Eesti Maaülikool
Estonian University of Life Sciences

PATHOGENIC AND ARBUSCULAR MYCORRHIZAL FUNGI IN POTATO FIELDS IN ESTONIA

PATOGEENSED JA ARBUSKULAARMÜKORIISSED SEENED EESTI KARTULIPÕLDUDEL

KAIRE LOIT

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

Väitekiri
filosoofiadoktori kraadi taotlemiseks
põllumajanduse erialal

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Opponent:	**Dr. Sari Timonen** University of Helsinki
Supervisors:	**Prof. Alar Astover** Estonian University of Life Sciences
	Prof. Leho Tedersoo University of Tartu
	Prof. Maarja Öpik University of Tartu
Reviewer:	**Dr. Mati Koppel** Estonian University of Life Sciences
Defense of the thesis:	Estonian University of Life Sciences, room D239, Fr. R. Kreutzwaldi 5, Tartu; International video conference in the BigBlueButton web room on June 22, 2021, at 10:15.

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following studies, presented in papers which are referred to in the text by their Roman numerals:

I **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). DOI: 10.15159/ar.18.063.

II **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, 10 (10), ARTN 1535. DOI: 10.3390/agronomy10101535.

III **Loit, K.**, Adamson, K., Bahram, M., Puusepp, R., Anslan, S., Kiiker, R., Drenkhan, R., Tedersoo, L. (2019). Relative Performance of MinION (Oxford Nanopore Technologies) versus Sequel (Pacific Biosciences) Third-Generation Sequencing Instruments in Identification of Agricultural and Forest Fungal Pathogens. Applied and Environmental Microbiology, 85 (21), ARTN e01368-19. DOI: 10.1128/AEM.01368-19.

The contribution of Kaire Loit to the papers was the following:

Paper	Idea and study design	Experiments	Data analysis	Manuscript writing
I	ERP, MÖ	ERP, MK, MÖ	**KL**, LS, TK	AA, ERP, **KL**, LS, MÖ, TK
II	ERP, **KL**, LS, LT, MÖ	ERP, **KL**, LS	AA, ERP, **KL**, LS, LT, MÖ	AA, ERP, **KL**, LS, LT, MÖ
III[1]	KA, **KL**, LT, MB, RP	KA, **KL**, LT, MB, RP	KA, **KL**, LT, MB, RP	KA, **KL**, LT, MB, RD, RK, RP, SA

AA – Alar Astover; ERP – Eve Runno-Paurson; KA – Kalev Adamson; **KL – Kaire Loit**; LS – Liina Soonvald; LT – Leho Tedersoo; MB – Mohammad Bahram; MK – Martin Kukk; MÖ – Maarja Öpik; RD – Rein Drenkhan; RK – Rasmus Puusepp; RP – Rasmus Puusepp; SA – Sten Anslan; TK – Tanel Kaart.

[1] Only the MinION-based identification of agricultural pathogens is handled within this thesis.

ABBREVIATIONS

AM fungi	Arbuscular mycorrhizal fungi
BBCH-scale	Decimal scale of plant growth stages
BEM	Before emergence
CC	Cover crop
cDNA	Complementary DNA
CFS	Conventional farming system
Corg	Organic carbon
DNA	Deoxyribonucleic acid
FL	Flowering
GLM	Generalized linear model
HCL	Hydrochloric acid
HTS	High-throughput sequencing
IP	Inoculum potential
IPM	Integrated pest management
KOH	Potassium hydroxide
LAMP	Loop-mediated isothermal amplification
M	Manure
N	Nitrogen
NS	Not significant
OFS	Organic farming system
OTU	Operational taxonomic unit
P	Phosphorus
PCR	Polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
SSC	Senescence

1. INTRODUCTION

Soil fungi have fundamental ecological roles as decomposers, mutualists, or pathogens of plants. Roots of agricultural crops are hosts of different microorganisms. These include many harmful, often soilborne, pathogens, as well as beneficial organisms, such as plant growth and health-promoting arbuscular mycorrhizal (AM) fungi (Smith and Read 2008). In agroecosystems, fungal plant diseases can be a major limitation determining agricultural productivity (Raaijmakers et al. 2009). Diseases caused by soilborne plant pathogens tend to produce only one infection cycle per host cycle, the infection starts when the roots of the newly planted crop encounter the propagules of the pathogen embedded in the soil. Therefore, soilborne fungal pathogens are challenging to manage, as they can remain in the soil for years (Bebber et al. 2014). The most important group of beneficial soil fungi in agricultural soils are AM fungi that play vital roles in improving plant performance (Frac et al. 2018; Smith and Read 2008) and protecting plants against soilborne pathogens (Pozo et al. 2015; Thirkell et al. 2017; Whipps 2004). In agricultural fields, beneficial and detrimental microbial communities are affected by several abiotic factors like soil type (Berg and Smalla 2009; Tkacz et al. 2020), pH (Soonvald et al. 2020; Tedersoo et al. 2014; Tedersoo et al. 2020 a), and biotic factors such as plant developmental stage (Hannula et al. 2012; Jia et al. 2020), plant health (Ginnan et al. 2020), as well as anthropogenic factors like crop rotation (Detheridge et al. 2016), agricultural practices (Benitez et al. 2016; Sharma-Poudyal et al. 2017), fertilization (Geisseler and Scow 2014), and pesticide application (Kalia and Gosal 2011), but crop selection of its fungal community in agroecosystems remains poorly understood.

Potato (*Solanum tuberosum* L.) ranks first as a non-cereal food crop for human consumption worldwide (FAO 2009). According to FAO (2009), the average global potato consumption per capita per year reached 34.2 kg in 2013, whereas the average potato consumption in Europe was 82.6 kg per capita per year. Potato yields more food more rapidly with less arable land needed than any other crop (Devaux et al. 2019). Still, depending on several factors, the average potato yield varies from 1 to 60 tons of fresh tubers per hectare in the world (Ritchie and Roser 2019). In 2018, the global average potato yield per hectare was 21 tons, whereas the average potato yield in Estonia was 17 tons (Ritchie and Roser 2019). In potato

production, multiple sequential agrotechnical operations have to be carried out during the growing period, which can have a high impact on soil health. Moreover, potato production is one of the most dependent on chemical pesticides (Birch et al. 2012) compared to other crops. In turn, pesticides have the potential to impact the environment negatively. As soil harbors microorganisms that are beneficial or harmful to plants, sustainable crop production requires intimate knowledge of both pathogenic and beneficial fungi. In order to develop novel approaches for enhancing sustainable agriculture, it is essential to fill the knowledge gap about the soil biota that offer important ecosystem services. Microbial pathogens cause enormous losses to potato production and can be limiting factors in sustainable agricultural production (Secor and Gudmestad 1999). Climate change and increased global trade of planting material have led to rapid emergence and adaptation of new crop pathogens in agroecosystems, including potato production, which has led to new agricultural challenges.

Conventional farming uses synthetic fungicides to control diseases. In organic potato production, only copper (Cu)-based pesticides have been allowed to be used against diseases. However, the use of copper may be banned in organic farming in the near future (Lamichhane et al. 2018). During recent years, because of the hazardous nature of synthetic pesticides (incl. insecticides, fungicides, and herbicides), numerous active substances of pesticides have been banned from use within the European Union (EU). Due to fewer active ingredients of pesticides, preserving potato productivity and profitability while controlling potato diseases is becoming increasingly difficult (Birch et al. 2012). Regardless of climate, cultivated potatoes are constantly exposed to various pathogens that pose a serious threat to potato production worldwide (Adolf et al. 2020; Burton 1989; Fiers et al. 2012; Hooker 1981). Among these, crops may also need protection against putative newly emerging fungal diseases (Tiwari et al. 2020).

Interest in utilizing beneficial soil biota as natural resources instead of chemical pesticides is growing (Köhl et al. 2016). All soils in which crops are cultivated already contain distinct arbuscular mycorrhizal (AM) fungal communities and most crops naturally become colonized by AM fungi independently from inoculation (Rúa et al. 2016). Several studies have shown that the potato plant responds well to artificial AM fungal inoculation (Bhattarai and Mishra 1984; Cesaro et al. 2008; Douds et al.

