimuste suh-tes tolerantsemaks ning nende pH-optimum on laiem. Sellegipoolest on igal liigil oma eelistus mulla pH suhtes ja nad peavad kohanema ümb-ritseva keskkonnaga. Seened on tuntud oma plastilisuse poolest ja muu-davad oma kasvu ja levikut vastavalt muutuvatele keskkonnale. Patogee-nide kooslust mõjutas peamiselt mulla lämmastiku sisaldus. Lämmastik on võib olla patogeenidele piiratud ressurss. Piisav kogus lämmastikku keskkonnas võimaldab patogeenidel kergemini lämmastikku omastada, aidates seeläbi kaasa nende levikule. Lisaks on lämmastik vajalik taimede

kasvuks ja arenguks. Kui taimedel on lämmastikupuudus, on nad haigustekitajatele vastuvõtlikumad. Ent vastupidiselt võib lämmastiku kättesaadavus aktiveerida ka taime kaitsemehhanismid ja sellest tulenevalt mõjutada patogeenide kooslusi. Arbuskulaarse mükoriisa seente kooslus oli see-eest mulla keemilistest omadustest nõrgalt mõjutatud.

Orgaanika lisamine mineraalsele väetisele kui ka alternatiivne orgaaniline väetis ei mõjutanud juuri asustavate seente üldist liigirikkust ega liigilist koosseisu. Samas täheldati muutusi patogeenide ja arbuskulaarmükorriissete seente koosluses. Vastupidiselt esitatud hüpoteesile tõusis patogeenide ning vähenes arbuskulaar-mükoriissete seente liigirikkus töötlustes, kus kasutati orgaanilisi väetiseid. Samas ei tähenda kõrgem patogeenide liigirikkus alati taimede haigestumise kasvu. Vastupidi, suurem konkurents toitainetele ja elupaikadele, võib vähendada teatud patogeenirühmade domineerimist ja seega ka haiguste esinemissagedust. Peale selle võis orgaanilise väetise lisamine suurendada fakultatiivsete patogeenide hulka, mis orgaanilise aine lisandudes muudavad oma toitumistüüpi ja muutuvad saprotroofseteks. Arbuskulaar-mükoriissete seente liigirikkuse vähenemise põhjus võib peituda omakorda selles, et orgaanika lisamisel suurenes toitainete hulk mullas ning lihtsustus toitainete omastamine taime juurte kaudu. See omakorda muutis sümbioosi AM-seentega ebavajalikuks.

Lisaks ei ole väetamine ainus tegur, mis võib mõjutada seenekooslusi. Tihti on muutused kooslustes põhjustatud mitme teguri koosmõjust. Antud doktoritöö tulemused näitasid, et olulisem tegur võib olla hoopis uuritav põllukultuur ise. Nimelt, kui väetamise ja põllukultuuri mõju uuriti samas mudelis, siis taime liigi mõju prevaleeris väetamise üle. Liigirikkus ja mitmekesisus oli suurim nisu ning madalaim kartuli juurtes. Samuti erines põllukultuuride juurtes seente liigiline koosseis, ent erinevused oli väiksemad teraviljade vahel. Üheks põhjuseks võib olla taimede fülogeneetiline taust. Kartul on kaheiduleheliste hulka kuuluv ühe-aastane rohttaim. Nisu ja oder kuuluvad üheiduleheliste kõrreliste hulka. Lisaks erineb uuritavate taimede juurte anatoomia ja füsioloogia. Kartulitaimede juurestik koosneb adventiivjuurest ning pigem vähe hargnevatest lateraaljuurtest. Vastupidiselt kartulile on teraviljade narmasjuurestik mitut tüüpi hargnemisega ning suurema pindalaga, see võimaldab rohkematel mikroorganismidel seonduda juure pinnale. Lisaks erinevad taimedel juureeritised. Juureeritised on orgaanilised ühendid, mida mikroorganismid saavad kasutada toiduallikana või nad võivad toimida sig-

naalmolekulidena. Seetõttu saavad juureeritised nii soodustada kui ka tõrjuda mikroorganismide seondumist juurepinnale. Juureeritised võivad erineda ka sortide vahel. Käesoleva doktoritöö tulemused näitasid ka, et kartulisortide juurtes erines seente liigirikkus ja kooslus. Sageli on taime juurtest eralduvad ühendid seotud taime kaitsemehhanismidega, see võib omakorda seletada erinevusi patogeenide koosluses.

Uurimistöö tulemused parandavad teadmisi taime ja seente vastastikmõjude kohta ning põllumajanduse mõjust seenekooslustele. Teadmine, kuidas põllumajandus mõjutab seenekooslusi, on kasulik nii teadlastele kui põllumajandustootjatele, kuna see võimaldab paljutõotavat strateegiat nii mulla kvaliteedi kui ka taimede tootlikkuse parandamiseks. Ent ilma põhimõttelise teadmiseta, kuidas seened reageerivad erinevatele häiringutele lokaalsel tasandil, pole võimalik selliseid jätkusuutlikke meetodeid arendada.

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ORIGINAL PUBLICATIONS

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ELSEVIER

The role of long-term mineral and organic fertilisation treatment in changing pathogen and symbiont community composition in soil

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A R T I C L E   I N F O

A B S T R A C T

Application of organic fertilisers to soil prevents erosion, improves fertility and may suppress certain soil-borne plant pathogens, but it is still unclear how different trophic groups of fungi and oomycetes respond to long-term fertilisation treatment. The objective of the study was to examine the effect of different fertilisation regimes on fungal and oomycete pathogen- and mycorrhizal symbiont diversity and community structure in both soil and roots, using PacBio SMRT sequencing. The field experiment included three fertilisation treatments that have been applied since 1989: nitrogen fertilisation (WOM), nitrogen fertilisation with manure amendment (FYM) and alternative organic fertilisation (AOF), each applied at five different rates. Soil samples were collected three times during the growing season, while root samples were collected during the flowering stage. There was no influence of the studied variables on soil and root pathogen richness. Contrary to our hypothesis, pathogen relative abundance in both soil and roots was significantly higher in plots with the AOF treatment. Furthermore, richness and relative abundance of arbuscular mycorrhizal (AM) fungi decreased significantly in the AOF treatment. Permutational analysis of variance (PERMANOVA) demonstrated the effect of fertilisation treatment on pathogen community composition in both soil and roots. Our findings indicate that organic fertilisers may not always benefit soil microbial community composition. Therefore, further studies are needed to understand how fertilisation affects mycorrhizal mutualists and pathogens.

1. Introduction

Organic fertilisers are produced by natural processes, and therefore considered more sustainable than inorganic fertilisers, as essential mineral nutrients are reused. Moreover, the application of organic fertilisers improves overall soil structure, in contrast to mineral fertilisers which mainly improve plant nutrient content and growth (Mäder et al., 2002). By promoting natural soil processes, organic fertilisers enhance soil microbial biomass (García-Gil et al., 2000; Wei et al., 2017). Higher microbial biomass supports soil microbial competition, which in turn prevents the dominance of small number of microorganisms, and contributes to suppressing soil-borne pathogens (Liu et al., 2016; Pérez-Piqueres et al., 2006). In addition, organic fertilisers have been shown to promote arbuscular mycorrhizal (AM) fungi (Verbruggen et al., 2010). In most agricultural plants, AM fungi provide some resistance to soil-borne pathogens, as well as improve nutrient uptake and plant

growth (Smith and Read, 2008; Smith and Smith, 2011). Thus, organic fertilisers represent a sustainable method for controlling certain plant diseases.

So far, the suppressive effect of organic fertilisers on specific plant pathogens has been shown in studies using single pathogen species, for example *Fusarium oxysporum* f. sp. *lactucae* (Gilardi et al., 2016) and *Ralstonia solanacearum* (Liu et al., 2016). In addition, studies have examined the effect of fertilisers on fungal communities (Paungfoo-Lonhienne et al., 2015; Sun et al., 2016). However, there is limited knowledge about the response of pathogen communities to different fertilisation treatments.

Understanding the effect of mineral and organic fertilisation on diversity of pathogens and mutualists may enable the development of more sustainable agricultural practices that benefit crop production and quality, as well as soil health. The objective of the present study was to determine the response of diversity and community structure of plant

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pathogens and mycorrhizal symbionts across three fertilisation treatments (nitrogen fertilisation (WOM), nitrogen fertilisation with farmyard manure amendment (FYM) and alternative organic fertilisation (AOF) and five application rates per treatment. We hypothesised that: 1) pathogen richness is lower, but Glomeromycota richness is higher, with organic fertilisation treatment; 2) increasing mineral fertiliser rate promotes pathogen richness, while decreasing the richness of Glomeromycota; and 3) pathogen and Glomeromycota community composition changes in response to fertilisation treatment and fertiliser application rate.

2. Materials and methods

2.1. The IOSDV experiment and sample collection

The International Organic Nitrogen Long-Term Field Experiment (IOSDV) is located in Tartu, Estonia (58°22.5′N, 26°39.8′E). The site is in a transitional climate zone between maritime and continental climate. In 2014, the area had a mean annual precipitation of 592 mm; the average temperature was $-1.4\,°C$ in winter, 6.3 °C in spring, 16.8 °C in summer and 7.3 °C in autumn (Estonian Weather Service, 2015). The soil of the field site is *Fragic Glossic Retisol* associated with *Stagnic Luvisol* (IUSS WG WRB 2015), with sandy loam texture and a humus layer of 27–32 cm. The three-field crop rotation experiment, with a potato–spring wheat–spring barley rotation, was established in 1989 to study the effect of different mineral and organic fertiliser application rates on field crops and soil properties in a long-term field experiment (Astover et al., 2016). The field experiment has a systematic block design with three replicates that included the following treatments: mineral fertilisation (WOM), mineral fertilisation with farmyard manure amendment (FYM) and alternative organic fertilisation (AOF, Table S1). Mineral fertilisation and manure treatment was further divided into five subplots (10 × 5 m) corresponding to the mineral fertiliser (ammonium nitrate) rates used (N-0, N-40, N-80, N-120, N-160 kg ha^{-1}). The five subplots of alternative organic fertilisation treatment were as follows: N0, PS (pulp sludge compost used in 2008–2011), C-200, C-275, C-350. The latter three are waste compost from separated food and green waste, as well as category III animal by-products in 2012–2014 respectively corresponding to three application rates with total N 200, 275 and 350 kg ha^{-1}. Soil tillage included ploughing (depth 18–20 cm) in autumn (October 2013), two-fold cultivation in spring (April 2014) before seeding, and sub-soiling (April 2014). Organic fertilisers were applied to soil surface in autumn and inserted with ploughing. Mineral fertilisers were spread and mixed with the soil during spring cultivation. Farmyard manure (40 t ha^{-1}) was applied every third year in autumn before planting potato. Potato was planted at a rate of one seed per 0.2 m, with 0.7 m spacing between them. No irrigation was used. The exact timing of applied pesticide treatments is shown in Table S2. Soil samples were collected from a potato field plots in 2014 three times over the vegetation period: 14 days after planting (29.05.2014); at the early plant flowering stage (27.07.2014) and before harvesting (12.09.2014). Each sample consisted of three subsamples, which were taken randomly from each subplot at 10 cm soil layer using a clean shovel. The subsamples were pooled, dried and mixed thoroughly following Tedersoo et al. (2014). The dried samples were stored in $-20\,°C$ for molecular analyses. Root samples were collected from plants of the Dutch potato variety 'Manitou' simultaneously with soil samples at the early flowering stage BBCH60 (cf. Hack et al., 2001). Each root sample consisted of three subsamples, each comprising an entire root system of a single plant individual. Roots were collected with a clean shovel from the 10–15 cm soil layer in each subplot. The roots were washed, cut into pieces of approximately 0.5 cm in length and kept in $-20\,°C$ for molecular analyses.

2.2. Soil chemical analysis

Composite samples were collected at a depth of 0–20 cm in spring before fertiliser application. From each plot, eight subsamples were taken and pooled to obtain the sample for each plot. Soil chemical analyses were conducted to determine the amount of N_{total}, $C_{organic}$, plant available phosphorus ($P_{available}$) and soil pH. The N_{total} was measured using Kjeldahl method (van Reeuwijk, 2002), $C_{organic}$ was determined using Tjurin method (Vorobyova, 1998), and $P_{available}$ was evaluated the ammonium lactate (AL) method (Egnér et al., 1960). The soil pH reaction was determined in 1 M KCl solution.

2.3. Molecular methods

DNA was extracted from 0.2 g of soil and 0.075 g roots using the PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA, USA). We made the following modifications to the manufacturer's protocol: a) samples were homogenised by bead beating for 5 min at 30 Hz with three 3 mm autoclaved steel beads with MixerMill MM400 (Retsch, Haan, Germany); and b) final elution was performed twice with 50 μl of Solution C6. PCR was performed using three forward primers ITS1ngs, ITS1Fngs (Oja et al., 2015), ITS1Oo (Riit et al., 2016) and a reverse primer ITS4ngs (Tedersoo et al., 2014). Both forward and reverse primers were tagged with one of the 93 identifiers (MIDs, 10–12 bases). All samples were amplified in duplicate using a reaction mixture consisting of 1 μl DNA, 0.5 μl each of the primers (20 pmol), 5 μl 5xHOT FIREPol Blend Mastermix (Solis Biodyne, Tartu, Estonia) and 18 μl double-distilled water in thermal cyclers. The cycling conditions for PCR amplification were as follows: initial 15 min at 95 °C; 28 cycles of 30 s at 95 °C, 30 s at 55 °C, 1 min at 72 °C; and a final cycle of 10 min at 72 °C. By contrast, PCR of root samples was carried out with 30 amplification cycles. DNA samples showing no visible band were reamplified using 33 cycles. The duplicate PCR samples were pooled, and their relative quantity was estimated by running 5 μl DNA on 1% agarose gel stained with ethidium bromide (Sigma-Aldrich, St Louis, MO, USA) and visualised with Bio-Vision 3026 WL (Vilber Lourmet, Torcy, France). We used negative (for DNA extraction and PCR) and positive controls (fungal specimen MURU6028 or oomycete mock community OoMix; Tedersoo et al., 2018) throughout the experiment. The amplicons were purified with FavorPrep PCR Clean Kit (FavorGen Biotech, Vienna, Austria). The libraries were prepared using PacBio (Pacific Biosciences, Menlo Park, CA, USA) amplicon library preparation protocol and loaded to two SMRT cells using the MagBead method. The libraries were sequenced using the PacBio RSII instrument using P6-C4 chemistry following the manufacturer's protocol.

2.4. Bioinformatics

Bioinformatics analyses were performed using the PipeCraft 1.0 analysis platform (Anslan et al., 2017). At first, circular consensus sequences from RSII data using default settings of pbccs 2.02 software (github.com/PacificBiosciences/unamity) were created. This was followed by quality filtering and demultiplexing using the following options of mothur 1.36.1 (Schloss et al., 2009): qwindowaverage = 30, qwindowsize = 50, minlength = 50, maxambig = 0, maxhomop = 13, pdiffs = 1, bdiffs = 1, tdiffs = 2. Putative chimeric sequences were removed using uchime de novo filtering (Edgar et al., 2011) and reference-based filtering against UNITE reference dataset v7.0 (Kõljalg et al., 2016). Prior to clustering, flanking SSU and LSU regions were removed using ITSx v1.011 (Bengtsson-Palme et al., 2013). The extracted ITS reads were clustered at 98% sequence similarity threshold using usearch algorithm (Edgar, 2010). Singleton operational taxonomic units (OTU) were removed from further analyses. For taxonomic assignment, a representative sequence from each OTU was selected for BLASTn search against the UNITE and GenBank database. We considered e-values < e − 50 of BLASTn search results and query

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coverage > 50% reliable to assign the taxonomy. The taxonomy of OTUs was assigned based on the consensus taxonomy assignment. If at least eight of ten BLAST hits agreed on the same taxonomy level, it will give the value to this taxonomy level.

Raw sequence data are publicly available through Sequence Read Archive, BioProject PRJNA530662.