2007; Hijri 2016; McArthur and Knowles 1993; Yao et al. 2002). Promising results have been reported about the benefit of AM fungal inoculation on tuber yield (Liu et al. 2018; Loján et al. 2017; Wu et al. 2013) and bio-protection against soilborne pathogens of potato (Bradley et al. 2015; Gallou et al. 2011; Ismail et al. 2013; Loján et al. 2017). Previous research has mainly focused on in vitro studies, which cannot be directly applied to open field conditions. Knowledge about how current agricultural practices can increase the diversity and abundance of indigenous AM fungal communities in potato cultivation and thereby changes in the AM fungal success independently from inoculation are still lacking.

Monitoring plant health and detecting plant diseases early are essential for reducing pathogen spread and facilitating effective management practices (Aslam et al. 2017; Baldi and La Porta 2020). Classical microbiology, visual inspection-, and DNA-based methods provide complementary information for detecting plant pathogens from symptomatic samples (Martinelli et al. 2015). Detecting plant pathogens in the latent phase is necessary to prevent disease epidemics. However, detection of pathogens from asymptomatic materials, such as potato tubers can be extremely difficult since very low numbers of pathogen propagules are present (Baldi and La Porta 2020; Donoso and Valenzuela 2018; Schaad and Frederick 2002). Molecular techniques for rapid and accurate identification of plant pathogens based on the polymerase chain reaction (PCR) such as conventional PCR and real-time quantitative PCR (qPCR) have considerably enhanced plant pathogen diagnosis (Donoso and Valenzuela 2018; Martinelli et al. 2015; Paul et al. 2020; Schaad and Frederick 2002). Still, they do not allow determination directly in the field (Baldi and La Porta 2020; Donoso and Valenzuela 2018). The loop-mediated isothermal amplification (LAMP) method has been the most widely explored application suitable for in-field testing (Le and Vu 2017; Paul et al. 2020). Recently, real-time loop-mediated isothermal amplification (Real-Time LAMP) plant disease diagnostic method (Ristaino et al. 2020) was reported. However, although LAMP assays can be used for rapid diagnosis of pathogens directly in the field, this method is available for detection of specific pathogens, and a larger number of primers per target must be designed compared with the conventional PCR approach (Duan et al. 2014; Le and Vu 2017). In-field rapid diagnosis of untargeted plant pathogens or pathogen complexes is still lagging behind. One of the greatest challenges in eradicating newly emergent crop diseases is the lack of advances in technology application, such as field-ready DNA sequencing systems that

could help to detect pathogen species in a short period of time and thus help to overcome pest control challenges in agriculture.

In this thesis, the author focuses on the diversity, abundance, and community composition of putative pathogenic fungi and symbiotic AM fungi associating with potato. The author characterized AM fungal colonization of potato roots in field-grown potato plants and determined AM fungal inoculum potential (IP) in organic and conventional management systems using a trap plant bioassay. Additionally, the author developed ultrarapid protocols for diagnostics of plant pathogens.

2. LITERATURE REVIEW

2.1. Arbuscular mycorrhizal fungi in potato production

Arbuscular mycorrhizal (AM) fungi are a widespread group of filamentous fungi that form symbiotic relationships with roots of most terrestrial plants, including potato (Douds et al. 2007; Senés-Guerrero et al. 2014). The ecosystem services provided by AM fungi (Delavaux et al. 2017; Gianinazzi et al. 2010; Smith and Smith 2011) make them an important group of soil biota that can potentially be important tools for sustainable agricultural production. Still, the diversity and abundance of AM fungi in agroecosystems are affected by agricultural intensification (Banerjee et al. 2019; Bowles et al. 2017; Verbruggen and Kiers 2010). In addition to an adverse effect of agrochemicals, several studies have demonstrated that AM fungal abundance declines in response to nitrogen (N) and phosphorus (P) fertilization (Liu et al. 2020; Treseder 2004; Verbruggen et al. 2013). Thus, in the high-input potato production system, benefits provided by AM fungi may not realize. Previous inoculation studies have reported high levels of AM fungal colonization in potato roots (Buysens et al. 2017; Davies et al. 2005; Douds et al. 2007; Duffy and Cassells 2000). Still, a study of AM fungal communities in low-input potato fields in the Peruvian Andes (Senés-Guerrero et al. 2014) showed that potato fields harbor high AM fungal diversity.

Regardless of evidence that AM fungi could act as an efficient biocontrol agent against potato pathogens, some studies performed in high-input agricultural fields show that relatively small fractions of the potato root systems are colonized by indigenous AM fungi (Cesaro et al. 2008). Although previous studies indicate that organic fertilizers have a positive impact on AM fungi (Gryndler et al. 2006; Jansa et al. 2006; Yu et al. 2013), the effects of organic compounds differ according to the chemical composition of the substrate (Ravnskov et al. 1999), and farmyard manure may cause soil nutrient enrichment, adversely affecting mycorrhizal fungi (Gosling et al. 2006; Soonvald et al. 2019, 2020). In addition to management impact to AM fungi, several authors have indicated that crops differ in their ability to respond to AM fungal colonization and harbor distinct AM fungal communities in their roots (An et al. 2010; Baon et al. 1993; García de León et al. 2020; Mao et al. 2014). Moreover, studies with potato (Cesaro et al. 2008; Hijri 2016; Senés-Guerrero

et al. 2014) have found that potato cultivars are colonized by different AM fungi. Although several AM fungi respond differently to agricultural inputs, little is known about the host genotype impact on the community assembly of AM fungi in field soil where many kinds of factors such as tillage, pesticide use, and variations in newly bred crop cultivars might influence both plants and indigenous AM fungi.

2.2. Phytopathogenic fungi in potato production

Fungal plant pathogens exert considerable pressure on agricultural productivity and threaten global food security (Kettles and Luna 2019). The highest crop losses are associated with emergent pests like the Colorado potato beetle (Burton 1989) and pathogens such as the oomycete *Phytophthora infestans*, the fungal pathogen *Verticillium dahliae*, and the bacterial pathogen *Dickeya solani* (Blin et al. 2021; Kettles and Luna 2019; Savary et al. 2019). Their spread among continents has accelerated due to the global trade of agricultural products (McDonald and Stukenbrock 2016; West 2014), including the increased movement of seed potatoes (Blin et al. 2021; Frost et al. 2013).

Since the rate of worldwide transportation of agricultural products (incl. imported seeds) increases, managing crop losses by use of pesticides continues to be an essential tool. Still, due to integrated pest management (IPM) principles, fungicides should be used as an alternative when preventive actions have been insufficient and the applications are based on disease management thresholds (Jalli et al. 2020). While chemical control is a useful tool for managing some soilborne crop diseases (Bolton et al. 2010; Liu et al. 2021), it is difficult to establish disease management thresholds for soilborne pathogens. Compared to aerial diseases, soilborne diseases are caused by pathogens hidden in the soil (Fiers et al. 2012), and their symptoms may not appear until the later stages of crop maturity (DeShields et al. 2018). Thus, frequent monitoring of both well-known and emergent soilborne pathogens in the plant root zone is crucial for selecting appropriate control measures in a timely manner. Furthermore, soilborne fungal pathogen pressure, one characteristic of soil health, poses challenges to potato production worldwide (Hills et al. 2020; Jeger et al. 1996). Thus, focusing on the spread of soilborne pathogens in potato cropping systems has become even more critical.

For airborne polycyclic pathogens, such as *Phytophthora infestans*, *Alternaria* spp., and *Phoma* spp., it is well known that variations in fungal composition are significantly related to seasonality (Campos and Ortiz 2020; Qi et al. 2020; Tordoni et al. 2021), and disease development can be related to the life cycle of crop plants (Brachaczek et al. 2016; Escuredo et al. 2019). By contrast, our understanding of how soilborne microbial communities differ during crop plant life cycle remains limited and most pathological studies are focused on the pathogen population structure of a single causal agent among multiple potato diseases.

Recently, Zimudzi et al. (2018) studied inter-and intra-seasonal variation of fungal communities associated with the potato rhizosphere in subtropical conditions. They found that the relative abundance of prevailing OTUs (operational taxonomic units) varied from tuber initiation to senescence stage. Also, the potato peel and rhizosphere fungal communities were different from pre-plant communities. Moreover, potato plants recruited specific fungi at different plant growth stages, with *Didymella glomerata* (syn. *Phoma glomerata*) being the most prevalent in the rhizosphere at tuber initiation and the relative abundance declined with plant age. Generally, *D. glomerata* is considered a widespread opportunistic plant pathogenic fungus colonizing different crop species (Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Retrieved December 10, 2020, from https://nt.ars-grin.gov/fungaldatabases/). However, to our knowledge, *D. glomerata* has found rarely to cause disease in members of the family *Solanaceae*. Despite that, several pathogenic species of this genus (*Phoma* sensu lato) attack numerous crop plants, including potato. Among these, *P. exigua* var. *foveata* has been recorded as a potato gangrene pathogen (Deb et al. 2020; Fungal Databases, U.S. National Fungus Collections, ARS, USDA).

The survey of Dean et al. (2012), which enlisted the top 10 economically and scientifically important fungal pathogens, showed that potato is susceptible to one or more *Colletotrichum* species (Dean et al. 2012). Nitzan et al. (2008) showed that the severity of soil- and tuber-borne potato black dot disease, caused by *Colletotrichum coccodes*, is more related to the concentration of soilborne, not tuber-borne inoculum. Andrivon et al. (1997) and Tian et al. (2019) have confirmed wide geographical distribution of *C. coccodes* and proved that most widely grown cultivars of potato are susceptible to the pathogen.

Among other worldwide distributed diseases, *Fusarium* dry rot, caused by different *Fusarium* species is a common storage disease in potatoes worldwide (Heltoft et al. 2015). None of the potato cultivars have yet been found to be fully resistant to the whole *Fusarium* species complex, but their susceptibility to different *Fusarium* species varies (Esfahani 2005; Tiwari et al. 2020). Heltoft et al. (2015) indicated that early-maturing cultivars are much more susceptible to *F. sambucinum* than late-maturing cultivars.

A widespread soilborne plant pathogen, *Rhizoctonia solani* (teleomorph: *Thanatephorus cucumeris*), causes stem canker, tuber black scurf, and tuber malformation in potato, is responsible for significant crop losses in different climate zones (Das et al. 2014; Zrenner et al. 2020), and can spread through infected plant material (Elmer 2001). A crop rotation study conducted in New Zealand showed that *Rhizoctonia solani* was the primary soilborne disease observed. The incidence of this disease was more significant in potato monoculture, compared to 4-year crop rotation (Wright et al. 2017). By contrast, a potato rhizosphere community analysis conducted in South Africa (Zimudzi et al. 2018) showed that the relative abundance of *Rhizoctonia solani* was relatively low compared to other soilborne pathogenic species. A study conducted in different potato-growing areas in France (Campion et al. 2003) showed that no variation in susceptibility to sclerotial formation was observed among five potato cultivars. Mostly, attention has been paid to the biological diversity of *Rhizoctonia solani*, rather than its relative abundance (Lehtonen et al. 2007; Salamone and Okubara 2020).

The oomycete pathogen *Phytophthora infestans* causes the serious potato disease known as late blight, that was responsible for the Irish potato famine in the middle of the nineteenth century (Adolf et al. 2020) and still continues to be the main biotic constraint of potato production. However, multiple other fungal pathogens infect the potato plant. The fungal species *Alternaria solani* and *Alternaria alternata* are widespread soil-, seed-, and air-borne pathogens causing early blight on potatoes (Hausladen and Leiminger 2007; Landschoot et al. 2017).

2.3. Methods for rapid detection of pathogenic fungi

In crop production, monitoring crop health with rapid detection of pathogens at the beginning of infection is crucial to reduce disease spread and facilitate effective management practices. Several crop pathogens can be recognized from symptoms determined on infected tissues by a trained specialist. However, similar symptoms can be caused by different disease agents, commonly resulting in misdiagnosis of the pathogen. That can also be the case for classical culturing methods followed by microscopy techniques (Ray et al. 2017). Culture-based molecular identification methods have enabled detection of rare and novel pathogens (Oo et al. 2017; Tomlinson et al. 2010), yet these methods are slow and may lack the ability to detect several pathogens simultaneously (Hyde et al. 2013). Besides, the vast majority of fungal species are unculturable or could be cultured using specific substrates and conditions. Methods based on antigen-antibody bindings such as enzyme-linked immunosorbent assay (ELISA) do not require pure isolation of the pathogen, being applicable to biotrophic as well as necrotrophic pathogens (Mancini et al. 2016). These assays are widely used as screening tools to assess the phytosanitary status of plants (Charlermroj et al. 2014). However, due to low sensitivity to bacteria and fungi, and the lack of species-specific antibodies, the application of ELISA is more used for virus detection (Boonham et al. 2014; Colin 1998; Fang and Ramasamy 2015; Luchi et al. 2020).

Technical advances in molecular methods have greatly facilitated accurate identification of disease-causing agents from infected crops or soil without the need for a culturing step (Martinelli et al. 2015; Nilsson et al. 2014; Ristaino et al. 2020). PCR, a selective DNA-based technology, is a relatively fast approach that allows detection of fungal DNA, offering an alternative to culture-based methods (Ray et al. 2017). The qPCR is a more appropriate tool for quantification of plant pathogens in latent infection phase (Luchi et al. 2016). To date, compared with PCR assays, the LAMP is a more recent technology with fewer preparation steps (Mancini et al. 2016). Although LAMP can be very effective for the detection of pathogen species (Niessen and Vogel 2010; Ristaino et al. 2020), it is still available for detection of specific pathogens, and a larger number of primers per target must be designed compared with PCR (Duan et al. 2014; Le and Vu 2017). In the past, the restriction fragment length polymorphism (RFLP) analysis has been generally used in population genetic studies of plant pathogens such as *Phytophthora infestans* (Runno-Paurson

et al. 2010) and for identification of *Phytophthora isolates* to species level (Martin and Tooley 2004).

Of the DNA sequence analysis methods, Sanger sequencing technology is considered as a first-generation tool in sequencing genetic material (Barba et al. 2014; Van Dijk et al. 2014). The Sanger method is still in use in basic plant pathology for detecting single plant pathogen species from fungal isolates. Moreover, in Sanger sequencing, species-specific PCR primers enable the detection of DNA of the target fungal pathogen species from symptomatic plant tissue samples (Drenkhan et al. 2016). In comparison with Sanger sequencing, second-generation high-throughput (HTS) techniques, such as 454 pyrosequencing, have been used in the past decade, improving our understanding of fungal diversity in complex environments (Tedersoo et al. 2020b), and allowing a novel pathogen detection from plant samples (Adams et al. 2009; Prigigallo et al. 2016). At present, Illumina sequencing is by far the most widely used HTS method (Tedersoo et al. 2018), and also the best option for tracking plant pathogens that are present in the plant sample (Doonan et al. 2017; Leff et al. 2017; Rezki et al. 2016). However, high-throughput detection of plant pathogens in a time-efficient manner remains a challenging task. A powerful computing infrastructure, appropriate reference datasets, and skilled bioinformatics staff are needed. Of the third-generation sequencing methods, the portable phone-sized DNA sequencing device MinION (Oxford Nanopore Technologies) has received much attention because of its small size and possibility of rapid analysis at reasonable cost. The MinION device can be used for rapidly sequencing full-length DNA, cDNA, and RNA molecules (Fellers et al. 2019). In plants, Badial et al. (2018) first demonstrated that plant-pathogenic bacteria and viruses can be detected using MinION. Hu et al. (2019) demonstrated that MinION can be efficient for detection of fungal pathogens of cereals. Chalupowicz et al. (2019) showed that the MinION platform provides a rapid possibility specifically to diagnose plant diseases with visible disease symptoms caused by viruses, bacteria, and fungi. These protocols usually take a full day or more to process the sample and analyze data.

3. AIMS OF THE STUDY

In order to develop novel approaches to enhance sustainable agriculture, it is essential to fill the knowledge gaps about the soil biota that offer important ecosystem services. Microbial pathogens cause enormous losses to potato production and thus deserve increased attention. Still, the vast majority of roots of crop plants, including potato, are associated with beneficial organisms such as root endophytes, rhizosphere saprotrophs and, in particular, AM fungi. Little is known about mycorrhizal dependence of crop plants in habitats under external anthropogenic management. Knowing the diversity of the beneficial and plant pathogenic communities at different growth stages in high-input potato production system helps to design more optimal solutions to reduce the negative impact of intensive agriculture on the environment. In order to benefit from microbial interactions and use them in integrated disease management, it is important to find fast and accurate methods for the detection of pathogenic fungi. The objectives of the study were as follows:

1. To characterize, how do the conventional and organic farming systems influence potato root AM fungal colonization (**Paper I**).