2.5. Functional assignment

To study the effect of fertilisation regimes on pathogen diversity, we focused on pathogens that infect crops grown in the experimental site, i.e. potato, barley, and wheat. OTUs were assigned to trophic groups based on their species-level identification according to the United States Department of Agriculture, Agricultural Research Service (https://nt. ars-grin.gov/fungaldatabases/; Table S3). Oomycetes assigned to genus level (except *Lagenidium*) were all considered pathogenic (Lévesque, 2011). Also, fungal genera *Alternaria*, *Fusarium* and *Phoma* were considered as pathogens. Besides some saprotrophic and endophytic species, these genera comprise multiple pathogens, and therefore, members of these genera were considered generalist pathogens (Aoki et al., 2014; Aveskamp et al., 2008; Thomma, 2003). All OTUs belonging to the phylum Glomeromycota were considered as arbuscular mycorrhizal (Table S3).

2.6. Statistical analysis

We used linear regression between obtained OTUs and the square root of the number of obtained sequences as a proxy for OTU richness to control for variation in sequencing depth (Bálint et al., 2016; Tedersoo et al., 2014). In the case of significant regression, standardised residuals were used as a proxy for taxonomic richness (of pathogens, Glomeromycota, *Pythium*, *Phoma* and *Fusarium*). If the regression was insignificant, the analyses were carried out with number of obtained OTUs (genus *Rhizoctonia* species richness in roots). The relative pathogen abundance was calculated as the number of pathogen sequences in each sample divided by the total number of fungal sequences in each sample. Differences in richness and relative abundance were tested using GLM analysis with backward elimination procedure (α = 0.05; Statistica 12.0, Palo Alto, CA, USA). Explanatory variables included the fixed factors treatment, fertiliser application rate (nested inside treatment) and time, time × treatment, and block as a random factor. In roots the time variable was excluded due to only one sampling point. We considered soil variables $C_{organic}$, N_{total}, P, pH and C:N ratio as covariates. All covariates, except pH and C:N ratio were log-transformed before analysis. Covariates were excluded stepwise to retain only significant covariates with explanatory variables. In addition, GLM analysis was applied to test the effect of fertilisation on soil variables. Individual comparisons among means were carried out using Tukey's unequal N HSD *post-hoc* test.

The response of pathogen and symbiont community composition to experimental factors and covariates was tested with PERMANOVA + implemented in PRIMER-e using Hellinger distance (Anderson et al., 2008). In the PERMANOVA design, the fixed factors included treatment with three levels with fertiliser rate nested within treatment (five levels) and sampling time with three levels. In roots the time variable was excluded due to only one sampling point. We also included block as a random factor. Soil variables and square-root of sequences were considered as covariates. PERMANOVA tests were carried out by using backward elimination of covariates and interaction terms accounting for < 1% of variance to retain only significant covariates. PERMANOVA tests were performed by using the type I sum of squares with 9999 permutations under a reduced model. Non-metric multidimensional scaling (NMDS) plots were generated on Hellinger-standardised matrices using Euclidean distance to visualise differences between experimental variables. The NMDS was performed in R software (R Development Team, http://www. R-project.org) using the "metaMDS"

function in "vegan" and "ggplot2". OTU abundance was visualised using Krona tools (Ondov et al., 2011).

3. Results

3.1. Identification of pathogens and symbionts

A total of 54,701 quality-filtered sequences were obtained from 177 soil and root samples, with an average number of 309 sequences per sample (Table S3). These reads were assigned to 1044 OTUs with an average number of 80 OTUs per sample. The most abundant phylum was Ascomycota, accounting for 33.3% of OTUs, followed by Basidiomycota (17.2%) and Oomycota (9.7%). Altogether 3.9% of OTUs were assigned to Glomeromycota (Fig. S1). Both in soil and roots, *Pythium* was the most abundant pathogen genus, comprising 54.2% of pathogen OTUs in soil (Fig. S2) and 57.9% in roots (Fig. S3). In soil, the three most abundant pathogen OTUs (OTU0001, OTU0065 and OTU0067), all belonging to genus *Pythium*, were respectively present in 98.4%, 98.4% and 96.2% of samples. Also, in roots, the three most abundant OTUs belonged to *Pythium* (OTU0004, OTU0001 and OTU0065), occurring in 95.5%, 86.4% and 79.5% of samples, respectively.

Of Glomeromycota, Glomerales was the most abundant order, prevailing both in soil (37%, Fig. S4) and roots (55.6%, Fig. S5). In soil, the three most frequent symbiont OTUs (OTU0549, OTU0948 and OTU0842) belonged to the order Diversiporales (represented in 27% of samples), followed by Glomerales (12%) and Paraglomerales (11.2%). In roots, the three most frequently detected symbiont OTUs (OTU0549, OTU1111, OTU0948) belonged respectively to the orders Diversisporales (40.9%), Glomerales (36.4%) and Glomerales (22.7%).

3.2. Richness and relative abundance of pathogens and mutualists in soil

The relative pathogen abundance in soil was significantly affected by fertiliser treatment (Table 1, Fig. 1), with the lowest value in WOM and the highest in AOF treatment (Table S4). In addition, *post-hoc* analyses revealed that relative pathogen abundance was significantly greater in the AOF fertiliser application rate C350 treatment, compared to the WOM fertiliser application rate N120 treatment (Table S4). The relative abundance patterns of *Pythium* spp. reflected that of overall pathogens (Fig. 1, Table S5). Time had the strongest influence on the relative abundance of *Phoma* spp. (teleomorph *Didymella* spp.), the second most abundant pathogen genus, and this influence increased during the growing season (Table S5).

The third most abundant genus, *Fusarium* spp., varied in abundance among treatments. The relative abundance of *Fusarium* was highest in the WOM treatment and lowest in the AOF treatment (Fig. 1). *Fusarium* spp. were influenced by fertiliser application rate, with significantly greater relative abundance in the WOM N120 treatment, compared to WOM N0, FYM N80, AOF PS, and AOF C200 treatments. This observation is opposite to those of overall pathogen- and *Pythium* relative abundance (Table S5).

In soil, none of the explanatory variables significantly influenced overall pathogen richness (Table 2) or that of *Pythium* or *Phoma* (Table S7). However, species richness of *Fusarium* varied significantly among treatments and fertilisation rates (Table S7). The highest species richness was found in the WOM treatment, and the lowest in the AOF treatment. More specifically, *Fusarium* species richness was significantly greater in the fertiliser application rate WOM N120 treatment, compared to WOM N0, FYM N80, AOF C200 and AOF C275 treatments.

The relative abundance of Glomeromycota varied significantly among treatments (Table 1), with the highest value in the WOM treatment and the lowest in the AOF treatment (Table S4). Moreover, the time × treatment interaction had a significant effect on Glomeromycota relative abundance, the FYM plots at the flowering stage harbouring greater Glomeromycota relative abundance, compared to
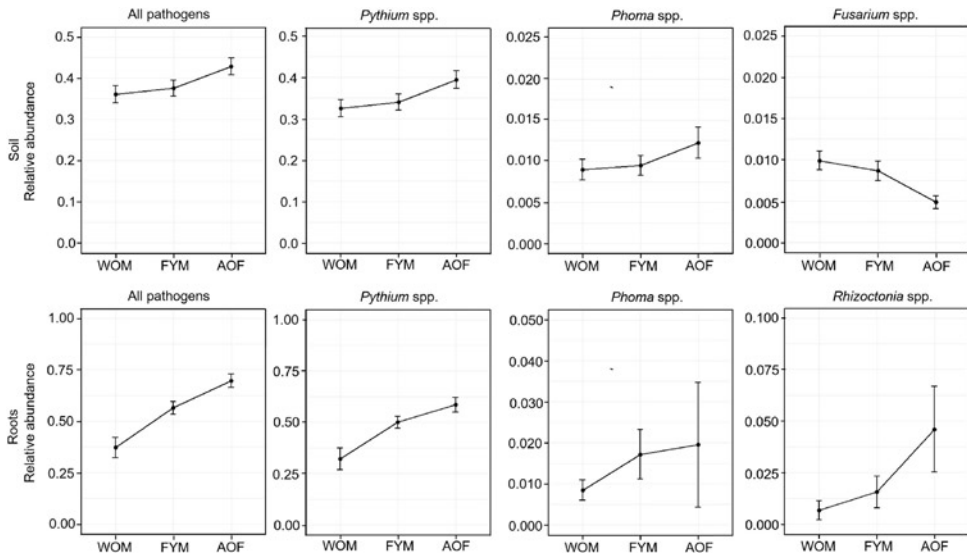
Fig. 1. Effect of different fertilisation treatments on overall pathogen relative abundance as well as a relative abundance of the three most abundant pathogen genera, in soil and roots. WOM, mineral fertilisation; FYM, mineral fertilisation combined with farmyard manure; AOF, alternative organic fertilisation.

that of the flowering stage in WOM and AOF plots, as well as in harvested WOM and FYM plots. The results of *post-hoc* analyses are shown in Table S4.

Treatment, time, their interaction and block all significantly influenced Glomeromycota species richness in soil (Table 2, Table S6). The WOM treatment harboured highest Glomeromycota richness, whereas lowest richness was observed in the AOF treatment (Table S6). Furthermore, estimating the effect of sampling time, Glomeromycota richness was highest during the plant flowering stage. The time × treatment interaction showed the greatest Glomeromycota OTU richness before planting in FYM plots, compared to each sampling time point in WOM plots, as well as during the flowering stage in AOF plots and harvested FYM and AOF plots.

Soil variables were also important predictors of Glomeromycota OTU richness and relative abundance. Only pH had an overall negative effect on Glomeromycota richness (adjusted $R^2 = 0.027$, $F_{1,109} = 6.083$, $P = 0.015$), while its relative abundance was negatively

affected by pH (adjusted $R^2 = 0.016$, $F_{1,106} = 3.974$, $P = 0.049$), $C_{organic}$ (adjusted $R^2 = 0.024$, $F_{1,106} = 5.290$, $P = 0.023$) and N_{total} (adjusted $R^2 = 0.017$, $F_{1,106} = 4.175$, $P = 0.043$). However, C:N ratio (adjusted $R^2 = 0.018$, $F_{1,106} = 4.267$, $P = 0.041$) had a positive influence on Glomeromycota relative abundance.

3.3. Richness and relative abundance of pathogens and mutualists in roots

In roots the relative abundance of pathogens was affected by treatment (Table 1, Fig. 1), which explained 42.8% of the variance in our GLM. In roots, the relative abundance of pathogens was lowest in the WOM treatment and highest in the AOF treatment (Table S4). The pattern also applied to the relative abundance of *Pythium* (Fig. 1, Table S5). The relative abundance of *Phoma* was unaffected by any variables, whereas the third most abundant pathogen in roots, *Rhizoctonia* was positively affected by $P_{available}$ (adjusted $R^2 = 0.083$, $F_{1,26} = 4.998$, $P = 0.034$).

Table 1
Results[†] of general linear models estimating the effect of treatment, fertiliser application rate (nested within treatment) and time on relative abundance of pathogens and Glomeromycota in soil and roots.

Sample type	Variable	df	Pathogens			Glomeromycota		
			R^2_{adj}	F	P	R^2_{adj}	F	P
Soil	Treatment	2	0.034	3.754	0.026*[‡]	0.027	3.612	0.030*
	Fertiliser rate	12	0.101	2.347	0.010*	0.058	2.057	0.026*
	Time	2	0	0.210	0.811	0.017	2.733	0.069
	Block	2	0	0.317	0.317	0.017	2.756	0.068
	Time × Treatment	4	0.012	1.613	0.176	0.041	2.997	0.022*
Roots	Treatment	2	0.428	16.001	0.001**	0.224	8.600	0.001**
	Fertiliser rate	12	0	0.766	0.678	0	1.005	0.471
	Block	2	0	0.794	0.462	0.085	4.218	0.025*

[†] *df*: degrees of freedom; R^2_{adj}: adjusted R^2; *F*: F-statistic, *P*: calculated probability.
[‡] **$P < 0.01$; *$P < 0.05$.

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Table 2

Results[†] of general linear models estimating the effect of treatment, fertiliser application rate (nested within treatment) and time on taxonomic richness of pathogens and Glomeromycota in soil and roots.

Sample type	Variable	df	Pathogens			Glomeromycota		
			R^2_{adj}	F	P	R^2_{adj}	F	P
Soil	Treatment	2	0.024	2.438	0.092	0.059	6.478	0.002**[‡]
	Fertiliser rate	12	0	0.422	0.952	0.011	1.500	0.135
	Time	2	0.006	1.302	0.276	0.044	5.197	0.007**
	Block	2	0.006	1.304	0.276	0.062	6.802	0.002**
	Time × Treatment	4	0	0.178	0.949	0.028	2.549	0.043*
Roots	Treatment	2	0.160	6.039	0.007**	0.223	7.036	0.003**
	Fertiliser rate	12	0.085	1.723	0.119	0	0.656	0.776
	Block	2	0	0.817	0.453	0.034	2.146	0.137

[†] df: degrees of freedom; R^2_{adj}: adjusted R^2; F: F statistic, P: calculated probability.

[‡] **$P < 0.01$; *$P < 0.05$.

Fig. 2. Nonmetric multidimensional scaling (NMDS) plot on Euclidean distance representing the differences in soil pathogen community composition (a) between fertilisation treatments, and (b) with time in a reduced two-dimensional space. WOM, mineral fertilisation; FYM, mineral fertilisation combined with farmyard manure; AOF, alternative organic fertilisation; 1. time point, 14 days after planting; 2. time point, plant flowering stage; 3. time point, before harvesting.

$P_{available}$ positively affected both total pathogen richness (adjusted $R^2 = 0.089$, $F_{1,26} = 6.659$, $P = 0.016$) and *Pythium* OTU richness (adjusted $R^2 = 0.061$, $F_{1,26} = 5.309$, $P = 0.029$), while *Phoma* richness did not show any significant effect towards any of the studied variables, whereas *Rhizoctonia* richness was positively affected by $C_{organic}$ (adjusted $R^2 = 0.076$, $F_{1,25} = 4.728$, $P = 0.039$) and N_{total} (adjusted $R^2 = 0.109$, $F_{1,25} = 6.283$, $P = 0.019$).

Both relative abundance and richness of Glomeromycota in roots were significantly affected by treatment (Tables 1, 2). Opposite to pathogens, the highest values occurred in the WOM treatment, and the lowest values in the AOF treatment (Tables S4, S6), with no residual effects of edaphic predictors.

3.4. Taxonomic composition

PERMANOVA revealed that fertilisation treatment accounted for the largest source of variation (10.1%) in pathogen community composition in soil (Table 3), with significant differences among all treatment types (Table S8). In addition, there was a significant effect of time on pathogen community structure that accounted for 6.0% of the community variation in soil, with the first sampling time showing significant differences compared to the other two sampling times (Table

S8). All soil variables and interactions among main factors accounted for < 1% of the variation in pathogen composition. The NMDS analysis confirmed the results of PERMANOVA (Fig. 2).

Across all factors, only treatment and fertiliser application rate had a significant effect on Glomeromycota composition in soil (Table 3). However, the pairwise analysis revealed no significantly different distance groups across fertilisation treatments (Table S8). Fertiliser application rate had the strongest effect on Glomeromycota community composition within the WOM treatment (Table S8).

In roots, only fertilisation treatment altered pathogen composition (Table 3, Fig. 3). Pathogen community in the WOM treatment significantly differed from both the FYM and the AOF treatment (Table S8). None of the studied variables had a significant effect on the Glomeromycota community structure in roots (Table 3).

3.5. Soil properties

Fertilisation treatment caused significant changes in soil parameters (Table S9). Soil pH was lower in the WOM treatment than in the soil with organic fertiliser treatments (FYM and AOF; $F_{2,28} = 693.93$, $P < 0.001$), ranging from 5.5 to 7.1 across treatments. Soil N_{total} concentration ranged from 0.04% to 0.12% in soil, being highest in the
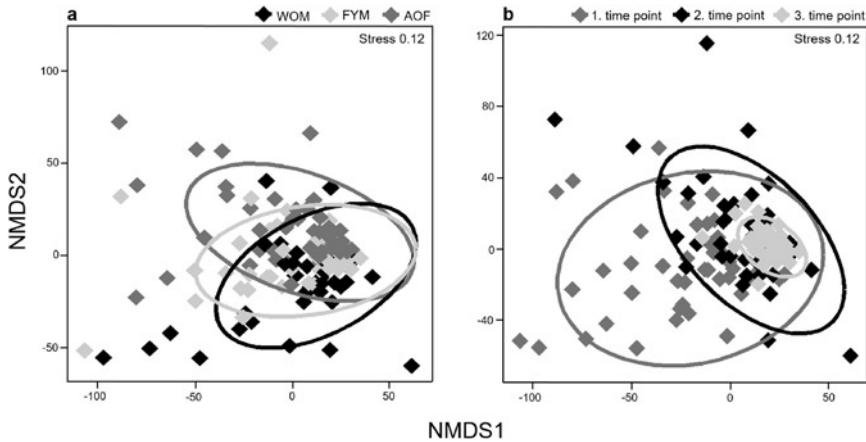
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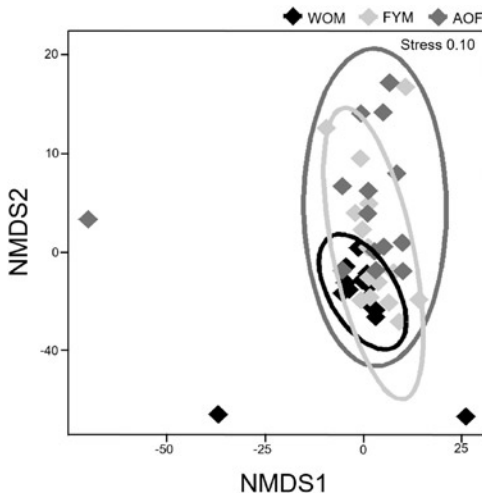
Fig. 3. Nonmetric multidimensional scaling (NMDS) plot on Euclidean distance representing the differences in root pathogen community composition in a reduced two-dimensional space. WOM, mineral fertilisation; FYM, mineral fertilisation combined with farmyard manure; AOF, alternative organic fertilisation.

FYM treatment, followed by the AOF treatment ($F_{2,28} = 42.75$, $P < 0.001$). Concentration of $C_{organic}$ followed the same pattern with values ranging from 0.94% to 1.45% in soil ($F_{2,28} = 60.416$, $P < 0.001$). Conversely, the C:N ratio was highest in the WOM treatment and lowest in the FYM treatment ($F_{2,28} = 14.789$, $P < 0.001$). The C:N ratio varied widely between samples ranging from 11.66 to 21.22. The highest soil $P_{available}$ occurred in the AOF treatment and the lowest in the WOM treatment ($F_{2,28} = 0.944$, $P < 0.001$). $P_{available}$ concentration varied from 36.5 to 139.0 mg kg^{-1} soil. In addition, fertiliser rate within treatment had a significant effect on soil pH. Higher fertiliser application rate significantly reduced soil pH in the WOM and FYM treatments, whereas the opposite trend was observed in the AOF treatment ($F_{4,8} = 9.370$, $P < 0.001$). The results of *post-hoc* analyses are shown in Table S9.

4. Discussion

In this study, we examined the response of root- and soil-inhabiting pathogens and mutualists to different fertilisers and rates of fertiliser application using metabarcoding as an identification tool. Plant

pathologists have begun implementing these high-throughput sequencing methods relatively recently (Sapkota and Nicolaisen, 2015; Tedersoo et al., 2019). The characterisation of pathogen communities commonly relies on sequencing internal transcribed spacer 1 (ITS1) or internal transcribed spacer 2 (ITS2; Agler et al., 2016; Bainard et al., 2017; Rezki et al., 2016). Identification of oomycetes is more challenging due to their relatively low biomass and poor performance of fungal-specific and general primers (Riit et al., 2016). Furthermore, the short ITS1 or ITS2 fragments separately may present limited resolution for species-level separation of taxa, or generate spurious taxa, when sequencing errors accumulate (Tedersoo et al., 2018). Here we implemented both fungal and oomycete primers, as well as the PacBio RSII platform, to sequence the full-length ITS (ITS1-5.8S-ITS2) region for more accurate classification (Tedersoo et al., 2018) of both mycorrhizal fungi and various pathogens. The most abundant pathogens included *Pythium* spp. Although there are few studies investigating both pathogenic fungal and oomycete communities, our results are in line with previous reports showing relatively high abundance of *Pythium* spp. compared to other groups (Rojas et al., 2017; Sapkota and Nicolaisen, 2018). *Pythium* species are common soil microbes, most of them being saprotrophs decomposing organic matter (Agrios, 2005). However, pathogenic *Pythium* species can cause damping off and wilting in a large number of plant species under favourable conditions (Agrios, 2005). Therefore, distinction between trophic groups may not always be straightforward, as it can depend on the plant and fungal species, cultivars and strains, plant stress, microbiome and soil variables, and especially a combination of these (Martin and Loper, 1999; Termorshuizen and Jeger, 2008). Although we use the full ITS region, we may still be unable to distinguish between taxa with pathogenic and saprotrophic properties.

The abundance of the symbiotic phylum Glomeromycota was generally low and definite assessments are difficult to make. However, the majority of detected OTUs belonged to the order Glomerales. Our data corroborate with previous findings, showing the dominance of Glomerales both in agricultural soils (Higo et al., 2015) and natural habitats (Hiiesalu et al., 2012; Rodríguez-Echeverría et al., 2017).

Pathogen richness in soil did not respond to any of the studied variables, although pathogen relative abundance was influenced both by fertilisation treatment and fertiliser application rate. Interestingly, the relative abundance was highest in the AOF treatment but lowest in the WOM treatment. This pattern also applied to the relative abundance of *Pythium*. The AOF treatment consists mainly of organic compounds, beneficial for pathogens that may act as facultative saprotrophs. The increase of *Pythium* spp. after incorporating plant tissue to soil has been also shown by Manici et al. (2004). Plant pathogens may have persisted in organic debris and increased their relative abundance in the AOF plots. This is in agreement with the increase of pathogen relative abundance coinciding with higher AOF fertiliser application rates. Therefore, future studies should also consider sequencing the organic compost to account the effect of fertiliser on pathogen abundance.

Table 3

Results[†] of PERMANOVA models testing the difference in pathogen and Glomeromycota community composition in soils and roots under different fertilisation treatment, fertiliser application rate (nested within treatment) and time.

Sample type	Variable	df	Pathogens			Glomeromycota		
			R^2_{adj}	Pseudo-F	P	R^2_{adj}	Pseudo-F	P
Soil	Treatment	2	0.101	9.697	0.001**[‡]	0.014	1.667	0.044*
	Fertiliser rate	12	0.024	1.579	0.001**	0.058	1.444	0.009**
	Time	2	0.060	6.324	0.001**	0	1.190	0.240
	Block	2	0.006	1.753	0.005**	0	1.104	0.305
Roots	Treatment	2	0.428	15.978	0.001**	0.045	1.824	0.058
	Fertiliser rate	12	0	0.762	0.681	0	1.097	0.309
	Block	2	0	0.794	0.461	0.004	1.208	0.305

[†] *df*: degrees of freedom; R^2_{adj}: adjusted R^2; *F*: pseudo-F statistic, *P*: calculated probability.
[‡] **$P < 0.01$; *$P < 0.05$.

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However, greater pathogen relative abundance may not always lead to higher disease incidence (Ros et al., 2005; Widmer et al., 1998). A metastudy by Bonanomi et al. (2010) concluded that the effect of organic compounds on pathogens depends on organic matter decomposition and it varies among pathogen species. Furthermore, the effect of organic fertilisation on pathogens could be context-dependent (Bongiorno et al., 2019; Hu et al., 2017).

Compared to the overall pathogen community, both species richness and relative abundance of *Fusarium* were highest in the WOM treatment and lowest in the AOF treatment. The decrease in *Fusarium* abundance could be related to the higher N_{total} and pH values in AOF plots. Previous studies have shown the sensitivity of *Fusarium* spp. to increases in ammonia and pH (Deltour et al., 2017; Huang et al., 2019; Zakaria, 1980).

None of the studied variables affected root-inhabiting pathogen richness, although their relative abundance was highest in the AOF treatment. Comparing pathogen composition in soil and roots suggests that only a few pathogenic OTUs dominate in both habitats. Higher abundance of these OTUs in soil also increases their probability of colonisation success in the roots.

In soil, pathogen community composition was influenced by the fertilisation treatment and to a lesser extent by the sampling time. A possible underlying mechanism for the differences in pathogen composition could be ascribed to facultative saprotrophs. AOF plots containing organic compounds, provide a habitat and support species, that can obtain their nutrition from dead and decaying matter (Bonanomi et al., 2006). Time-dependent change in pathogen community composition could be related to replacement of pathogens susceptible to the previous host. At the IOSDV experimental site, crops are grown in continuous rotation. The temporal effect and nested pattern seen in the NMDS plot (Fig. 2) might indicate a change in community composition from pathogens with broad host ranges to pathogens more specific to potato. This is in agreement with Bainard et al. (2017) who showed that crop rotation and previous crop influence overall fungal as well as pathogen community composition in soil.

Both species richness and relative abundance of AM fungi were affected by fertiliser treatment. Both variables declined in the AOF treatment, while the taxonomic composition remained unaffected. Mineral fertilisers usually reduce the growth of AM fungi in soil (reviewed by Verbruggen and Toby Kiers, 2010). However, a certain subset of AM fungal taxa could be less sensitive to intensive farming systems (Hijri et al., 2006).

The most abundant order in our study was Glomerales, which have been shown to be more tolerant towards nutrient addition (Ryan and Graham, 2018; Sommermann et al., 2018). Although not due to synthetic fertilisers, the amount of N_{total} and $P_{available}$ was the highest in the AOF plots, a possible reason for the present study's inconsistency with previous studies. Both nutrients in higher concentration have been shown to inhibit the growth of AM fungi (Treseder, 2008; Detheridge et al., 2016). However, Ryan and Graham (2018) summarised that until the functioning of AM fungi under high fertilisation has been adequately evaluated, the true effects of fertilisers on AM fungi are largely unknown. The significant changes dependent on the time × treatment interaction could be related to differences in rate of establishment and dispersal capacity of AM fungal taxa. The WOM and FYM treatments showed the highest species richness and relative abundance during the flowering stage, whereas the AOF treatment showed the opposite pattern.

Our results revealed that concentrations of most nutrients increased in organic fertilisation treatments (FYM and AOF). Cattle manure was an important source of organic carbon, as the amount of $C_{organic}$ was highest in the FYM treatment. As summarised by Liu et al. (2006), $C_{organic}$ is one of the main contributors to soil health by not only improving soil nutrient level but also amending soil physical properties and water holding capacity. N_{total} followed the same pattern as $C_{organic}$, having the highest concentration in soils under the FYM treatment.

However, plants do not always benefit from high loads of nitrogen. Van Bruggen et al. (2015) noted that high concentrations of soil nitrogen, especially nitrate (NO_3^-), promote the spread of wilt- and rot-causing diseases. Both in the WOM and FYM treatments, increasing fertiliser application rate reduced soil pH. Ammonium nitrate containing fertilisers are known to promote soil acidification (Barak et al., 1997). Thus, reduction of soil pH is probably mostly due to the nitrogen fertiliser used in the study.

5. Conclusions

So far, only a few studies have explored the impact of different fertilisation treatments and application rates on both pathogens and mutualists simultaneously. Our results indicate that pathogen relative abundance may be enhanced by organic fertilisation, despite the minimal changes in pathogen taxonomic richness across fertilisation treatments. This suggests survival of plant pathogens in organic fertilisers, which we attribute to the prevalence of facultative saprotrophs. In contrast to pathogens, AM fungal species richness and relative abundance were lower in the AOF treatment, both in soil and roots. Considering that previous studies have shown opposite patterns (i.e. decrease of pathogens and increase of AM fungi in organic fertilisation), our results suggest a context-dependent response of different microbial guilds to fertilisation treatments. Furthermore, our study highlights the importance of agricultural management practices on the soil microbiome, as well as the need to evaluate the effects of fertilisation treatments on communities of both pathogens and mutualists.

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Characterising the effect of crop species and fertilisation treatment on root fungal communities

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Information about the root mycobiome may improve the overall quality of the plants and contribute to a valuable strategy to enhance sustainable agriculture. Therefore, we assessed differences in fungal community diversity and composition in the roots of potato, wheat and barley grown under mineral nitrogen fertilisation at five rates, with and without farmyard manure amendment. The same factorial combination of treatments has been used since 1989. Species richness and diversity, as well as community composition, of different fungal guilds were characterised using Illumina MiSeq sequencing of the ITS2 region. Crop species was the main factor determining overall fungal richness and diversity, with wheat showing the highest, and potato the lowest, richness and diversity. Pathogen diversity indices were highest in wheat plots amended with farmyard manure, whereas the lowest values were observed for potato roots. Fertilisation treatments and the interaction between crop species and fertilisation had the strongest impact on arbuscular mycorrhiza and saprotroph diversity. Crop species also determined the composition of the overall fungal community and that of fungal guilds, whereas fertilisation treatment had only a minor effect. This study highlights crop species as the main driver in shaping root fungal diversity and composition under the same environmental conditions.

The continuing challenge in agriculture is to keep increasing crop production in an environmentally sustainable manner[1,2]. In order to achieve this, one possible approach is to harness the benefits of plant-associated microbes[3,4]. Diverse plant root systems create a heterogeneous environment for microorganisms that play an important role in plant health and fitness[5]. Beneficial microorganisms improve plant nutrient uptake, liberate nutrients from organic matter and induce plant systemic resistance, whereas pathogens suppress the plant immune system and cause diseases[6]. Studying the plant–microbial interactions presents a possibility to find plant genotypes that facilitate beneficial microbial interactions, which could allow the reduction of fertiliser inputs and pesticide use[7]. However, most studies so far have focused on bacterial communities[8–11] in spite of the importance of fungi in soil processes and plant nutrition and pathogenesis.

Wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) are among the most broadly cultivated cereals and are an important source of minerals and vitamins[12]. Potato (*Solanum tuberosum* L.) is one of the most widely grown vegetables in the world, ranking as the third most important food crop[13]. A diverse microbiome consisting of beneficial microorganisms can play an important role in sustainably increasing the yield of these economically important crops[14,15]. Studies have shown that plants may change their microbiome depending on genotype, plant root system, developmental stage and the ecosystem they inhabit[16–18]. However, we lack a comparative and comprehensive understanding of how different crops shape their microbiome.

Furthermore, we have contradictory knowledge on how different agricultural practices structure the microbiome of crops. In general, it has been shown that organic management diversifies soil microbial community composition, whereas mineral fertilisation decreases community diversity[19–21]. However, contrasting results have been reported[22,23]. Similarly, research on root fungal communities and how they respond to different fertilisation treatments has been inconsistent[24–26]. To our knowledge, only few studies have compared the root fungal community structure of different crop species under the same field environment and its response to different fertilisation practices. Wemheuer et al.[27] determined the effect of mowing and fertilisation on endophytic fungal communities

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Barley		Potato		Wheat	
WOM	FYM	WOM	FYM	WOM	FYM
N160	N160	N160	N160	N160	N160
N120	N120	N120	N120	N120	N120
N80	N80	N80	N80	N80	N80
N40	N40	N40	N40	N40	N40
N0	N0	N0	N0	N0	N0

Figure 1. Schematic representation of one replicate block. Each block is divided into three plots planted with barley, potato and wheat, respectively. Each plot comprised two main plots treated with either mineral nitrogen fertilisation (WOM) or mineral nitrogen fertilisation combined with farmyard manure 40 t h^{-1} (FYM). The mineral nitrogen fertilisation was applied in five different application rates (N). The numbers refer to the application rate according to total N (0, 40, 80, 120, 160 kg ha^{-1}).

in three grassland species, in a long-term field experiment. However, they assessed the effect of management practices on fungal communities in aerial parts of plants. Hartman et al.[28] studied the effect of fertilisation and tillage on soil- and root microbiota in a multifactor field experiment, but the study focused only on wheat.

The objective of this study was to assess differences in fungal community diversity and composition in the roots of potato, spring wheat and spring barley under different fertilisation treatments. The crops were grown in rotation under mineral nitrogen fertilisation and mineral nitrogen fertilisation combined with farmyard manure treatment. Nitrogen fertiliser was applied at five different rates. We hypothesised that crop species influence the community composition of diverse fungal guilds (pathogens, arbuscular mycorrhiza, and saprotrophs). We also tested the hypothesis that plots treated with organic manure support higher fungal richness and diversity, and reduce pathogen occurrence, compared to plots treated only with mineral nitrogen fertilisation.