   Hypothesis: Potato roots have greater AM fungal colonization levels under organic farming than conventional farming.

2. To assess differences in AM fungal and putative pathogenic community composition, richness, and relative abundance both within roots and in soil among potato cultivars (**Paper II**).

   Hypothesis: AM fungal and pathogenic fungal community composition, richness, and relative abundance in roots and soil differ among plant growth stages and across potato cultivars.

3. To test the suitability of a portable sequencer, MinION, for ultrarapid plant pathogen diagnostics (**Paper III**).

4. MATERIALS AND METHODS

4.1. Study sites

Individual studies were conducted in agricultural experimental fields in Estonia in 2010 (**I**) and 2014 (**II**). For study **I**, the field site was located in Tartu, Estonia (58°22'N, 26°40'E; Figure 1). The field experiment was established in 2008 as a part of a 5-year crop rotation experiment with two treatments in the organic farming system (OFS) and four treatments in the conventional farming system (CFS), in which systematically arranged treatment blocks were replicated four times. In both organic management treatments, oilseed rape (*Brassica napus* L.) as a winter cover crop (CC) was used for green manure. Composted cattle manure (M)

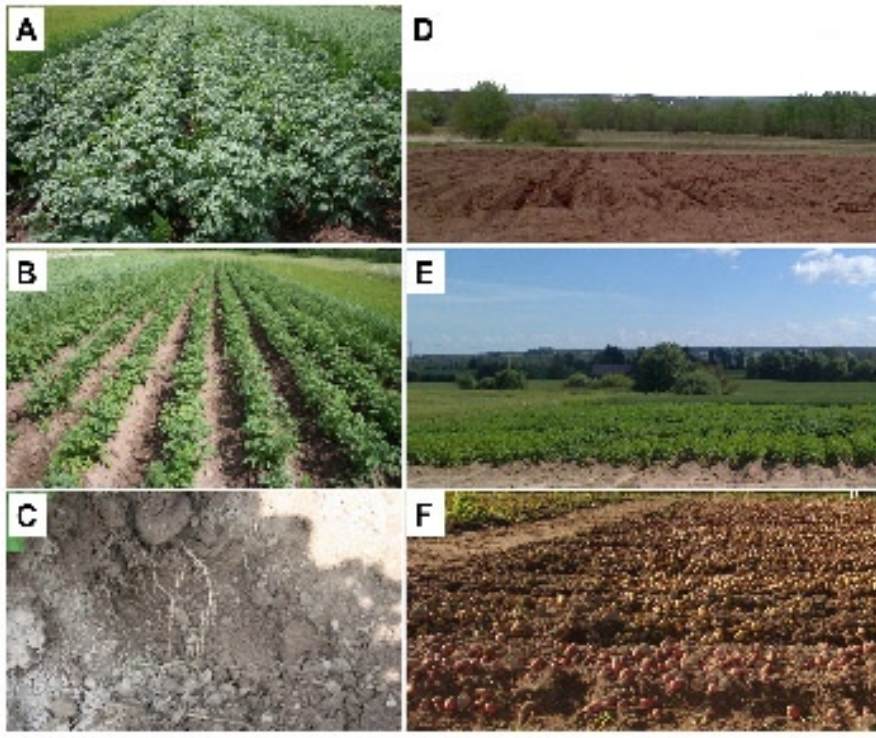

Figure 1. Photos of 5-year crop rotation experiment (**I**): treatment block in the conventional farming system (A), treatment block in the organic farming system (B), and potato roots in sampling (C). Photos of potato field experiment (**II**): after planting (D), at flowering stage (E), and after harvesting (F). Photos by E. Runno-Paurson (A, B, C) and K. Loit (D, E, F).

was added to the second organic treatment (CC + M). In the organic management system, neither organic nor synthetic pesticides were used. In the conventional management system, treatments with four fertilizer application rates were used: $N_0P_0K_0$ – control system with no additional fertilizers used, $N_{50}P_{25}K_{95}$, $N_{100}P_{25}K_{95}$, and $N_{150}P_{25}K_{95}$ – systems with different N rates used. In addition to synthetic fertilizers, several synthetic pesticides were used equally in all conventional treatment plots. At the study site, red clover (*Trifolium pratense* L.) and barley (*Hordeum vulgare* L.) were grown in succession since 2008. In the experiment, the potato cultivar Reet was evaluated.

Study **II** was performed at Einola Farm (58°17'N, 26°43'E; Figure 1) in Reola, Estonia. The study was conducted under a conventionally managed farming system from May to September 2014. At the study site, winter wheat (*Triticum aestivum* L.), spring wheat (*T. aestivum* L.), rapeseed (*Brassica napus* L.), spring wheat, and potato were grown in succession since 2010. Twenty-one cultivars of potato were grown in a completed randomized block design with three replicate plots per cultivar, each containing twenty-three certified seed tubers that were planted on 15 May 2014. During the growing period, several synthetic fertilizers and pesticides were used equally in all replicate plots. In the experiment, the following cultivars were evaluated: Arielle, Erika, Ranomy, Esmee, Viviana, Glorietta, Catania, Solist, Champion, Madeleine, Mariska, Concordia, Antonia, Karlena, Laudine, Fontane, Bellefleur, Rosagold, Excellency, Manitou, and Merlot.

For study **III**, the main objective was to test MinION protocols for ultra-rapid pathogen identification. The third-generation HTS methods on potato (*Solanum tuberosum* L.) and cucumber (*Cucumis sativa* L.) tissue samples with disease symptoms were tested (Table 1).

Table 1. Details of potato and cucumber samples.

Run	Sample	Disease symptom	Collection	
			locality	date
ONT2i	*S. tuberosum* / tuber	Black sclerotia on tuber	Õssu	30.08.2018
ONT2j	*S. tuberosum* / leaf	Dark circular lesions on leaves	Tartu	16.05.2019
ONT2k	*C. sativa* / roots	Wilted roots	Eistvere	20.05.2019
ONT2l	*C. sativa* / leaf	White powdery spots on leaves	Luunja	22.05.2019
ONT2n	*S. tuberosum* / stem	Dark brown lesions on stem	Roiu	30.05.2019

4.2. Sampling

The root systems of three individual plants from each replicate plot were collected using a clean shovel (**I**, **II**). Each root sample consisted of the entire root system of three randomly chosen individual plants (**I**, **II**). Root samples were collected during different phenological development stages of a potato: the early flowering stage (BBCH-scale 60–62) (**I**, **II**) and senescence (BBCH-scale 93–95) (**II**). At each sampling time, together with roots, adjacent soil was collected from each plot (**II**). Also, during planting, bulk soil samples were collected near potato tubers (**II**). Each sample consisted of three subsamples of soil taken randomly from the potato root zone at 0–25 cm depth (**II**). For study **II**, a total of 315 samples were collected.

For study **I**, potato plants of the locally bred potato cultivar Reet were sampled during the early flowering stage (BBCH-scale 60–62) in July 2010. In total, 72 composite root samples of all treatments of OFS and CFS were collected. Root samples were dried and preserved airtight at room temperature until further processing. For study **II**, root samples were transported to the laboratory in a cooler, washed three times with deionized water to remove residual soil, dried at 50 °C for 24 h as described in (García de León et al. 2016), and stored air-tight at room temperature until molecular analysis.

4.3. Assessment of AM fungal root colonization

Root samples from both field and pot experiment (**I**) were stained with Trypan blue according to Koske and Gemma (1989). Roots were cleared in 10% Potassium hydroxide (KOH), acidified with 1% hydrochloric acid (HCl) and stained with 0.01% trypan blue in lactoglycerol. Total root length colonized by AM fungi was estimated using the magnified grid-line intersections method (McGonigle et al. 1990), by scoring 120 fields of view per sample under the compound microscope at 400 × magnification, as described in (Uibopuu et al. 2012).

4.4. Trap plant bioassay

Narrowleaf plantain (*Plantago lanceolata* L.), as an AM host plant commonly used in bioassay experiments, was used to evaluate the mycorrhizal inoculum potential IP of field soil (**I**). Three soil samples were collected

randomly from each of the four replicate plots of all treatments before soil tillage in spring 2011 and stored in darkness at 10 °C until use. Before the use in experiment, the three soil samples per plot were pooled and handled as one composite sample. Each composite sample was thoroughly mixed with autoclaved sand in a 1:1 v/v ratio to improve drainage and aeration of the soil mixture. Narrowleaf plantain seeds were germinated in Petri dishes on moist filter paper, following (Uibopuu et al. 2012). Three seedlings per pot were planted in January 2011 in plastic pots (13 × 15 cm, depth × diameter). One seedling per pot was retained after four weeks of growth. Plants were kept in a greenhouse under controlled conditions, watered as needed with tap water and grown for 3 months. The infectivity bioassay of Moorman and Reeves (1979) was used to quantify the relative density of colonizing propagules of AM fungi.