Materials and methods

Field experiment and sample collection. The study was conducted at the field trial site located in Tartu, Estonia (58° 22.5′ N, 26° 39.8′ E). The climate here is characterised as a transitional climate zone between maritime and continental. In 2016, the mean annual temperature was 6.7 °C, and had annual rainfall of 696 mm[29]. The soil at the experimental site is classified as Fragic Glossic Retisol associated with Stagnic Luvisol (IUSS WG WRB 2015), with a sandy loam texture.

The field experiment was arranged in a split-block design, with three replicates (Fig. 1). The treatments constituted a factorial combination of three crops, two fertilisation treatment levels and five mineral fertiliser application rates (the same crop rotation and fertilisation treatments have been used since 1989). Crops were arranged in strips across the fertilisation treatments representing the main plots, and the five nitrogen application rates as subplots (10 × 5 m). The crops studied were potato (cultivar 'Manitou'), spring wheat (cultivar 'Vinjett') and spring barley (cultivar 'Anni'). The fertilisation treatments included mineral nitrogen fertilisation (without manure, hereafter WOM) and mineral nitrogen fertilisation combined with 40 t ha^{-1} of farmyard manure (hereafter FYM). The five nitrogen fertiliser application rates were 0 (N0), 40 (N40), 80 (N80), 120 (N120) and 160 (N160) kg ha^{-1}, and were applied to and mixed with the soil as ammonium nitrate during spring cultivation. Farmyard manure was applied to FYM potato plots in autumn before potato planting. An overview of treatments is provided in Supplementary Table S1. Wheat and barley root samples were collected on 20 July 2016. Due to the later planting, the potato samples were collected on 9 August 2016. Using a clean shovel, three root samples were collected from the 10–15 cm soil layer in each subplot. Each root sample consisted of the entire root system of three randomly chosen individual plants. The roots were cleaned from the soil, dried at 70 °C for 48 h, and stored dry at room temperature until molecular analysis[30].

Soil chemical analysis. In spring, before fertiliser application, eight subsamples, 20 cm in depth, were collected from each plot. All samples were air-dried, sieved to < 2 mm and pooled to obtain the composite sample for each plot. Soil chemical analyses were carried out to assess the amount of total nitrogen (N_{total}), organic carbon ($C_{organic}$), plant-available phosphorus ($P_{available}$) and soil potassium (K), and the soil pH level. N_{total} was measured using the Kjeldahl method[31] and $C_{organic}$ was measured using the Tjurin method[32]. The ammonium lactate method[33] was used to determine the $P_{available}$ and K. The soil pH was determined in 1 M KCl solution.

Molecular analysis. DNA was extracted from 75 mg of roots using the PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA, USA). We made the following modifications to the manufacturer's protocol: 1) root samples were homogenised by bead beating with a MixerMill MM400 (Retsch, Haan, Germany) for 3 min at

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30 Hz with three 3 mm autoclaved steel beads; and 2) the final elution was performed twice with 50 µl of Solution C6. PCR was performed using ITS3-Mix1-5 (CANCGATGAAGAACGYRG)[34] and ITS3Oo (AGTATGYYT GTATCAGTGTC)[35] forward primers and the degenerate reverse primer ITS4ngs (CCTCCSCTTATTGATATG C)[34]. The reverse primer was tagged with one of the 93 identifiers (MIDs, 10–12 bases). Each PCR mix contained 1 µl of DNA, 0.5 µl of each primer (20 pmol), 5 µl of 5xHOT FIREPol Blend Mastermix (Solis Biodyne, Tartu, Estonia) and 18 µl of PCR grade water (Solis Biodyne, Tartu, Estonia). Samples were run in duplicate on an Eppendorf Mastercycler (Hamburg, Germany) under the following conditions: initial 15 min at 95 °C, 25 cycles of 30 s at 95 °C, 30 s at 55 °C, 1 min at 72 °C, and a final cycle of 10 min at 72 °C. The products were visualised on 1% agarose gel stained with ethidium bromide to confirm successful amplification. We used PCR grade water as a negative control, and *Lentinula edodes* dry material as a positive control throughout the experiment. The duplicate PCR products were pooled, purified with a FavorPrep PCR Clean Kit (FavorGen Biotech Corporation, Vienna, Austria), and their concentrations were measured using a Qubit (Invitrogen, Life Technologies, CA, USA). Samples were sequenced on an Illumina MiSeq system (2 × 300 bp, Estonian Genome Centre, University of Tartu).

Bioinformatics. Bioinformatic analyses were performed using the PipeCraft analysis platform[36]. The paired-end reads were quality-trimmed and assembled using vsearch v 1.1.11[37]. The resulting sequences were demultiplexed using mothur v1.36.1[38]. Chimeras were checked using de novo and reference-based (UNITE v7.2)[39] methods as implemented in vsearch[37]. ITSx 1.0.9[40] was used to remove flanking gene fragments and extract the full-length ITS2 region. The high-quality sequences were then clustered into operational taxonomic units (OTUs) at a 97% sequence similarity threshold with CD-Hit v4.6[41]. Singleton OTUs were removed from further analyses. For taxonomic assignment, a representative sequence from each OTU was selected for BLASTn search (word size = 11; gap open = 5; gap extension = 2; reward = 2; penalty = − 3)[42] against the UNITE v7.2[39] database. We conservatively considered BLASTn search results with an e-value < e−50 reliable enough to taxonomically assign OTUs. The taxonomy of OTUs was assigned based on the consensus taxonomic assignment taking into consideration the ten best BLAST hits when at least eight agreed on the same taxonomic level. The raw data of this study are publicly available through the Sequence Read Archive, BioProject PRJNA541805.

Functional assignment. Fungal guilds of OTUs were classified using FUNGuild[43]. Where OTU fungal guild had the assignment of a plant pathogen, these were assigned as plant pathogens, (2) The guilds "plant saprotroph", "soil saprotroph", "dung saprotroph" and "undefined saprotroph" were merged into saprotrophic fungi. All arbuscular mycorrhizal fungi (AMF) were assigned as plant symbionts. For this study, we used the confidence rankings "probable" and "highly probable". However, one exception was made: (1) In FunGuild, the genera *Alternaria*, *Fusarium* and *Phoma* are assigned both as plant pathogens and saprotrophs with a confidence ranking of "possible". However, these genera are well-known soilborne fungi with split ecology[44]. Therefore, we decided also to include *Alternaria* spp., *Fusarium* spp. and *Phoma* spp. in our analysis. OTUs that were not assigned as pathogens by FUNGuild, but considered as pathogens of potato, wheat and barley, and reported in Europe according to the Agricultural Research Service of the United States Department of Agriculture (https:// nt.ars-grin.gov/fungaldatabases/), were additionally assigned as pathogens. As an exception, we removed *Clonostachys* spp. from the pathogen list assigned by FUNGuild, due to its known use in agriculture as a biocontrol agent[45,46].

Statistical analysis. We used two ecological measures—species richness and Simpson index—to study the α-diversity of root fungal communities. Species richness was calculated based on the linear regression of OTU richness and the square root of the number of sequences to account for differences in sequencing depth[34,47]. The Simpson (1-λ) index was calculated using Primer + software on standardised and transformed tables (square-root transformation for overall fungal and arbuscular mycorrhizal abundance, and fourth-root transformation for pathogen and saprotroph abundance)[48]. A linear mixed-effects model (LMER) was used to test the effect of explanatory variables on fungal diversity indices (package "car" and "lme4" in R 3.6.0, R Development Team, https://www.R-project.org). The fixed factors included in the model were crop species, fertilisation treatment and fertiliser application rate. Replication block was included as a random factor. All tests were carried out using type II Wald Chi-Square tests. The "emmeans" package for R was used to perform the post hoc Tukey test for pairwise comparisons between variable categories. The significance threshold value was set at $P < 0.05$. In addition, LMER-analysis was applied to test the effect of fertilisation on soil chemical properties. All soil variables, except pH, were log-transformed before analysis. The model included two fixed factors (fertilisation treatment, fertiliser application rate) and one random factor (replication block). There was no significant interaction between fertilisation treatment and fertiliser application rate for any soil variable. Therefore, pairwise analysis for fertiliser application rate was conducted within the fertilisation treatment group.

As implemented in PRIMER 7 (PRIMER-E, Auckland, New Zealand), PERMANOVA +[49] with 9999 permutations, Monte Carlo tests, and pooling under a reduced model was used to compare the variability of fungal community composition, as well as of separate fungal guilds across experimental factors. The accompanying adjusted R^2 value was calculated in R using the function RsquareAdj in the package "vegan". These results were highlighted by a canonical analysis of principal coordinates (CAP)[50]. The read abundance data was standardised (by samples) and transformed (square-root transformation for overall fungal abundance, and fourth-root transformation for pathogen and saprotroph abundance) before calculating the Bray–Curtis similarity index. Due to multiple zero values in the data matrix, the analysis for AMF community composition was carried out using a modified Gower log10 resemblance matrix[51]. To test the effect of soil properties on root fungal community composition, we used the non-parametric multivariate regression DistLM[52] in PERMANOVA + based on the abovementioned

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| | WOM | | | | | FYM | | | | | |
	N0	N40	N80	N120	N160	N0	N40	N80	N120	N160	Pr(> Chisq)†
pH	6.12^a (±0.06)	6.02^{ab} (±0.06)	5.96^{abc} (±0.07)	5.76^{bc} (±0.09)	5.68^c (±0.08)	6.24 (±0.06)	6.23 (±0.10)	6.19 (±0.99)	6.10 (±0.10)	6.03 (±0.11)	<0.001***
C_{org}	0.98 (±0.03)	0.98 (±0.43)	1.01 (±0.02)	1.00 (±0.01)	0.98 (±0.02)	1.23 (±0.03)	1.26 (±0.04)	1.28 (±0.03)	1.30 (±0.03)	1.31 (±0.02)	<0.001***
N_{tot}	0.06 (±0.03)	0.07 (±0.04)	0.07 (±0.02)	0.07 (±0.01)	0.07 (±0.02)	0.09 (±0.01)	0.09 (±0.01)	0.11 (±0.01)	0.09 (±0.01)	0.10 (±0.00)	<0.001***
P	56.33^a (±3.14)	47.11^{ab} (±2.34)	44.89^b (±2.29)	44.00^b (±3.01)	45.89^b (±2.27)	100.00^a (±6.11)	93.38^{ab} (±6.95)	85.89^b (±4.53)	84.22^b (±3.38)	87.00^b (±3.44)	<0.001***
K	92.22^a (±5.18)	76.78^b (±3.80)	77.44^b (±2.96)	75.33^b (±2.37)	80.22^{ab} (±2.63)	178.56 (±9.89)	168.25 (±11.03)	164.78 (±7.12)	157.22 (±7.00)	167.44 (±10.66)	<0.001***

Table 1. Linear-mixed effects model examining the effect of fertilisation treatment (WOM, FYM) and fertiliser application rate (N) on soil chemical properties. WOM, mineral nitrogen fertilisation; FYM, mineral nitrogen fertilisation combined with farmyard manure amendment; N, fertiliser application rate. The number refers to the application rate according to total N (40, 80, 120, 160 kg ha⁻¹). Values are listed as mean ± standard error. Letters indicate statistical differences between soil chemical properties within the fertilisation treatment using Tukey post hoc test following linear-mixed effects models at $P < 0.05$. †Pr(> Chisq) indicates the statistical difference between fertilisation treatments. ***$P < 0.001$ of significance.

resemblance matrices. As recommended by Anderson et al.[49], at first we looked for multicollinearity among soil properties using Draftsman plots. This led to the exclusion of K from the analysis, as it was strongly correlated with C_{org} and P_{total}. Models were generated using the BEST procedure, and the best fitting model was identified using the corrected Akaike's Information Criterion (AICc). P values were calculated using 9999 permutations.

A stacked bar chart was created in R using the package "ggplot", and Venn diagrams created using the package "VennDiagram".

Results

Soil properties. Fertilisation treatment and fertiliser application rate both significantly influenced soil chemical properties (Table 1). Soil pH was significantly lower in the WOM than in the FYM treatment ($\chi^2 = 22.673$, $P < 0.001$). Within the WOM treatment, higher fertiliser application rate significantly reduced soil pH. Soil $C_{organic}$ ($\chi^2 = 225.936$, $P < 0.001$), N_{total} ($\chi^2 = 59.018$, $P < 0.001$), $P_{available}$ ($\chi^2 = 340.889$, $P < 0.001$) and K ($\chi^2 = 596.995$, $P < 0.001$) were significantly higher in the FYM treatment. Furthermore, fertiliser application rate had a significant effect on soil $P_{available}$ content within both fertilisation treatments. Within WOM and FYM treatments, N0 plots harboured significantly higher $P_{available}$ concentration compared to N80, N120 and N160 plots. In addition, within the WOM treatment, N0 plots harboured significantly higher K content compared to N40, N80 and N120 plots.

Identification of fungi. Illumina sequencing of 89 samples yielded 841,519 (mean: 9455; range: 920–18,532) reads that were assigned to 2112 OTUs (Supplementary Table S2). Altogether, 844 OTUs overlapped between roots of the three studied crops (Fig. 2A), and 1514 OTUs between two of the studied fertilisation treatments (Fig. 2B). In potato roots, 37.3% of the sequences remained unidentified. Basidiomycota and Ascomycota accounted for 32.0% and 19.8% of sequences in potato roots, respectively (Fig. 2C). Ascomycota was the most abundant phylum in both wheat and barley roots, comprising 50.8% and 65.5% of sequences, respectively (Fig. 2C). Unidentified fungal sequences represented 26.0% of sequences in wheat and 15.8% in barley. The third most abundant sequences in wheat belonged to Basidiomycota with 15.5% and in barley to unidentified sequences with 13.5%. With regard to fertilisation treatment, Ascomycota was the most abundant phylum in both treatments, accounting for 43.0% and 52.5% of sequences in WOM and FYM treatments, respectively. In WOM, this was followed by unidentified sequences (19.7%) and unidentified fungi (18.3%, Fig. 2D). In FYM, this was followed by unidentified fungi (15.8%) and unidentified sequences (14.9%, Fig. 2D). Of all sequences, 27.2% were assigned to putative pathogens, 9.7% to saprotrophs, 16.6% to taxa with both pathogenic and saprotrophic features, and 0.7% to AMF symbionts. Of the ten most abundant OTUs in different crop species, at least half of these were pathogens (Table 2).

Overall fungal species richness and diversity. Both overall fungal species richness ($P < 0.001$, Table 3) and diversity ($P < 0.001$, Table 4) differed among crop species. Species richness and diversity were highest in wheat roots and lowest in potato roots (Supplementary Table S3). PERMANOVA analysis showed that crop species ($P < 0.001$, adjusted $R^2 = 0.362$) and fertilisation treatment ($P < 0.001$, adjusted $R^2 = 0.025$, Supplementary Table S4) were the main factors determining the differences in fungal community composition. These results were confirmed by CAP analysis (Fig. 3A). DistLM marginal tests showed that when considered individually, each of the studied soil properties had a significant effect on fungal community composition ($P < 0.05$, Table 5). The best fitting model was achieved using the combination of pH and $C_{organic}$, and accounted for 11.3% of the variation in the data cloud (Table 5).

Pathogenic fungi. *Gaeumannomyces* spp. (22.2%), *Rhizoctonia* spp. (teleomorph: *Thanatephorus* spp., 19.7%) and *Phoma* spp. (10.4%) were the most abundant pathogen genera. Crop species affected both pathogen
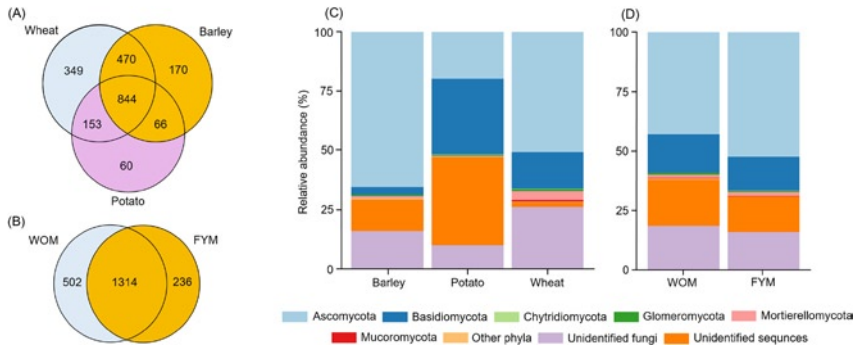
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Figure 2. Venn diagram showing the amount of shared and unique OTUs between the roots of three crop species (**A**) and two fertilisation treatments (**B**). Taxonomic composition of root fungal communities in different crop species (**C**) and fertilisation treatments (**D**). Unidentified fungi are represented by sequences that were assigned only at kingdom level, whereas unidentified sequences represent sequences with no match. WOM, mineral nitrogen fertilisation; FYM, mineral nitrogen fertilisation combined with farmyard manure 40 t h^{-1}.

	Potato				Wheat				Barley			
	OTU	Taxonomy	Fungal guild	%	OTU	Taxonomy	Fungal guild	%	OTU	Taxonomy	Fungal guild	%
1	Otu0536	Unidentified	Unassigned	26.8	Otu2329	*Phoma* spp.	Pathogen/Saprotroph	7.1	Otu1303	*Gaeumannomyces* spp.	Pathogen	21.9
2	Otu0930	*Rhizoctonia* spp.	Pathogen/Saprotroph	21.4	Otu1760	*Bolbitaceae* spp.	Saprotroph	5.7	Otu0164	Unidentified	Unassigned	12.9
3	Otu3047	*Colletotrichum coccodes*	Pathogen	6.2	Otu2602	*Microdochium bolleyi*	Pathogen	5.4	Otu2467	*Magnaporthaceae* spp.	Pathogen	9.6
4	Otu0364	*Thanatephorus cucumeris*	Pathogen/Saprotroph	4.7	Otu3413	*Cladosporium herbarum*	Pathogen	5.1	Otu2602	*Microdochium bolleyi*	Pathogen	5.2
5	Otu2704	Fungi	Unassigned	4.4	Otu3648	*Sordariomycetes* spp.	Unassigned	4.6	Otu3648	*Sordariomycetes* spp.	Unassigned	4.5
6	Otu0714	Unidentified	Unassigned	3.1	Otu0522	Fungi	Unassigned	3.4	Otu2329	*Phoma* spp.	Pathogen/Saprotroph	3.7
7	Otu2329	*Phoma* spp.	Pathogen/Saprotroph	2.6	Otu1363	*Exophiala equine*	Saprotroph	2.4	Otu0522	Fungi	Unassigned	3.2
8	Otu2581	*Gibellulopsis nigrescens*	Pathogen	2.3	Otu1954	Fungi	Unassigned	2.2	Otu2664	*Magnaporthaceae* spp.	Pathogen	2.5
9	Otu1943	Fungi	Unassigned	1.9	Otu1525	*Lasiosphaeriaceae* spp.	Saprotroph	2.0	Otu3413	*Cladosporium herbarum*	Pathogen	2.4
10	Otu0920	*Ceratobasidiaceae* spp.	Unassigned	1.7	Otu3288	*Fusarium* spp.	Pathogen/Saprotroph	1.6	Otu1525	*Lasiosphaeriaceae* spp.	Saprotroph	1.5

Table 2. Relative abundance of 10 most abundant OTUs in the roots of potato, wheat and barley.

species richness ($P < 0.001$, Table 3) and diversity ($P < 0.001$, Table 4). Both measures were highest in wheat roots and lowest in potato roots (Supplementary Table S5). Highest pathogen richness was in unfertilised (N0) wheat roots, and lowest pathogen richness in unfertilised (N0) potato roots (Supplementary Table S5). In general, pathogen richness was higher in the FYM plots ($P = 0.003$, Table 3). Furthermore, potato grown both in the FYM and WOM plots had significantly lower pathogen diversity compared both wheat and barley grown both in thein either WOM and or FYM plots (Supplementary Table S5).

Crop species was the main variable explaining the variation ($P < 0.001$, adjusted R^2 = 0.407) in community composition, while other variables had only a minor contribution (Supplementary Table S4). Following the pattern for the total fungal community, the pathogen community composition was substantially different among all crop species (Fig. 3B). Soil pH and N$_{total}$ were statistically significant in DistLM marginal tests, but each variable explained less than 8% of the variation (Table 5). Furthermore, the most fitting model resulted from combining pH and N$_{total}$, and accounted for 11.1% of the variation (Table 5).

	df	Overall		Pathogens		AMF		Saprotrophs	
		Chi-Square value	Pr(> Chisq)ᵃ	Chi-Square value	Pr(> Chisq)	Chi-Square value	Pr(> Chisq)	Chi-Square value	Pr(> Chisq)
Crop	2	313.393	<0.001***	209.578	<0.001***	3.577	0.167	528.726	<0.001***
Treatment	1	2.082	0.149	4.577	0.032*	19.374	<0.001***	8.894	0.003**
Nᵇ	4	5.783	0.216	3.026	0.553	3.812	0.432	14.469	0.006**
Crop × TREATMENT	2	4.409	0.110	3.053	0.217	8.894	0.012*	8.931	0.011*
Crop × N	8	9.202	0.326	19.928	0.010*	9.723	0.285	6.086	0.638
Treatment × N	4	9.108	0.058	6.087	0.192	5.641	0.228	10.049	0.040*
Crop × treatment × N	8	13.324	0.101	7.432	0.491	11.090	0.197	14.430	0.071

Table 3. Results of linear mixed effect models estimating the effect of crop species, fertilisation treatment, fertiliser application rate and their interaction on species richness for all root fungi, pathogens, arbuscular mycorrhizal fungi and saprotrophs. *AMF* arbuscular mycorrhizal fungi, *df* degrees of freedom. ***$P < 0.001$ of significance; **$P < 0.01$ level of significance; *$P < 0.05$ level of significance. ᵃPr(> Chisq) associated probability value corresponding to the test that all of the predictors are simultaneously equal to zero. ᵇN fertiliser application rate.

	df	All fungi		Pathogens		AMF		Saprotrophs	
		Chi-Square value	Pr(> Chisq)ᵃ	Chi-Square value	Pr(> Chisq)	Chi-Square value	Pr(> Chisq)	Chi-Square value	Pr(> Chisq)
Crop	2	289.651	<0.001***	148.581	<0.001***	9.331	0.009**	253.923	<0.001***
Treatment	1	0.220	0.639	1.182	0.277	8.194	0.004**	0.520	0.471
Nᵇ	4	1.872	0.759	2.946	0.567	3.802	0.433	3.099	0.541
Crop × Treatment	2	5.891	0.053	13.691	0.001**	14.951	<0.001***	11.483	0.003**
Crop × N	8	9.858	0.275	11.419	0.179	14.988	0.059	2.745	0.949
Treatment × N	4	3.798	0.434	5.420	0.247	4.111	0.391	10.051	0.040*
Crop × Treatment × N	8	4.109	0.847	4.564	0.803	10.033	0.263	7.016	0.535

Table 4. Results of linear mixed effect models estimating the effect of crop species, fertilisation treatment, fertiliser application rate and their interaction on inverse Simpson diversity index for all root fungi, pathogens, arbuscular mycorrhizal fungi and saprotrophs. *AMF* arbuscular mycorrhizal fungi, *df* degrees of freedom. ***$P < 0.001$ of significance; **$P < 0.01$ level of significance. ᵃPr(> Chisq) associated probability value corresponding to the test that all of the predictors are simultaneously equal to zero. ᵇN fertiliser application rate.

Arbuscular mycorrhizal fungi. Of AMF, Glomeraceae was the most abundant order (81.2%), with the genus *Rhizophagus* (11.3%) dominating. AMF species richness was significantly affected by fertilisation treatment ($P < 0.001$) and crop x fertilisation treatment interaction ($P = 0.012$, Table 3). The roots of potato grown in the FYM plots showed a significant reduction in AMF richness compared to the roots of potato, barley and wheat grown in WOM plots (Supplementary Table S6). Diversity was significantly different between crop species ($P = 0.009$), fertilisation treatment ($P = 0.004$) and their interaction ($P < 0.001$, Table 4). Potato grown in FYM plots had significantly lower AMF diversity compared to any other crop and fertiliser treatment combination (Supplementary Table S6).

Crop species had a significant effect on AMF community composition, explaining 4.7% of the variation ($P = 0.001$, Supplementary Table S4). Other factors had a minor contribution to AMF community variation (Supplementary Table S4). Furthermore, CAP analysis showed only weak clustering of crop species and fertilisation treatment (Fig. 3C). DistLM marginal tests showed that pH, $C_{organic}$ and $P_{available}$ were significant soil properties in explaining AMF community composition (Table 5). However, the best model included only $P_{available}$, and explained 3.7% of the total variation (Table 5).

Saprotrophic fungi. *Rhizoctonia* spp. (32.7%), *Phoma* spp. (17.2%) and *Fusarium* spp. (9.9%) were the most abundant genera. Saprotroph species richness and diversity were significantly affected by crop species and treatment interaction ($P < 0.001$, Table 3, Table 4). Wheat grown in FYM plots harboured significantly higher saprotroph richness compared to any other crop and fertiliser treatment combination (Supplementary Table S7). Independent of crop species, saprotroph richness was the lowest in WOM plots treated with the highest fertiliser application rate (N160) (Supplementary Table S7). Saprotroph diversity was the highest in wheat and barley

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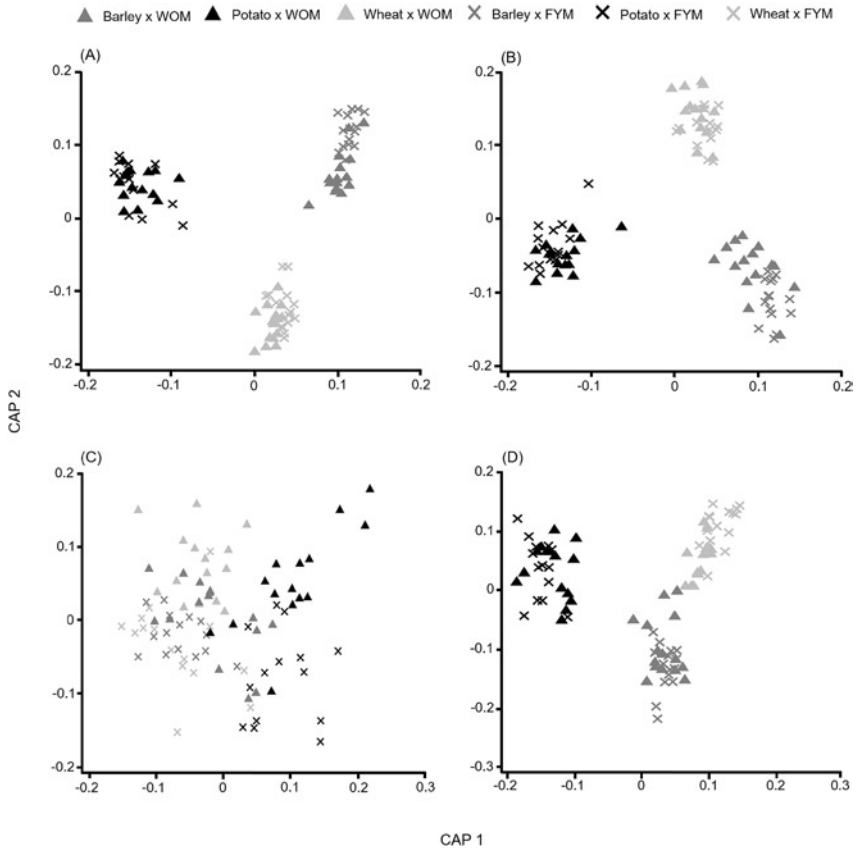
Figure 3. Canonical analysis of principal coordinates (CAP) based on Bray–Curtis similarity matrix (all fungi, pathogens, saprotrophs) and on modified Gower log10 matrix (arbuscular mycorrhiza) to model the effect of crop and treatment for overall fungal (**A**), pathogen (**B**), arbuscular mycorrhizal (**C**) and saprotroph (**D**) community composition. WOM, mineral nitrogen fertilisation; FYM, mineral nitrogen fertilisation combined with farmyard manure 40 t h⁻¹.

grown in the FYM plots, whereas the lowest values were observed in potato grown both in WOM and FYM plots (Supplementary Table S7).

PERMANOVA analysis showed a significant effect of crop species ($P < 0.001$, adjusted $R^2 = 0.275$) and fertilisation treatment ($P = 0.007$, adjusted $R^2 = 0.012$) on saprotroph community composition (Supplementary Table S4). According to CAP analysis, considerably different saprotroph community compositions were observed depending on both crop species and fertilisation treatment (Fig. 3D). DistLM analysis showed a significant effect of each soil variable on saprotroph community composition in marginal tests (Table 5). However, each variable explained less than 5% of the variation. Moreover, the best model included only pH and $C_{organic}$ as predictors and explained 7.8% of the total variation (Table 5).

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Marginal tests	Overall			Pathogens			AMF			Saprotrophs		
	Pseudo-F[a]	P[b]	Prop[c]	Pseudo-F	P	Prop	Pseudo-F	P	Prop	Pseudo-F	P	Prop
pH	6.278	<0.001***	0.067	7.183	<0.001***	0.076	2.487	0.002**	0.028	4.007	<0.001***	0.044
$C_{organic}$	3.454	0.002**	0.038	2.006	0.051	0.023	2.408	0.002**	0.0267	2.371	0.016*	0.027
N_{total}	3.738	0.001**	0.041	3.044	0.009**	0.034	1.321	0.112	0.015	2.965	0.006**	0.033
$P_{available}$	3.340	0.003**	0.037	1.928	0.063	0.022	3.347	<0.001***	0.037	2.154	0.029*	0.024
	Variables	AICc[d]	R²[e]	Variables	AICc	R²	Variables	AICc	R²	Variables	AICc	R²
Best overall solution	pH and $C_{organic}$	680.91	0.113	pH and N_{total}	632.78	0.110	P	−51.64	0.037	pH and $C_{organic}$	652.41	0.078

Table 5. Results of the distance-based linear model (DistLM) analysis estimating the effect of soil chemical properties for overall, pathogen, arbuscular mycorrhizal fungal and saprotroph community composition. AMF, arbuscular mycorrhizal fungi. [a]Pseudo-F statistic for testing the general null hypothesis of no relation. [b]P P value. [c]Prop Proportion of explained variation for each variable. [d]AICc Akaike corrected value. [e]R^2 Proportion of explained variation for the model. ***$P < 0.001$ of significance; **$P < 0.01$ level of significance; *$P < 0.05$ level of significance.

Discussion

We documented the patterns of root fungal communities in response to three crop species, two types of fertilisation treatment and five fertiliser application rates. In support of our first hypothesis, fungal community diversity and composition differed substantially among crop species, indicating that agricultural plant species shape their root mycobiome. How plants affect their fungal communities can be related to differences in root traits and root exudates[53,54]. Plants produce root exudates that vary between plant species and thus establish a unique root microbe community[55]. These differences are more significant between phylogenetically distant species[56].

Moreover, roots also secrete root border cells and mucilage, both of which can vary between plant species[57,58]. Koroney et al.[59] showed that there are galactan-containing polymers in potato mucilage. In wheat roots, the abundance of galactan-containing polymers has been observed to be relatively low[60]; thus galactan-containing polymer content may be one cause for the differences in root microbe communities between cereals and potato, observed in our study. In addition, plant root architecture can influence microbial communities both directly and indirectly[5]. Both overall fungal- and pathogen diversity were greatest in wheat, followed by barley and potato. The higher fungal diversity in cereals, compared with potato, may be related to their more differentiated root structure[61] or phylogenetic effects[62]. Cereals have strong fibrous root systems, which branch throughout the life of the plant[63–65], whereas the potato root system is considered shallow and sparse[66]. Furthermore, wheat plants exhibit a higher total volume of roots, compared to potato[67]. Therefore, the greater root surface area of cereals may provide more adhesion sites for fungi.

Our study revealed a relatively high frequency of pathogens compared with previous studies in agricultural fields[68] and forests[69]. The particularly high abundance of pathogens on barley may be related to crop rotation. The most abundant OTU in barley was identified as *Gaeumannomyces* spp., which are common root disease agents in various cereals. In our study, barley followed wheat in crop rotation. Having suitable plant hosts in rotation across two consecutive years may have allowed the accumulation of pathogens. These results are consistent with Chen et al.[70] and Song et al.[71], who showed the effect of continuous cropping on pathogen increase. Different crop species in the rotation that do not share common pathogens can help to break the life cycle of plant pathogens and hinder their establishment in the field over time[72].