4.5. Molecular analyses

Study II The total DNA was extracted from powdered root and soil samples with 75 mg 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), according to manufacturer's instructions with the following modifications: 1) samples were homogenized 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. To identify a wide variety of fungal genera, the ITS2 region was amplified using ITS3mix1-5 (CANCGATGAAGAACGYRG; Tedersoo et al. 2014) forward primers and a degenerate reverse primer ITS4ngs (CCTCCSCTTATTGATATGC; (Tedersoo et al. 2014), which was tagged with one of the multiplex identifiers (Tedersoo et al. 2014). The PCR products were sequenced using an Illumina MiSeq instrument (2 × 300 bp) at the Estonian Genome Center (University of Tartu, Tartu, Estonia) (**II**).

Study III For optimizing the DNA extraction protocols, 20 mg of infected tissue of plant samples was used. For DNA extraction, two technical replicates in 2-ml microcentrifuge tubes that contained either 100 µl Phire plant direct PCR kit lysis buffer (Thermo Fisher Scentific) or 500 µl $(NH_4)_2SO_4$-based lysis buffer (0.8 M Tris-HCl, 0.2 M $(NH_4)_2SO_4$, 0.2% (wt/vol) Tween 20; Solis BioDyne) were used. To shorten the DNA extraction time, lysis steps, tissue disruption, centrifugation, and incubation time were reduced as follows: the Phire lysis protocol was shortened by reduc-

ing the step of lysis to 2-5 min, preceded by tissue disruption using bead beating (5 min at 30 Hz) or a mortar and pestle in liquid N_2, followed by brief centrifugation at 5,000 × g, incubation at 30 °C for 5 min, and a final centrifugation at 11,000 × g for 1 min. The $(NH_4)_2SO_4$ lysis included incubation time reduced to 5 min, followed by incubation at 98 °C for 2 min. The DNA was concentrated from lysate using the FavorPrep gel/PCR purification kit (Favorgen Biotech Corp., Vienna, Austria) by following the manufacturer's instructions, except including centrifugation steps for 1 min and final elution using 50 µl water. Some samples were further subjected to Agencourt AMPure XP bead purification (Beckman Coulter, East Windsor, NJ, USA) to remove DNA fragments of < 500 bases. DNA concentration was measured using QUBIT. For library preparation, the SQK-RAD004 kit was used with manufacturer's protocols followed, except for a shortened fragmentation time at 30 °C for 30 s.

4.6. Bioinformatics

Study II Illumina sequencing resulted in 772,326 reads that were quality-filtered and assigned to samples using mothur 1.34.4 (Schloss et al. 2009) (average quality over 15 bp ≥ 26, and no ambiguities allowed). The quality-trimmed data were assembled using PANDAseq Assembler (Masella et al. 2012), with a minimum overlap of 15 bp, and demultiplexed in mothur. Potential chimeric sequences were removed using USEARCH 7.0.1090 (Edgar 2010). The remaining chimeric sequences, where full primer strings were detected inside the reads, were removed using PipeCraft built-in module (removal of multiprimer artefacts) (Anslan et al. 2017). The ITS2 subregion was extracted using ITSx 1.0.9 (Bengtsson-Palme et al. 2013), and clustered using a 97% similarity threshold in CD-HIT (Fu et al. 2012). 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 (Kõljalg et al. 2016). 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 (Tedersoo et al. 2014).

Study III For the MinION instrument, amplicon library preparation was performed using the ligation kit SQK-RAD004 and followed the manufacturer's protocols, except for a shortened fragmentation time at 30 °C of 30 s. Sequencing was performed in the laboratory at room temperature, connecting the MinION device to a plugged-in, internet-connected laptop computer with four processors and 32 GB RAM. MinION runs were performed on R9.4 flow cells, and the data were downloaded after obtaining sufficient amounts of passed reads (Table 2). The passed FASTA-formatted reads were further subjected to bioinformatics analysis using PipeCraft (Anslan et al. 2017). The options in PipeCraft included demultiplexing of metabarcoding reads allowing no mismatches to the barcode, followed by BLASTn search using default settings. Given the poor overall sequence quality, traditional MOTU-based approaches are not applicable to the MinION data; therefore, reads were mapped based on their best matches to database sequences in the UNITE reference database by following the principles of previous nanopore sequencing studies (Benítez-Páez et al. 2016; Kerkhof et al. 2017).

Table 2. Details of the rapid pathogen diagnostics experiments using the MinION instrument [a]

Sample / Run (min)	DNA extraction method (min)	DNA Purification (min)	No. of Raw / passed reads	Bioinformatics: quality-filtered reads (min)	Sequencing flow cell usage
ONT2i (50)	BB, Phire lysis (15)	FavorPrep (25)	1,142 / 436	97 (40)	New
ONT2j (5)	LN, Phire lysis (40)	FavorPrep (60)	20,000 / 9,974	6,750 (409)	New
ONT2k (30)	LN, Phire lysis (40)	FavorPrep (55)	5,044 / 4,046	849 (79)	2nd use
ONT2l (5)	LN, Phire lysis (35)	FavorPrep (55)	2,000 / 1420	669 (132)	3rd use
ONT2m (60)	LN, Phire lysis (35)	FavorPrep (55), AMPure (30)	1,074 / 223	104 (10)	4th use
ONT2n (4)	LN, $(NH_4)_2SO_4$ lysis (20)	FavorPrep (20), AMPure (30)	4,000 / 2,457	1,236 (97)	New

BB – bead beating; LN – liquid N_2.

[a] Library preparation and data interpretation took 15 min and 5 min, respectively.

4.7. Functional assignment

Study II Based on taxonomic assignments, OTUs were parsed to one of the following functional groups of fungal guilds: plant pathogenic fungi and arbuscular mycorrhizal fungi, based on FUNGuild (Nguyen et al. 2016). 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.

4.8. Statistical analyses

For **study I**, normal distribution (*Shapiro-Wilk test*) and equality of variances in treatment groups (*Levene test*) of data were explored. Then, the non-parametric *Kruskal-Wallis rank sum test* was conducted to determine the differences in treatments followed by the *Dunn's test* for pairwise comparison. For the *Dunn's test*, the P-values were adjusted according to the *Bonferroni correction*. When comparing only two groups, the non-parametric *Wilcoxon signed-rank test* was used. To study the relationship between soil parameters and AM fungal colonization, *Pearson's correlation analysis* was carried out.

For **study II**, multiple ecological measures, such as relative abundance of AM fungi and species richness, relative abundance, and community composition were used to test the main determinants of putative pathogenic fungal richness in soil and roots. Species richness was calculated based on residuals of OTU richness in relation to the square root of the number of sequences to account for differences in sequencing depth across samples (Bálint et al. 2016; Tedersoo et al. 2014). The relative abundance of AM fungi and putative fungal pathogens were 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 pathogenic fungal communities were tested using general linear model (GLM, Type III SS) followed by *Tukey* HSD post hoc tests comparing the means of standardized 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). 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). As implemented in PRIMER 7 (PRIMER-E, Auckland, New Zealand). PERMANOVA+ (Anderson et al. 2008) with 9999 permutations, under the reduced model, was used to compare the variability of pathogenic fungal community composition across explanatory variables.

For **study III**, the relative abundance of putative fungal pathogens was calculated as a number of sequences corresponding to the putative pathogenic OTU divided by the total number of fungal sequences for each sample.

5. RESULTS

5.1. Potato root AM fungal colonization

To determine how the natural AM fungal colonization in potato roots is affected by agricultural treatments, conventional and organic farming systems were compared. The AM fungal colonization level in roots of the potato cultivar Reet was higher in organic (OFS) than conventional farming system (CFS), though colonization levels were very low in both systems (Figure 2A). Individual treatments influenced root AM fungal colonization levels differently. AM fungal colonization was higher in cover crop and manure amendment (CC + M) treatment than synthetic nitrogen amendment treatments N_{50} and N_{150} (Figure 3A). There were no statistically significant differences among other treatments.

Correlation analysis revealed that potato root AM fungal colonization level was negatively correlated with soil phosphorus content (R = -0.50, P = 0.012, **I**) and positively with total annual C input to the soil (R = 0.46, P = 0.023; **I**). When exploring these relationships separately by management groups, the relationship between soil phosphorus content and potato root AM fungal colonization level remained negatively correlated with soil phosphorus content for organic (OFS) (R = -0.78, P = 0.022; **I**), but not for conventional farming system (CFS) (R = -0.11, P = 0.67; **I**). The relationship between total annual C input to soil and potato root AM fungal colonization level was significant neither for OFS (R = 0.26, P = 0.52; Table S3, **I**) nor CFS (R = -0.35, P = 0.17; Table S4, **I**). Unexpectedly, potato root AM fungal colonization level was negatively correlated with Corg in OFS (R = -0.76, P = 0.03; Table S3, **I**). There were no significant relationships between other soil chemical parameters and potato AM fungal colonization (**I**).

5.2. AM fungal inoculum potential in a potato field

Analyzing the AM fungal inoculum potential in a potato field revealed that root AM fungal colonization of the trap plant narrowleaf plantain (*Plantago lanceolata* L.) in the greenhouse trial was significantly greater (P < 0.001) than that of field-grown potato roots in both farming systems. The median AM fungal colonization levels in the narrowleaf plantain

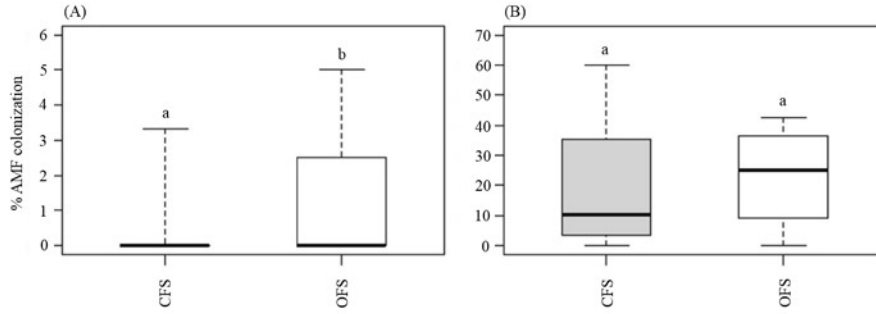

Figure 2. Median values of percent of AM fungal colonization of potato roots in conventional (CFS) and organic farming systems (OFS) in the field trial (A) and narrowleaf plantain roots grown in sand inoculated with soil from the same conventional and organic farming systems (B). Box plots indicate median (bold horizontal line), interquartile ranges (box), and minimum and maximum values (whiskers).

Different letters above the boxes indicate statistically significant differences at $P < 0.05$ among treatments (Wilcoxon rank sum test). Grey boxes – CFS, open boxes – OFS (**I**).

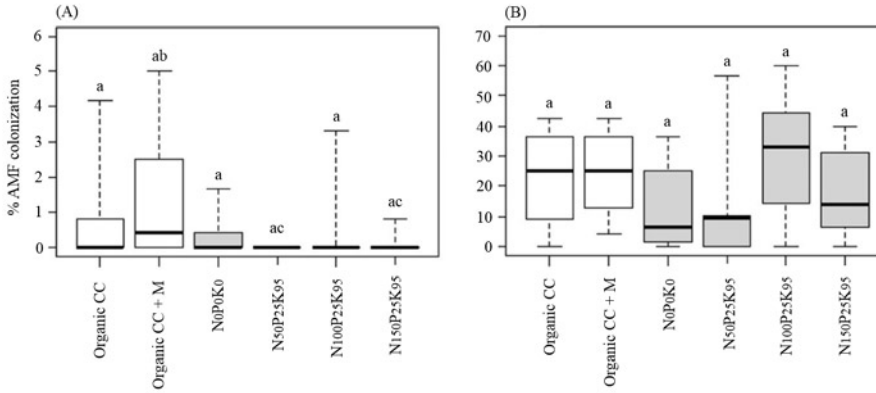

Figure 3. Percent of AM fungal colonization of potato roots in different agricultural treatments in the field trial (A) and of narrowleaf plantain roots in the greenhouse trial inoculated with soils from the same agricultural treatments in the field (B). Box plots indicate median (bold horizontal line), interquartile ranges (box), and minimum and maximum values (whiskers). Different letters above the boxes indicate statistically significant differences at $P < 0.05$ among treatments (Kruskal-Wallis rank sum test, Dunn test with Bonferroni correction as post hoc test).

Open boxes – organic farming systems, grey boxes – conventional farming systems. CC – farming system with cover crops, CC + M – farming system with cover crops and composted cattle manure, $N_0P_0K_0$ – control system with no additional fertilizers used, $N_{50}P_{25}K_{95}$, $N_{100}P_{25}K_{95}$, and $N_{150}P_{25}K_{95}$ – systems with different N rates used (**I**).

roots were 10.6% (range 0-60%) in CFS and 25.0% (range 0-42.5%) in OFS. The AM fungal colonization of narrowleaf plantain showed non-significant tendency to be higher in the OFS than in the CFS (Figure 2B). AM fungal root colonization values in soils from individual field treatments varied considerably, but with no significant differences between treatments (Figure 3B).

5.3. Relative abundance of AM fungi in the potato field

Sequencing of potato root and soil samples of twenty-one potato cultivars grown in a conventionally managed field showed that of all sequences, AM fungi accounted for 0.6%. In roots, neither sampling time nor variety affected relative abundance of AM fungi (Table 3). In soil, sampling time was the main factor describing relative abundance of AM fungi (Table 3), with significantly highest values at the senescence (SSC) stage and lowest values before potato plants emerged (BEM stage), and at flowering stage (FL)(Figure 4).

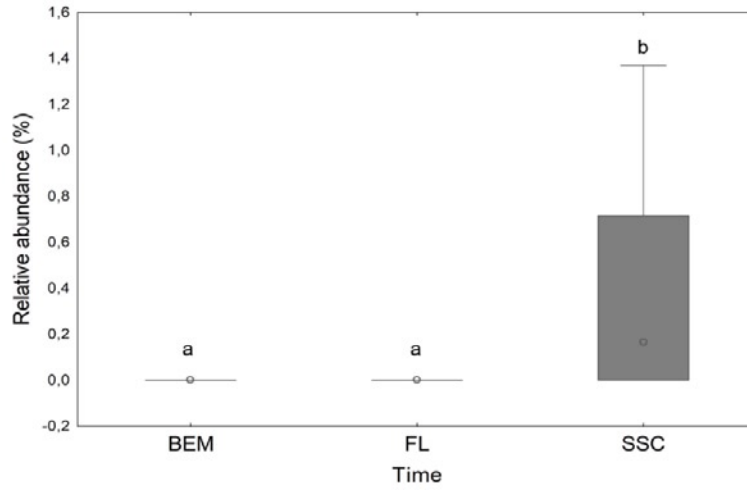

Figure 4. Relative abundance of AM fungi from all fungal sequences at different phenological plant growth stages in soil. Different letters above the plots indicate statistically significant differences at $P < 0.05$ among treatments. BEM – before emergence; FL – plant flowering stage; SSC – plant senescence.

Table 3. Effects of cultivar, sampling time, and their interaction on the relative abundance of AM fungi in soil and roots.

Sample type	Variable	df	R^2adj	*Pseudo F*	*P*
Roots	Cultivar	20	0	0.82	0.681
	Time	1	0.009	2.13	0.148
	Cultivar × Time	20	0	0.94	0.545
	Replication block	2	0.022	2.33	0.104
Soil	Cultivar	20	0	0.75	0.763
	Time	2	0.078	8.54	<0.001***
	Cultivar × Time	40	0	0.91	0.622
	Replication block	2	0.005	1.50	0.227

df – degrees of freedom; R^2adj – adjusted R^2; *Pseudo F* – pseudo-F statistic; *P* – probability value. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

5.4. Plant genotype, associated pathogens, and potato developmental phase

In roots, the potato cultivar was the main factor determining pathogen community composition ($P < 0.001$, R^2adj = 0.130; Table 4). Conversely, in soil, sampling time was the main factor describing fungal pathogen community composition (Table 4).

Table 4. Effects of cultivar, sampling time, and their interaction on the pathogenic fungal community composition in roots and soil.