Root symbiotic AMF accounted for < 1% of sequences, which is in accordance with previous studies showing a low amount of Glomeromycota rRNA genes in the roots of crop plants[73]. While the AMF assemblages were similar between wheat and barley, potato showed greater differences. Plant host could be the major determinant affecting root AMF communities[74], but this may also be related to differences in root structure, phylogenetic distance, or our three-week interval between sampling events.

Saprotrophs also showed distinct communities in roots of crop species. In line with this study, Francioli et al.[75] have shown plant species is the main factor in shaping the root-associated saprophytic fungal community. They argued that the variation between communities may be driven by differences in C:N ratio and root lignin content. Furthermore, Mariotte et al.[76] highlighted the importance of different organic inputs in decomposer communities. Therefore, saprotrophs may have developed plant tissue specificity, allowing the development of distinct saprotroph communities in the crop roots. It is also possible that some of these saprotrophs act as pathogens in certain plant species or fertilisation treatments, which may favour their accumulation in specific plant taxa.

The total fungal community showed no response to fertilisation treatments. In previous studies, both inorganic and organic nitrogen fertilisation have demonstrated substantial effects on fungal diversity and composition in agricultural plants[77,78]. Furthermore, in this study soil, chemical properties were significantly different between WOM and FYM plots. However, soil properties had a relatively weak effect in determining fungal community composition. This may be related to a lower fungal sensitivity towards changes in soil properties[79–81]. It is possible that after several years of fertilisation at our field site, the local fungal communities had been selected to tolerate high levels of fertilisation and continuous disturbance (tillage), and therefore, here, fertilisation type and application rate play minor roles in shaping the root fungal microbiome. A stable fungal community in response to long-term fertiliser amendment has also been observed by Marschner et al.[82] and Ai et al.[83].

Pathogen richness and diversity were higher in the roots of wheat and barley grown in FYM plots. Farmyard manure amendments may result in a more eutrophic environment[84,85] and, together with the more complex root structure of wheat and barley provide a more suitable habitat for pathogens. This assumption is supported by the fact that potato roots harboured the lowest pathogen richness and diversity.

In general, the lowest AMF diversity was observed in FYM plots, whereas soil nutrient levels, including $P_{available}$, were highest in FYM plots. Studies have shown that higher phosphorus concentration can decrease AMF colonisation in roots and may cause a shift in soil AMF community composition[86,87]. It is possible that in WOM plots, AMF mediated nutrient acquisition for the crops. However, in FYM plots, the manure amendment may have saturated soil nutrient concentrations, reducing AMF diversity. This assumption is supported by the DistLM analysis, which suggested that $P_{available}$ is the only soil variable influencing AMF community composition. Nutrient saturation may also explain the lowest AMF richness and diversity in potato grown in FYM plots since these plots had the most recent farmyard manure amendment.

Saprotrophs were generally more diverse in FYM plots. Results showing an increase in saprotroph diversity in manure-amendment-treated fields have also been reported in other recent studies[88,89]. Saprotrophs are important for decomposing and mineralising organic matter in agricultural soils[44,90], and thus a positive relationship between soil organic matter and saprotroph richness and diversity may be expected. The three most abundant saprotroph taxa (*Rhizoctonia* spp., *Phoma* spp., *Fusarium* spp.) were also assigned as pathogens. Members of these genera are common soil inhabitants that become pathogenic under favourable conditions[44]. We speculate that although manure amendment itself did not affect pathogen communities in our study, its beneficial impact relies on the increase in fungi with saprotrophic characteristics. This is in agreement with earlier observations that several plant pathogens are viable on organic matter and increase their inocula due to a saprotrophic mode of nutrition[91,92].

Conclusion

Root fungal diversity and composition are strongly shaped by crop species, the effect of which prevails over that of fertilisation treatment. The relatively small effect of fertilisation treatment and fertiliser application rate may be explained by the stability of the local agricultural system after years of fertilisation. Therefore, our results indicate that within a conventional system, organic manure amendment does not enhance the root mycobiome. Although the root mycobiome remained relatively unaffected by fertilisation treatment, nitrogen fertilisation may affect bacteria, free-living soil fungi or soil conditions. To gain further insights into the interactions between agricultural management and microbiomes, future studies should be carried out on multi-crop experimental sites, at larger spatial scales, and include additional groups of microorganisms.

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Author contributions

L.S. and K.L. participated in the study design, collected the samples and performed laboratory analyses of root samples. A.A. performed the laboratory analyses for soil samples. L.S. performed statistical analyses. The first draft was written by L.S. and was revised by L.T. All authors commented on previous versions of the manuscript. A.A., E.R.-P., K.L. and L.T. substantially revised the work. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Temporal and Cultivar-Specific Effects on Potato Root and Soil Fungal Diversity

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Abstract: The soil fungal community plays an important role in determining plant growth and health. In this study, we investigated the fungal diversity and community composition in the roots and soil of 21 potato (*Solanum tuberosum* L.) cultivars using high-throughput sequencing at three different time points across the growing season. In soil and roots, the fungal richness and relative abundance of pathogens and saprotrophs were mainly affected by sampling time. While sampling time affected fungal composition in soil, root fungal communities were also significantly affected by cultivar. The cultivar had the strongest effect on diversity of pathogens and abundance of particular pathogen species. Our results demonstrate changes in soil and root fungal communities of potato over the growing season, as well as highlighting the importance of potato cultivar on root fungal communities and abundance of pathogens.

Keywords: *Solanum tuberosum*; agroecosystems; high-throughput sequencing; fungal guild; fungal diversity; host specificity; potato cultivars

1. Introduction

Modern agriculture has increased crop yields significantly [1]; however, it often relies on chemical fertilisers and pesticides [2], and thus can negatively impact the environment [3], including soil ecosystems [4–6]. Soils harbour millions of microorganism species, many of which form intimate associations with plant roots [7,8]. These associations may have direct, i.e., intimate mutualistic or pathogenic interactions with plants, or indirect effects, through the action of free-living microbes that affect nutrient availability in the surrounding environment [9]. Both biotrophic and saprotrophic fungi can affect plant productivity.

In agricultural fields, plant-associated and saprotrophic microbial communities are affected by different management practices such as crop rotation, fertilisation and tillage [10–14]. Different plant species and different growth stages may harbour distinct microbial communities [15–17]. Furthermore, plants modify their associated and surrounding microbial communities by exuding organic compounds into the surrounding environment, which act as nutrient sources for microbes [18,19]. Higher fertilisation rates may increase root exudation as well as microbial abundance.

Since breeding programs are usually conducted at high nutrient levels [8,20], it is likely that breeding affects microorganisms inhabiting the plant-soil interface. This may lead to changes in native soil communities, including beneficial plant microbes, as high nutrient conditions are substantially different from those environments where the interaction has evolved [20].

Potato (*Solanum tuberosum* L.) is one of the most important staple crops that can grow in broad climatic conditions, and had a global production of over 368 million tons in 2018 [21]. Regardless of climate, cultivated potatoes are constantly exposed to various pathogens that pose a serious threat to potato production worldwide [22–25]. Several studies have focused on individual pathogens of potato [22–24,26,27]. However, to our knowledge, there is a lack of community-level data regarding other putative hazardous soil-borne fungal pathogens, as well as genotypic differences in biotic stress resistance in potato plants. Screening and selecting plant genotypes that would resist pathogens, and even associate with beneficial microbes, may improve the health and yield of this important crop [20,28].

The objective of this study was to assess differences in fungal community structure, both within roots and in soil, of 21 potato cultivars. Our goal was to examine the relationship between plant genotype and associated fungal guilds (saprotrophic fungi, pathogenic fungi and arbuscular mycorrhizal fungi), as well as overall fungal community composition, over the growing season. We postulated two hypotheses: (1) fungal guilds differ in their responses to potato genotype, and (2) fungal guild composition and diversity differ among plant growth stages.

2. Materials and Methods

2.1. Study Site

The study site was located at Einola Farm (58°17′02.0″ N 26°43′19.6″ E) in Reola, Tartu County, Estonia. The study was conducted under a conventionally managed farming system from May to September 2014, according to the following practices: primary tillage by moldboard plowing (with straw addition) in late autumn, secondary tillage in early spring, seedbed preparation by harrowing and furrowing in early spring, and hilling, which was conducted three times during the growing season. No irrigation was used. The climate of the study area is characterised as a transitional climate zone between maritime and continental. In 2014, the mean annual temperature was 7.1 °C, with the annual rainfall being 592 mm [29]. At the study site, winter wheat (*Triticum aestivum* L.), spring wheat (*T. aestivum*), rapeseed (*Brassica napus* L.), spring wheat and potato have been grown in succession since 2010. Twenty-one cultivars of potato were grown in a randomised block design with three replicate plots per cultivar, each containing 23 certified seed tubers (Table S1) that were stored at 3–4 °C. Tubers were kept at 10–15 °C for three weeks before being planted into the bottom of the furrow on 15 May. The potato field was treated with foliar fungicides containing trifloxystrobin + tebuconazole (Glory 450 SC), amisulbrom (Leimay), mancozeb + metalaxyl M (Ridomil Gold MZ 68 WG), fluopicolide + propamocarb (Infinito) and cyazofamid (Ranman Top). Detailed information about field operations is shown in Table S2.

2.2. Soil Chemical Analysis

During planting, three subsamples of soil were collected from 0–20 cm depth from each plot. All samples were air-dried, sieved to <2 mm and pooled to obtain one composite sample for each plot. Soil chemical analyses were carried out to assess soil plant-available (ammonium lactate extraction method [30]) phosphorus (P), potassium (K), magnesium (Mg) and calcium (Ca) content, as well as soil pH_{KCl}. Total nitrogen (N) and carbon (C) content of air-dried samples was determined by dry combustion, using a varioMAX CNS elemental analyser (ELEMENTAR, Langenselbold, Germany). Soil parameters are indicated in Table S3.

2.3. Sampling and DNA Extraction

Root samples were collected at the early flowering stage (BBCH 60–62) and during senescence (BBCH 93–95) [31]. Cultivars were classified as early-, medium- or late-maturing, based on their maturity period. Maturity classes comprised eight early-, seven intermediate- and six late-maturing cultivars (See Table S1 for list of cultivars). Based on the different flowering times of potato cultivars, root samples were collected on 9 July, 16 July and 22 July. During the plant senescence stage (SSC), root samples of all cultivars were collected on 26 August. The root systems of three individual plants from each plot were randomly chosen and collected using a clean shovel. Roots were washed three times with deionised water to remove residual soil, dried at 50 °C for 24 h, as described in García de León et al. [32], and stored air-tight at room temperature until molecular analysis. Soil samples were collected three times over the study period: during planting (BEM), and again during flowering (FL) and senescence (SSC), simultaneously with root sampling. Each sample consisted of three subsamples of soil taken randomly from the potato root zone at 0–25 cm depth. At BEM, roots were not developed and therefore, these samples represent bulk soil. The subsamples were pooled and air-dried at <35 °C following Tedersoo et al. [33], mixed thoroughly and subjected to molecular analysis.

Total DNA was extracted from 0.075 g dry weight of roots and 0.2 g dry weight of soil, using the PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA), following manufacturer's instructions with the following modifications: (1) samples were homogenised via bead beating with three 3 mm autoclaved steel beads for 5 min at 30 Hz, using a MixerMill MM400 (Retsch, Haan, Germany); and (2) final elution was performed twice with 50 μL solution C6.

2.4. PCR Amplification and High-Throughput Sequencing

The ITS2 region was amplified using ITS3mix1-5 (mixture of six forward primers in equimolar concentration analogous to ITS3) and a degenerate reverse primer ITS4ngs, which was tagged with one of the 108 multiplex identifiers [33]. PCR amplification was performed in a 25 μL reaction volume, and consisted of 18 μL nuclease-free water, 5 μL 5× HOT FIREPol Blend Mastermix (10mM MgCl2) (Solis Biodyne, Tartu, Estonia), 0.5 μL of each primer (20 pmol) and 1 μL DNA extract. PCR was performed using Eppendorf 5341 and Eppendorf 6321 thermal cyclers (Eppendorf AG, Hamburg, Germany) in four replicates, under the following thermocycling conditions: 15 min at 95 °C, 35 cycles of 30 s at 95 °C, 30 s at 55 °C, 1 min at 72 °C and 10 min at 72 °C. PCR products were pooled, and their relative quantities were estimated by running 5 μL of amplicon DNA on 1% agarose gel (SeaKem LE Agarose, Lonza Group Ltd., Basel, Switzerland) for 15 min. PCR products were pooled for library preparation on the basis of relative band strength, as visualized on an agarose gel, which is indicative of amplicon concentration. To obtain sufficient PCR product, DNA samples yielding no visible band, and samples with a very strong band, were re-amplified by altering the number of cycles. The quantities of PCR products were normalised with SequalPrep Normalisation Plate Kit (Invitrogen, Carlsbad, CA, USA). The sequencing libraries were prepared using a Nextera XT kit (Illumina Inc., San Diego, CA, USA). All samples were sequenced on an Illumina MiSeq instrument at the Estonian Genome Center (University of Tartu, Tartu, Estonia).

2.5. Bioinformatics

Paired-end sequencing (2 × 300 bp) resulted in 772,326 paired reads. Sequencing reads were quality-filtered and assigned to samples using mothur 1.34.4 [34] (average quality over 15 bp ≥ 26, and no ambiguities allowed). The quality-trimmed data were assembled using PANDAseq Assembler [35], with a minimum overlap of 15 bp, and demultiplexed in mothur. Potential chimeric sequences were removed using USEARCH 7.0.1090 [36]. The remaining chimeric sequences, where full primer strings were detected inside the reads, were removed using PipeCraft in-built module (remove multiprimer artefacts) [37]. The ITS2 subregion was extracted using ITSx 1.0.9 [38], and clustered using a 97% similarity threshold in CD-HIT [39]. Singleton OTUs were removed from further

analyses. Representative sequences for BLASTn search were picked in mothur using the abundance method. In addition, BLASTn searches were performed for the representative sequence of each OTU against the UNITE reference dataset v7.0 [40]. OTUs were further checked and filtered based on BLASTn search values as well as positive and negative controls to remove contaminants, non-fungal OTUs, potential artefacts and index-switching errors. OTUs with 75%, 80%, 85%, 90%, 95%, and 97% sequence similarity thresholds were considered to represent the phylum, class, order, family, genus, and species level, respectively [33]. Based on taxonomic assignments, OTUs were parsed to one of the following functional groups of fungal guilds: plant pathogenic fungi, saprotrophic fungi and arbuscular mycorrhizal fungi, based on FUNGuild [41]. OTUs not assigned to plant pathogens by FUNGuild, but reported as potato pathogens according to the United States Department of Agriculture (USDA) Agricultural Research Service (U.S. National Fungus Collections Fungus-Host Database. Available online: https://nt.ars-grin.gov/fungaldatabases/), were additionally assigned as pathogens. The raw data of this study are available through the Sequence Read Archive, BioProject PRJNA638263.

2.6. Statistical Analysis

We calculated linear regression between a number of obtained OTUs per sample and square root of the obtained sequences per sample, in order to control for variation in sequencing depth [33]. The standardised residuals of OTU richness were used as a proxy for fungal richness. In roots, linear regression explained 51%, 40.9%, and 36% of variation in OTU richness of all fungi, pathogens, and saprotrophs, respectively. In soil, square-root of sequencing depth explained 72.1%, 20.6%, and 67.2% of variation in OTU richness of all fungi, pathogens and saprotrophs, respectively. The relative abundance of fungal guilds was calculated as a number of sequences corresponding to the particular guild divided by the total number of fungal sequences for each sample.

Differences in richness and relative abundance of total fungal communities, as well as those of fungal guilds, were tested using GLM (Type III SS) followed by Tukey HSD post hoc tests comparing the means of standardised residuals of OTU richness and relative abundance ($\alpha = 0.05$; Statistica 12.0, Palo Alto, CA, USA). Explanatory variables included potato cultivar, plant growth stage (fixed factor with levels BEM, FL, SSC), time × cultivar interaction, and replicate block (random factor with three levels). PERMANOVA+ [42], implemented in Primer 7 software (PRIMER-E, Auckland, New Zealand), was used to study the community composition of both the overall fungal community, as well as that of separate fungal guilds. Three samples (LK129, LK130 and LK131) were removed from soil total fungal and saprotroph datasets, as they contained only a few sequences and OTUs. PERMANOVA+ tests were carried out with 9999 permutations under the reduced model. The read abundance was standardised by samples and fourth-root transformed before calculating the Bray-Curtis dissimilarity index. Adjusted R^2 values were calculated using the function RsquareAdj in the package "vegan" in R 3.6.0 (R Development Team, http://www.R-project.org). Trends in fungal composition were visualised via non-metric multi-dimensional scaling (NMDS) ordinations, as implemented in the metaMDS function in both "vegan" and "ggplot" packages.