Sample type	Variable	df	R^2adj	*Pseudo F*	*P*
Roots	Cultivar	20	0.130	2.485	<0.001***
	Time	1	0.077	13.088	0.098
	Replication block	2	0.004	1.689	0.040*
	Cultivar × Time	20	0.004	1.378	0.004**
	Cultivar × Field	40	0	0.916	0.793
	Time × Field	2	0	1.088	0.369
Soil	Cultivar	20	0.004	1.135	0.144
	Time	2	0.102	9.125	0.004**
	Replication block	2	0.0109	2.513	0.001**
	Cultivar × Time	40	0	1.139	0.079
	Cultivar × Field	40	0	1.132	0.087
	Time × Field	4	0.003	1.438	0.053

df – degrees of freedom; R^2adj – adjusted R^2; *Pseudo F* – pseudo-F statistic; *P* – probability value. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

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 5), with higher relative pathogenic fungal abundance in the senescence (SSC) stage compared to flowering (FL) stage (Figure 5C). Cultivar also had a significant effect on pathogenic fungal abundance ($F_{20,82}$ = 1.81,

Table 5. Effects of cultivar, sampling time, and their interaction on the relative abundance of plant pathogenic fungi in soil and roots.

Sample type	Variable	df	R^2adj	*Pseudo F*	*P*
Roots	Cultivar	20	0.004	1.8	0.033*
	Time	1	0.296	66.8	<0.001***
	Cultivar × Time	20	0	1.1	0.333
	Replication block	2	0.048	7.0	0.002**
Soil	Cultivar	20	0.011	1.7	0.043*
	Time	2	0.250	37.7	<0.001***
	Cultivar × Time	40	0	1.4	0.068
	Replication block	2	0	0.8	0.459

df – degrees of freedom; R^2adj – adjusted R^2; *Pseudo F* – pseudo-F statistic; *P* – probability value. ***P < 0.001; **P < 0.01; *P < 0.05.

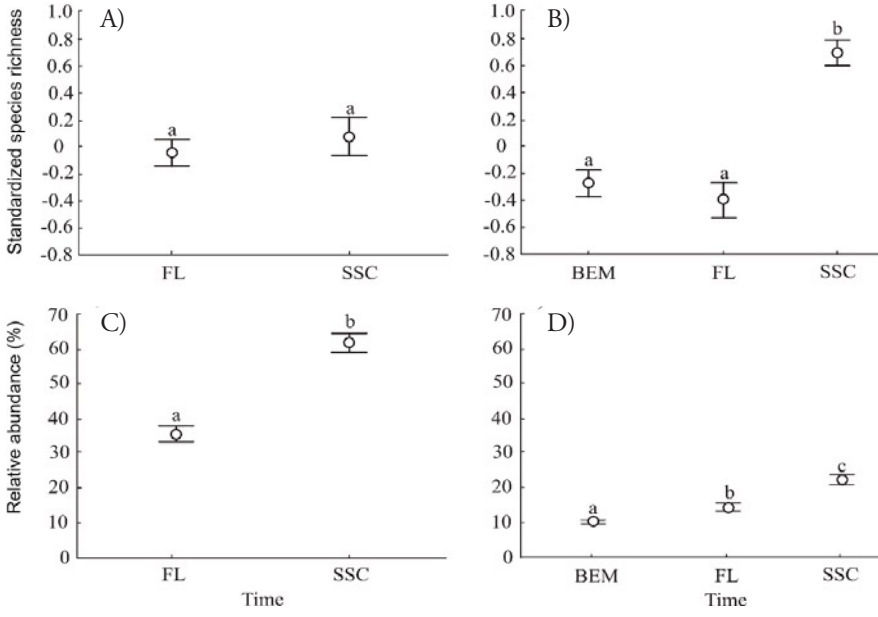

Figure 5. Results of a general linear model estimating the species richness and relative abundance of pathogenic fungi in root (A, C) and soil (B, D). Different letters above the plots indicate statistically significant differences at P < 0.05 among treatments.

R^2adj = 0.004, P = 0.033; Table 5), with Rosagold having a significantly higher fungal pathogen abundance than Catania (Table S8, **II**).

Sampling time had the strongest effect on soil pathogen abundance (Table 5), with highest values at the senescence (SSC) stage and lowest values before potato plants emerged (BEM stage) (Figure 5D). Cultivar had a significant effect on soil pathogenic fungal abundance ($F_{20,124}$ = 1.69, R^2adj = 0.011, P = 0.004; Table 4), which was non-significant in post hoc analyses.

In root samples, *Plectosphaerella cucumerina* (12.7%), *Microdochium* sp. (7.2%), *Fusarium* sp. (6.2%), and *Rhizoctonia* sp. (teleomorph: *Thanatephorus* sp., 5.2%) were the most abundant pathogen taxa (Figure 6A). Similarly to the 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), which peaked at the senescence (SSC) stage (Table 6). Cultivar also had a significant effect on *P. cucumerina* abundance. Post hoc analyses revealed a marginally greater *P. cucumerina* abundance (Table 6) in Rosagold and Esmee compared with Fontane (**II**). Of the other most dominant pathogenic fungi in potato roots, cultivar had the strongest influence on the relative abundance of

Table 6. Relative abundance (%) of the four most dominant pathogenic OTUs from potato roots and soil sampled in different time points.

OTU No.	Closest hit	Time			*P* values from GLM analysis		
		BEM	FL	SSC	Cult	Ti	Cult × Ti
Roots							
OTU_1087	*Plectosphaerella cucumerina*	NA	3.0 (±1.69)	22.6 (±1.69)	*	***	NS
OTU_578	*Microdochium* sp.	NA	6.9 (±0.80)	7.4 (±0.80)	NS	NS	NS
OTU_1330	*Fusarium* sp.	NA	5.2 (±0.9)	7.2 (±0.9)	NS	NS	NS
OTU_19032	*Rhizoctonia* sp.	NA	1.8 (±1.8)	8.7 (±1.8)	**	**	NS
Soil							
OTU_1179	*Gibellulopsis nigrescens*	3.6 (±0.5)	5.3 (±0.5)	6.6 (±0.5)	NS	***	NS
OTU_1087	*Plectosphaerella cucumerina*	1.0 (±0.3)	1.7 (±0.3)	4.6 (±0.3)	NS	***	NS
OTU_2317	*Didymellaceae* sp.	1.0 (±0.2)	1.2 (±0.2)	2.1 (±0.2)	NS	***	NS
OTU_1330	*Fusarium* sp.	0.7 (±0.1)	1.0 (±0.1)	1.0 (±0.1)	*	NS	NS

Ti – time; BEM – before emergence; FL – plant flowering stage; SSC – plant senescence; Cult – cultivar. ***P <0.001; **P < 0.01; *P < 0.05.

Rhizoctonia sp., that again increased with time (Table 6). Post hoc analyses revealed a marginally higher abundance of *Rhizoctonia* sp. 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* sp. (**II**).

In the soil samples, *Gibellulopsis nigrescens* (5.2%), *P. cucumerina* (2.4%), *Didymellaceae* (1.4%), and *Fusarium* sp. (0.9%) were the most abundant pathogen taxa (Figure 6B). The relative abundance of *G.nigerescens* was affected by sampling time ($F_{2,124}$=9.34, R^2adj = 0.071, $P < 0.001$; **II**), with lowest abundance before potato plants emerged (BEM stage) compared to flowering (FL) and senescence (SSC) stages (Table 6). This pattern also revealed for other most abundant pathogen taxa, *P. cucumerina* ($F_{2,124}$ = 32.70, R^2adj = 0.251, $P < 0.001$; **II**) and *Didymellaceae* sp. ($F_{2,124}$ = 10.5, R^2adj = 0.103, $P < 0.001$; **II**). The lowest *P. cucumerina* abundance was observed before plants emerged compared to flowering (FL) and senescence (SSC) stages (Table 6). The lowest *Didymellaceae* sp. abundance was observed at both before potato plants emerged (BEM) and at the flowering (FL) stage compared to the senescence (SSC) stage (Table 6). In contrast, cultivar was the only significant variable affecting *Fusarium* sp. abundance (Table 6), but post hoc analyses revealed that *Fusarium* sp. abundance in soil near the roots of the cultivar Merlot was only marginally higher than that of Karlena (**II**).