3. Results

The quality-filtered sequence dataset comprised 224,195 high quality, full length ITS2 reads in 315 samples, with an average number of 712 sequences per sample. These sequencing reads were assigned to 1655 fungal OTUs, with 80 OTUs in root samples and 110 OTUs in soil samples on average (Table S4). In roots, Mortierellaceae was the most abundant family observed in the FL stage, while Plectosphaerellaceae was the most dominant in the SSC stage (Figure 1A). In soil, Mortierellaceae prevailed in all growth stages (Figure 1B). In roots of each cultivar, Plectosphaerellaceae, Ceratobasidiaceae, Microdochiaceae and Nectriaceae Lasiosphaeriaceae, Filobasidiaceae and Nectriaceae were among the most predominant fungal families (Figure 2A). Mortierellaceae had the highest relative abundance in soil of each cultivar (Figure 2B).
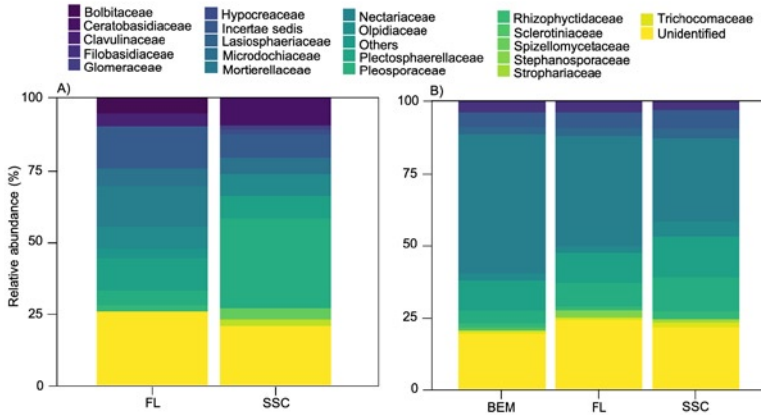
Figure 1. Taxonomic composition of root (**A**) and soil (**B**) fungal communities in different sampling times across all cultivars and replication blocks. BEM, before emergence; FL, flowering stage; SSC, senescence stage.
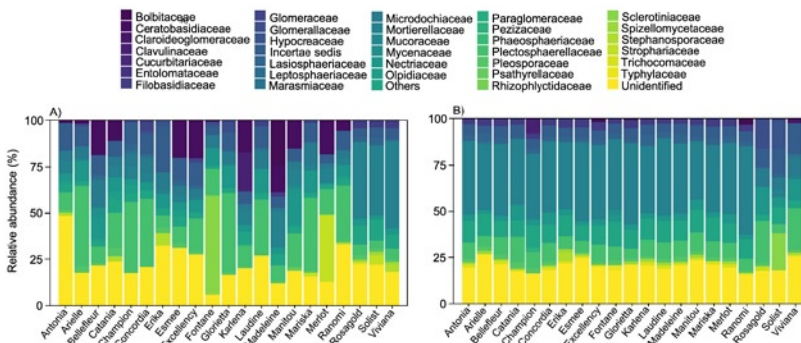

Figure 2. Taxonomic composition of root (**A**) and soil (**B**) fungal communities in different potato cultivars across all sampling times and replication blocks.

Of all sequences, 24.1% were assigned to plant pathogens, whereas saprotrophs and arbuscular mycorrhizal fungi accounted for 44.0% and 0.6% of sequences, respectively. Similar patterns occurred in root and soil samples. Due to their lack of detection from several samples, diversity patterns of arbuscular mycorrhizal fungi are not reported. Furthermore, no correlation studies between beneficial and pathogenic fungi were carried out.

3.1. Richness of Fungal Guilds

The highest proportion of variance in root fungal richness was explained by sampling time ($F_{1,82}$ = 43.75, R^2adj = 0.184, p < 0.001; Table 1), followed by the sampling time × cultivar interaction ($F_{20,82}$ = 2.85, R^2adj = 0.106, p < 0.001) and cultivar ($F_{20,82}$ = 2.20, R^2adj = 0.038, p = 0.007). Post hoc analyses showed that the cultivar Merlot had significantly higher total fungal richness than cultivars Concordia, Solist and Laudine (Figure 3a), and revealed that in the FL stage, Merlot had significantly higher root fungal richness than most other cultivars (except Manitou, Excellency, Glorietta, Viviana, Esmee, Erika and Arielle) (Table S5). There were no significant differences among pairwise comparisons during the SSC stage (Table S5). Soil fungal richness was explained only by sampling time ($F_{2,124}$ = 4.20, R^2adj = 0.032, p = 0.017; Table 1), with significantly higher values during the SSC stage than BEM stage (Table S5).

Table 1. Effects of cultivar, sampling time, and cultivar × sampling time interaction on the richness of all fungi, saprotrophic fungi, and plant pathogenic fungi in soil and roots.

Sample Type	Variable	df	All Fungi			Pathogens			Saprotrophs		
			R^2adj	Pseudo F	p	R^2adj	Pseudo F	p	R^2adj	Pseudo F	p
Soil											
	Cultivar	20	0.030	1.4	0.157	0	0.6	0.908	0.002	1.1	0.420
	Time	2	0.032	4.2	0.017 *	0.234	28.5	<0.001 ***	0.048	5.6	0.005 **
	Cultivar × Time	40	0	1.1	0.396	0	0.99	0.492	0	0.9	0.661
	Replication block	2	0	0.9	0.423	0	1.2	0.326	0	0.4	0.684
Roots											
	Cultivar	20	0.038	2.2	0.007 **	0.176	2.3	0.004 **	0.021	2.3	0.005 **
	Time	1	0.184	43.8	<0.001 ***	0	0.6	0.437	0.305	79.5	<0.001 ***
	Cultivar × Time	20	0.106	2.9	<0.001 ***	0	1.0	0.464	0.024	2.3	0.004 **
	Replication block	2	0	1.2	0.301	0.002	1.4	0.259	0	1.4	0.260

*** p < 0.001; ** p < 0.01; * p < 0.05. df, degrees of freedom; R^2adj, adjusted R^2; *Pseudo F*, pseudo-F statistic; p, calculated probability.

Root-pathogenic fungal richness was explained only by cultivar ($F_{20,82}$ = 2.30, R^2adj = 0.176, p = 0.004; Table 1). Post hoc analyses showed that the cultivar Glorietta had significantly higher pathogen richness than the cultivars Viviana, Concordia and Solist, with no significant differences among other pairwise comparisons (Table S6). In contrast, soil pathogen richness was explained only by sampling time ($F_{2,124}$ = 28.50, R^2adj = 0.234, p < 0.001; Table 1), with the SSC stage exhibiting higher richness compared to BEM and FL stages (Table S6, Figure S1).

Root saprotroph richness was highest in the FL stage ($F_{1,82}$ = 79.50, R^2adj = 0.305, p < 0.001; Table 1, Figure S1), with cultivar ($F_{20,82}$ = 2.30, R^2adj = 0.021, p = 0.005) and sampling time × cultivar interaction ($F_{20,82}$ = 2.30, R^2adj = 0.126, p = 0.004; Table 1) both showing significant effects. Post hoc analyses showed that the cultivar Merlot had significantly higher saprotroph richness than Concordia and Laudine, with no significant differences among other pairwise comparisons (Figure 3c). In contrast, soil saprotroph richness was explained only by sampling time ($F_{2,124}$ = 5.60, R^2adj = 0.048, p = 0.005; Table 1). The highest saprotroph richness was observed during the plant SSC stage, and the lowest value at the FL stage (post hoc, p = 0.003; Table S7).
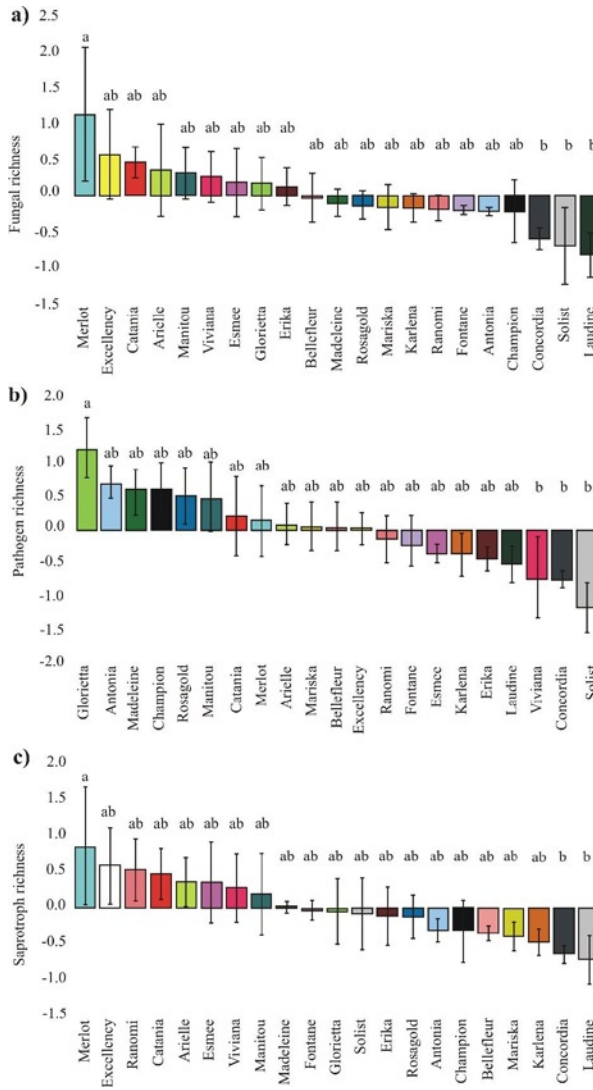
Figure 3. Results of a general linear model estimating the effect of cultivar for root (**a**) overall fungal, (**b**) pathogen, (**c**) saprotroph richness collected across all sampling times and replication blocks. Colours represent the cultivars. Different letters indicate statistically significant differences between cultivars (Tukey HSD post hoc tests, *p* < 0.05).

3.2. Plant Pathogen, and Saprotroph Abundance

The highest proportion of variance in relative pathogen abundance in roots was explained by sampling time ($F_{1,82}$ = 66.79, R^2adj = 0.296, *p* < 0.001; Table 2), with higher relative pathogen abundance

in the SSC stage compared to FL stage. Cultivar also had a significant effect ($F_{20,82} = 1.81$, R^2adj = 0.004, p = 0.033; Table 2), with Rosagold having a significantly higher fungal pathogen abundance than Catania (Table S8). Sampling time had the strongest effect on soil pathogen abundance ($F_{2,124}$ = 37.70, R^2adj = 0.250, p < 0.001; Table 2), with highest values at the SSC stage, and lowest values at the BEM stage (Table S8). Cultivar also had a significant effect on soil pathogen abundance ($F_{20,124}$ = 1.69, R^2adj = 0.011, p = 0.004; Table 2), which was non-significant in post hoc analyses (Table S9).

Table 2. Effects of cultivar, sampling time, and cultivar × sampling time interaction on the relative abundance of plant pathogenic fungi and saprotrophic fungi in soil and roots.

Sample Type	Variable	df	Pathogens			Saprotrophs		
			R^2_{adj}	*Pseudo F*	*p*	R^2_{adj}	*Pseudo F*	*p*
Soil								
	Cultivar	20	0.011	1.7	0.043 *	0.012	1.7	0.039 *
	Time	2	0.250	37.7	<0.001 ***	0.273	41.0	<0.001 ***
	Cultivar × Time	40	0	1.4	0.068	0	1.2	0.223
	Replication block	2	0	0.8	0.459	0	1.3	0.278
Roots								
	Cultivar	20	0.004	1.8	0.033 *	0.012	1.6	0.072
	Time	1	0.296	66.8	<0.001 ***	0.242	46.7	<0.001 ***
	Cultivar × Time	20	0	1.1	0.333	0	1.0	0.423
	Replication block	2	0.048	7.0	0.002 **	0.019	3.3	0.042 *

df, degrees of freedom; R^2_{adj}, adjusted R^2; *Pseudo F*, pseudo-F statistic; *p*, calculated probability. *** p < 0.001; ** p < 0.01; * p < 0.05.

The highest proportion of root saprotroph abundance was explained by sampling time ($F_{1,82}$ = 46.60, R^2adj = 0.242, p < 0.001; Table 2), with the plant FL stage exhibiting significantly higher saprotroph abundance. Furthermore, sampling time had a significant influence on soil saprotroph abundance ($F_{2,124}$ = 41.02, R^2adj = 0.273, p < 0.001) (Table 2). The highest saprotroph abundance was observed at the BEM stage, compared to both the FL (post hoc; p < 0.001; Table S9) and SSC stages (post hoc, p < 0.001; Table S9). Cultivar also had a weak but significant influence on soil saprotroph abundance ($F_{20,124}$ = 1.69, R^2adj = 0.012, p = 0.040; Table 2). Post hoc analysis revealed that the cultivar Viviana had significantly higher saprotroph abundance than Manitou (Table S9).

3.3. Factors Affecting the Abundance of Dominant Plant Pathogens

In root samples, *Plectosphaerella cucumerina* (12.7%), *Microdochium* spp. (7.2%), *Fusarium* spp. (6.2%) and *Rhizoctonia* spp. (teleomorph: *Thanatephorus* spp., 5.2%) were the most abundant pathogen taxa. Similarly to overall pathogen abundance in root samples, sampling time had the strongest influence on the relative abundance of *P. cucumerina* ($F_{1,82}$ = 66.40, R^2adj = 0.284, p < 0.001; Table S10), which peaked at the SSC stage. Cultivar had a minor effect on *P. cucumerina* abundance (Table S10). Post hoc analyses revealed a marginally greater abundance in Rosagold and Esmee compared to Fontane (Table S11). Cultivar had the strongest influence on the relative abundance of *Rhizoctonia* spp. ($F_{20,82}$ = 2.22, R^2adj = 0.140, p = 0.006; Table S10). Post hoc analyses revealed a marginally higher abundance of *Rhizoctonia* spp. in roots of the cultivars Merlot, Concordia, Solist, Manitou, Karlena and Glorietta, compared to those of Laudine, Rosagold, Excellency, Viviana, Bellefleur, Fontane, Antonia, Mariska, Madeleine, Champion, Esmee, Erika and Arielle, which showed almost no occurrence of *Rhizoctonia* spp. (Table S11). *Rhizoctonia* spp. relative abundance increased with time ($F_{1,82}$ = 7.40, R^2adj = 0.039, p = 0.007; Table S11).

In the soil samples, *Gibellulopsis nigrescens* (5.2%), *P. cucumerina* (2.4%), Didymellaceae (1.4%) and *Fusarium* spp. (0.9%) were the most abundant pathogen taxa. Similarily to overall pathogen abundance in root samples, sampling time had the strongest influence on the relative abundance of *G. nigrescens* ($F_{2,124}$=9.34, R^2adj = 0.071, p < 0.001; Table S10), with lowest abundance at the BEM stage, compared to FL and SSC stages (Table S12). Sampling time affected the relative abundance of *P. cucumerina* ($F_{2,124}$ = 32.70, R_2adj = 0.251, p < 0.001; Table S10) and Didymellaceae ($F_{2,124}$ = 10.5, R^2adj = 0.103,

$p < 0.001$; Table S10). The lowest *P. cucumerina* abundance was observed at the BEM compared to FL and SSC stages (Table S12). The lowest Didymellaceae abundance was observed at both BEM and FL stages compared to the SSC stage (Table S12). In contrast, cultivar was the only significant variable affecting *Fusarium* spp. abundance ($F_{20,124} = 1.70$, R^2adj = 0.060, $p = 0.040$; Table S10), but post hoc analyses revealed that *Fusarium* spp. abundance in soil near the roots of Merlot is only marginally higher than that of Karlena (Table S12).

3.4. Factors Affecting Fungal Community Composition

In roots, the cultivar was the main factor determining overall fungal ($p < 0.001$, adjusted $R^2 = 0.082$), pathogen ($p < 0.001$, adjusted $R^2 = 0.130$) and saprotroph ($p = 0.001$, adjusted $R^2 = 0.057$) community composition (Table 3). Conversely, in soil, sampling time was the main factor describing overall fungal ($p = 0.002$, adjusted $R^2 = 0.062$, Figure 4A), pathogen ($p = 0.004$, adjusted $R^2 = 0.102$, Figure 4B) and saprotroph ($p = 0.004$, adjusted $R^2 = 0.058$, Figure 4C) community composition (Table 3).

Table 3. Differences in total fungal, pathogenic and saprotrophic community composition in both soil and roots of different potato cultivars.

Sample Type	Variable	df	All Fungi			Pathogens			Saprotrophs		
			R²adj	Pseudo F	p	R²adj	Pseudo F	p	R²adj	Pseudo F	p
Soil											
	Cultivar	20	0.008	1.183	<0.001 ***	0.004	1.135	0.144	0.014	1.260	<0.001 ***
	Time	2	0.062	4.758	0.002 **	0.102	9.125	0.004 **	0.058	4.302	0.004 *
	Replication block	2	0.007	1.858	<0.001 ***	0.0109	2.513	0.001 **	0.004	1.467	0.012 *
	Cultivar × Time	40	0	1.007	0.409	0	1.139	0.079	0	0.954	0.845
	Cultivar × Replication block	40	0	1.016	0.299	0	1.132	0.087	0	0.981	0.655
	Time × Replication block	4	0.009	1.586	<0.001 ***	0.003	1.438	0.053	0.010	1.621	<0.001 ***
Roots											
	Cultivar	20	0.082	1.823	<0.001 ***	0.130	2.485	<0.001 ***	0.057	1.399	0.001 **
	Time	1	0.053	7.863	0.102	0.077	13.088	0.098	0.0331	4.994	0.105
	Replication block	2	0.012	2.223	<0.001 ***	0.004	1.689	0.040 *	0.009	1.938	0.003 **
	Cultivar × Time	20	0.013	1.392	<0.001 ***	0.004	1.378	0.004 **	0.001	1.280	0.004 **
	Cultivar × Replication block	40	0	1.020	0.362	0	0.916	0.793	0	1.179	0.011 *
	Time × Replication block	2	0	1.253	0.105	0	1.088	0.369	0	1.284	0.132

df, degrees of freedom; R^2adj, adjusted R^2; *Pseudo-F*, pseudo-F statistic; *p*, calculated probability. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.
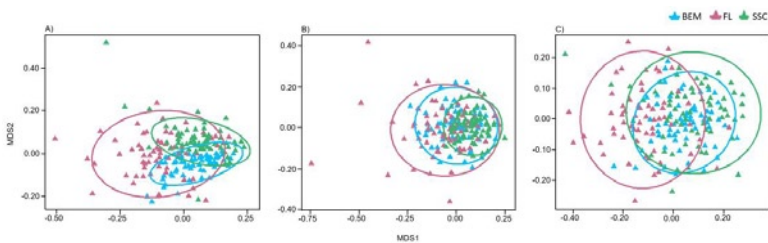
Figure 4. Non-metric multi-dimensional scaling, to model the effect of time on soil (**A**) overall fungal, (**B**) pathogen and (**C**) saprotroph community composition collected at different sampling points across all cultivars and replication blocks. BEM, before emergence; FL, flowering stage; SSC, senescence stage.

4. Discussion

4.1. Dominant Taxa

Plant soil offers habitat niches and root exudates as nutrients for microorganisms [43,44]. In the present study, root samples harboured only one fifth of the total OTUs detected, suggesting that a few microorganisms overcome plant defense mechanisms and inhabit plant roots. Ascomycota was the most abundant phylum detected in roots for each studied time point. Furthermore, ascomycetes prevailed in the roots of all potato cultivars. Ascomycota is the most diverse fungal phylum, comprising the majority of plant pathogens [45]. Furthermore, ascomycetes dominate as decomposers of organic matter in agro-ecosystems [46]. Mortierellomycota dominated in the soil—this phylum includes the genus *Mortierella*, mold-like decomposers that contribute to soil phosphorus cycling [47,48].

The observed dominant plant pathogens are common pathogens of potato, with a capability for saprophytic growth and infective spread to other hosts. *Plectosphaerella cucumerina* prevailed both in soil and root samples, which is in agreement with previous studies in Italy [49,50]. Although this pathogen causes wilting in potato [51], some studies have shown that *P. cucumerina* acts as a biocontrol agent against potato cyst nematodes [52]. Other abundant pathogens, belonging to genera such as *Fusarium* and *Rhizoctonia*, are common potato pathogens causing dry rot and black scurf, respectively. These soil-borne genera are among the most economically-important plant-pathogenic fungi, [26,53,54] that can survive saprophytically on crop residues in the absence of their hosts, growing rapidly when fresh organic matter is available [54]. Here we considered both genera to be tentatively pathogenic, although these groups contain non-pathogenic endophytes and saprotrophs and pathogens on hosts other than potato [55–57]. Limited species-level and *forma speciales*-level resolution of the ITS marker in *Fusarium* [58,59] and the paucity of SH-level functional reference data in both groups hamper our ability to distinguish effectively pathogenic organisms from closely related non-pathogenic taxa.

4.2. Seasonal Variation

Sampling time was the strongest variable shaping total fungal richness and composition, both in soil and roots. The role of temporal change in both bacterial and fungal communities has been observed in other studies [60–62]. In the present study, overall root-fungal richness was highest in the plant FL stage, whereas in soil samples, overall fungal richness peaked during the SSC stage. These results imply that in intensively managed agricultural soil during the early stages of plant growth, fungi mainly colonize healthy plant roots, which may provide high amounts of energy to the fungi. In later stages, when pathogens accumulate and plants senesce, nutrient flow ceases. In addition, changes in fungal community composition during the growing season may also contribute to the observed temporal dynamics, possibly due to climatic conditions, time since disturbance (ploughing) and interactions among fungal taxa. During the vegetative period, organic matter content decreases, and is replaced by root exudates. A previous study by Chaparro et al. [63] showed that the surrounding rhizosphere microbiome is affected by plant developmental stage, and is related to root exudation. Plants exude organic compounds into the surrounding environment [64], and microbes use these organic compounds as nutrients; and different microbial groups have distinct nutritional preferences [65]. Therefore, these qualitative changes in root exudation may cause differences in soil fungal diversity and community composition.

Saprotroph richness in roots and soil peaked during the plant FL and SSC stages, respectively, whereas saprotroph relative abundance in both soil and roots decreased in the plant SSC stage. Saprotroph richness-peaks in the FL stage could be attributed to increases in rhizodeposits [66]. In roots, plant FL stage exhibited significantly higher saprotroph relative abundance, whereas in soil, the highest saprotroph abundance was observed at the BEM stage. Thus, it can be expected that continuous tillage operations at the beginning of the growth period, and resource-rich spring wheat residues, being relatively fibrous with more long-term decomposition, promoted saprotroph abundance in the BEM stage.

The abundance of most dominant pathogens increased over the growing season. This is in agreement with the generally observed exponential increase in disease incidence of most crop pathogens over time during the growing season. It can be speculated that, in addition to changes in exudate patterns and root architecture, plants' resistance against pathogens decreases during ageing, allowing virulent pathogens to become prevalent [67].

4.3. Effect of Cultivars

In contrast to other fungal guild and habitat combinations, root pathogen richness was mainly affected by plant cultivar. Lowest pathogen richness was observed in the cultivars Viviana, Solist and Concordia, which are considered relatively resistant cultivars with medium nutrient demand (Europlant.biz, Danespo.com). Roots of the cultivar Glorietta comprised the highest pathogen richness, but this variety is not known to be particularly susceptible to pathogens. However, higher pathogen richness may not always be related to higher disease incidence and severity, but may instead lead to microbial competition in the rhizosphere and suppress the dominant pathogens [68].

The present study confirmed previous findings [69] regarding different cultivar susceptibility to *Rhizoctonia solani*. Our results reveal that some cultivars show almost no occurrence of *R. solani* when grown in the same soils where other cultivars became infected by this fungus. According to information provided by the breeding company Norika (Norika GmbH), the cultivar Merlot has high resistance to *Rhizoctonia* spp. Nevertheless, we found that Merlot showed marginally stronger abundance of *R. solani* than other cultivars. Furthermore, in this field trial, at the end of the growing period, plants with nests of small-sized tubers caused by *R. solani* were only observed on Merlot. Plant cultivar was the main factor affecting overall fungal, pathogen and saprotroph community composition in roots. It can be speculated that host genotype determined the fungal mycobiome recruited from the soil into the potato roots. Furthermore, a similar, consistent and weak, yet significant, effect of genotype on bacterial community composition in plant roots was observed in previous studies [70–72].

5. Conclusions

In this study, we assessed differences in fungal community structure in the roots and soil near the root zone of 21 potato cultivars, and among different plant growth stages. Potato cultivars (genotypes) developed distinctive fungal communities in their roots, with the background of similar fungal assemblages in soil showing temporal changes over the growing season. The abundances of fungal guild representatives in roots of potato cultivars, as well as fungal guild composition and diversity, showed temporal changes. The occurrence of major pathogens strongly varied among potato cultivars. Overall, our results demonstrate that in roots, cultivar was a primary factor determining overall fungal, pathogen and saprotroph community composition.

Conflicts of Interest: The authors declare no conflict of interest.

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2011 – 2014   University of Kiel – MSc studies in Biology
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1994 – 2006   Kadriorg German Gymnasium

Professional employment

2019 –   Estonian University of Life Sciences, Chief Specialist
2017 – 2019   Estonian University of Life Sciences, Junior Researcher
2014 – 2015   Environmental Board, Land Management Specialist

Professional training

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2019   Workshop on Multivariate Analysis in Ecology using PRIMER v7 and PERMANOVA+ – University of Massey, Auckland, New Zealand

2017   Visiting Scholar – Agroscope, Zurich, Switzerland

2017   NOVA PhD course Biological Control: "Microbial Interactions for Improved Plant Health" – Swedish University of Agricultural Sciences, Uppsala, Sweden

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1994 – 2006   Kadrioru Saksa Gümnaasium

Erialane teenistuskäik
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Erialane täiendkoolidtus
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2019   PRIMER v7 ja PERMANOVA+ tarkvara kasutamine mitmemõõtmelises statistikas – Massey Ülikool, Auckland, Uus Meremaa

2017   Külalisteadlane – Agroscope, Zürich, Šveits

2017   NOVA doktorikursus "Biokontroll: mikroobide ja taimede vaheline interaktsioon taime tervise parandamiseks" – Rootsi Põllumajandusülikool, Uppsala, Rootsi

2016   NOVA doktorikursus "Ülevaade keemilistest ja genoomikal põhinevatest meetoditest uurimaks peremees-mikroob vahelisi sümbiootilisi interaktsioone" – Kopenhaageni ülikool, Kopenhaagen, Taani

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3. Puidet, B., Mabon, R., Guibert, M., Kiiker, R., **Soonvald, L.**, Hong Le, V., Eikemo, H., Dewaegeneire, P., Saubeau, G., Chatot, C., Aurousseau, F., Cooke, D., Lees, A.K., Abuley, I., Hansen, J.G., Corbière, R., Leclerc, M., Andrivon, D. 2021. Examining phenotypic traits contributing to the spread in Northern European potato crops of EU_41_A2, a new clonal lineage of *Phytophthora infestans*. Phytopathology
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5. Põlme, S., Abarenkov, K., Nilsson, R.H., Lindahl, B.D., Engelbrecht Clemmensen, K., …, **Soonvald, L.**, et al. 2021. FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. Fungal Diversity 105, 1–16.
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8. **Soonvald, L.**, Loit, K., Runno-Paurson, E., Astover, A., Tedersoo, L. 2020. Characterising the effect of crop species and fertilisation treatment on root fungal communities. Scientific Reports 10: 18741
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9. Loit, K., **Soonvald, L.**, Astover, A., Runno-Paurson, E., Öpik, M., Tedersoo, L. 2020. Temporal and cultivar-specific effects on potato root and soil fungal diversity. Agronomy-Basel 10: 1535
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1. Loit, K., **Soonvald, L.**, Kukk, M., Astover, A., Runno-Paurson, E., Kaart, T., Öpik, M. 2018. The indigenous arbuscular mycorrhizal fungal colonisation potential in potato roots is affected by agricultural treatments. Agronomy Research 16 (2), 510–522.

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1. **Soonvald, L.**, Loit, K., Astover, A., Tedersoo, L. 2019. Assessment of plant pathogenic fungal and oomycete communities in the soil of a long-term fertilization experiment. International Congress of Plant Pathology (ICPP). Phytopathology 108, 90.

2. Loit, K., **Soonvald, L.**, Astover, A., Tedersoo, L. 2019. Molecular characterization of the pathogen and symbiotic fungal community composition in the rhizosphere of common European potato varieties. International Congress of Plant Pathology (ICPP). Phytopathology 108, 80.

3. **Soonvald, L.**, Loit, K., Runno-Paurson, E., Tedersoo, L. 2016. Molecular diversity of fungal communities in conventional arable soil. 38th New Phytologist Symposium "Colonization of the terrestrial environment". New Phytologist Trust 43.

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1. **Soonvald, L.**, Loit, K. 2017. Arbuskulaar-mükoriissere seente inokuleerimises tähtsus agroökoloogilise tehnoloogia meetodina. Luule Metspalu, Anne Luik, Elen Peetsman (Toim.). Teaduselt mahepõllumajandusele (139–145). SA Eesti Maaülikooli Mahekeskus.

2. Puidet, B., Kiiker, R., Loit, K., **Soonvald, L.**, Najdabbasi, N. 2017. Kartuli-lehemädaniku ja kuivlaiksuse areng maheviljeluseks soovitatud kartulisortidel. Luule Metspalu, Anne Luik, Elen Peetsman (Toim.). Teaduselt mahepõllumajandusele (123–129). SA Eesti Maaülikooli Mahekeskus.

VIIS VIIMAST KAITSMIST

MARGUS ARAK

CULTIVATION TECHNOLOGY FOR LOW-BUSH BLUEBERRY
CULTIVATION IN MILLED PEAT FIELD PLANTATIONS

AMMENDATUD FREESTURBAVÄLJADEL KASVATATAVA
AHTALEHISE MUSTIKA MASINVILJELUSTEHNOLOOGIA

Professor **Jüri Olt**
1. november 2021

LIINA JÜRISOO

DISTRIBUTION OF AND DAMAGES CAUSED BY DUTCH
ELM DISEASE AGENTS IN NORTHERN EUROPE

JALAKASURMA LEVIK JA KAHJUSTUSED PÕHJAEUROOPAS

Professor **Rein Drenkhan**
11. november 2021

HEIKI LILL

NOVEL APPLICATION PRINCIPLES FOR ENERGY STORAGE
TECHNOLOGIES IN NEARLY ZERO ENERGY BUILDINGS

ERINEVATE ENERGIA SALVESTUSTEHNOLOOGIATE UUDSED
RAKENDUSPÕHIMÕTTED LIGINULLENERGIAHOONETES

Teadur **Alo Allik**, professor **Andres Annuk**
24. november 2021

PIRET RAUDSEPP

POLYPHENOLIC COMPOSITION OF RHUBARB (*RHEUM RHAPONTICUM* L.)
AND BLACKCURRANT (*RIBES NIGRUM* L.), ANTIBACTERIAL AND FREE
RADICAL SCAVENGING PROPERTIES OF THESE PLANTS IN COMPARISON
WITH SOME OTHER FOOD PLANTS

HARILIKU RABARBERI (*RHEUM RHAPONTICUM* L.) JA MUSTA SÕSTRA
(*RIBES NIGRUM* L.) POLÜFENOOLNE KOOSTIS, NENDE TAIMEDE ANTI-
BAKTERIAALSE TOIME JA VABADE RADIKAALIDE SIDUMISE VÕIME
VÕRDLUS MÕNEDE TEISTE TOIDUTAIMEDEGA

Professor **Tõnu Püssa**, vanemteadur **Ave Kikas**
10. detsember 2021

INGRID BENDER

EFFECT OF ORGANIC MANAGEMENT METHODS ON YIELD
AND QUALITY OF CARROT AND ON WEEDS

MAHEVILJELUSE MEETODITE MÕJU PORGANDI SAAGILE JA
KVALITEEDILE NING UMBROHTUDELE

Prof. Emeritus **Anne Luik**, dotsent **Evelin Loit**,
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17. detsember 2021