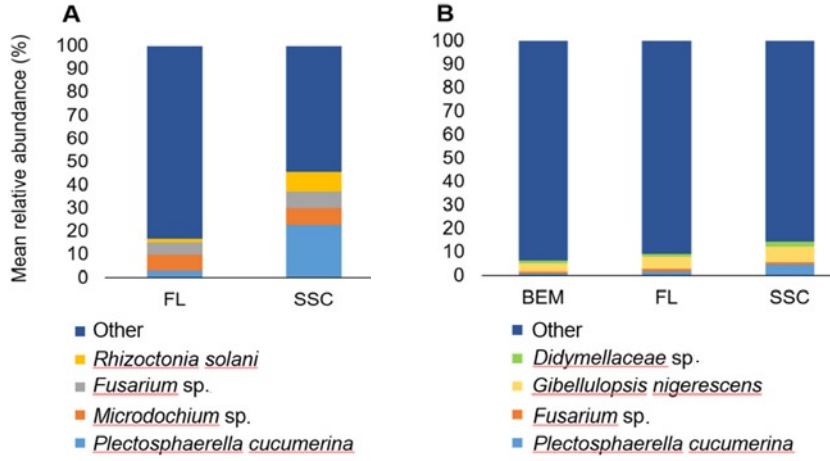

Figure 6. Taxonomic composition of fungal pathogens in roots (A) and soil (B) in different sampling times across all cultivars and replication blocks. BEM – before emergence; FL – flowering stage; SSC – senescence stage.

5.5. Pathogenic fungal richness

The highest proportion of variance in pathogenic fungal richness in roots was explained only by cultivar (Table 7). 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 (Figure 7). In contrast, the

Table 7. Effects of cultivar, sampling time, and their interaction on the richness of plant pathogenic fungi in soil and roots.

Sample type	Variable	df	R^2adj	*Pseudo F*	*P*
Roots	Cultivar	20	0.176	2.3	0.004**
	Time	1	0	0.6	0.437
	Cultivar × Time	20	0	1.0	0.464
	Replication block	2	0.002	1.4	0.259
Soil	Cultivar	20	0	0.6	0.908
	Time	2	0.234	28.5	<0.001***
	Cultivar × Time	40	0	0.99	0.492
	Replication block	2	0	1.2	0.326

df – degrees of freedom; R^2adj – adjusted R^2; *Pseudo F* – pseudo-F statistic; *P* – probability value. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

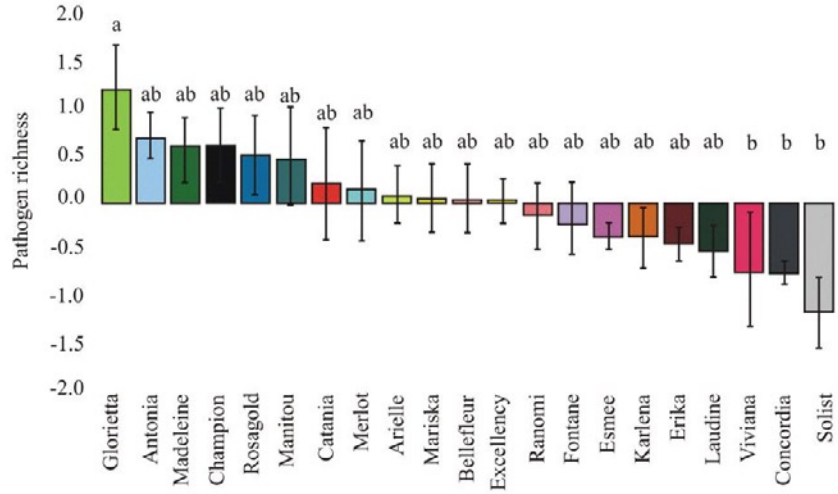

Figure 7. Results of a general linear model estimating the effect of cultivar for root fungal pathogen richness (indicated as standardized species richness) collected across all sampling times and replication blocks. Bars represent standard error. Different letters indicate statistically significant differences between cultivars (Tukey HSD post hoc tests, $P < 0.05$).

highest proportion of variance in soil pathogen richness was explained only by sampling time (Table 7), with the senescence (SSC) stage exhibiting higher richness compared to FL stage or time before potato plants emerged (Figure 5B).

5.6. MinION tool for ultrarapid pathogen identification

To test the suitability of the third-generation sequencing device MinION for rapid plant fungal pathogen detection, several ONT (Oxford Nanopore Technologies) MinION runs (i, j, k, l, m, n) were performed (Table 1). In particular, the lysis and centrifugation time in DNA extraction and purification protocols and sequencing time were reduced (Table 8). Depending on the time used for DNA extraction, DNA purification, sequencing, and bioinformatics, the minimum pathogen detection time for the plant tissue sample was 84 minutes, whereas the maximum speed indicates the most successful steps of pathogen diagnostics experiments (Figure 8).

For the first run ONT2i, bead beating combined with Phire lysis and FavorPrep purification was used to obtain DNA from a single diseased *S. tuberosum* tuber sample with *T. cucumeris* symptoms. After 150 min of

Table 8. Details of the rapid pathogen diagnostics experiments using the MinION instrument.

Sample/ Run (min)	Samples (n)	Detected pathogen / pathogen abundance	Disease symptom	Total analysis time (min)
ONT2i (50)	*Solanum tuberosum* tuber	*Thanatephorus cucumeris* (4.1%)	Black sclerotia on tuber	150
ONT2j (5)	*Solanum tuberosum* leaf	*Phytophthora infestans* (0.015%)	Dark circular lesions on leaves	534
ONT2k (30)	*Cucumis sativa* roots	*Fusarium* aff. *fujikuroi* (0.12%)	Wilted roots	224
ONT2l (5)	*Cucumis sativa* leaf	*Albugo laibachii* (0.2%)	White powdery spots on leaves	247
ONT2m (60)	*Cucumis sativa* leaf	None	White powdery spots on leaves	ND [a]
ONT2n (4)	*Solanum tuberosum* stem	*Rhizoctonia solani* (2.0%)	Dark brown lesions on stem	191

[a] ND, not determined because AMPure purification was performed on a subsequent day.

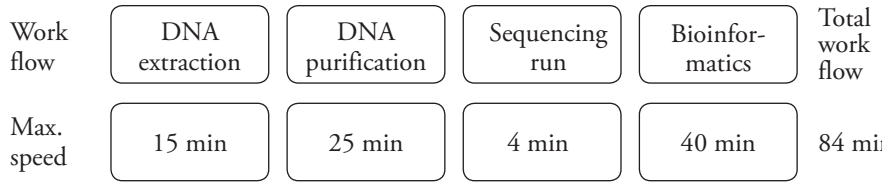

Figure 8. Schematic overview of maximum potential speed of rapid pathogen diagnostics in this study under optimal conditions using metagenomics approaches with the MinION instrument. Note that the time used here was compiled across the successful runs and does not indicate any individual analyses.

analyses, MinION sequencing revealed *Lycopersicon esculentum* (tomato; 72.2% of sequences), *S. tuberosum* (14.4%), and *T. cucumeris* (4.1%) as the only putative pathogens, which matched the symptoms and were confirmed by Sanger sequencing that revealed *T. cucumeris* (all four subsamples) and *Pyronemataceae* sp. (two subsamples; Ascomycota).

For the ONT2j run, potato plants inoculated with a suspension of *Phytophthora infestans* and *Boeremia exigua* isolates were used. With an analysis time of 534 minutes, due to contamination and absence or marginal abundance of the two inoculated pathogens, this MinION run was considered unsuccessful.

After 224 min of analyses, the ONT2k run of wilted cucumber (*Cucumis sativus* L.) roots was dominated by *L. esculentum*, a contaminant from the previous run (43.9%), followed by *C. sativus* (11.9%) and *C. melo* (11.8%). Apart from the pathogen *Fusarium* aff. *fujikuroi* (0.12%), the microbiome was dominated by bacteria *Rhodanobacter denitrificans* (3.8%) and *Pseudomonas* aff. *umsongensis* (1.6%), all of which were undetected in the previous run. Neither symptoms nor Sanger sequencing allowed to confirm that fusariosis caused wilting.

A diseased *C. sativus* leaf with white powdery infection that by symptoms defined as *Podosphaera xanthii* was used for the ONT2l run. After 247 min of analyses, MinION sequencing revealed that *C. sativus* (58.7%) and *C. melo* (27.5%) dominated, but only a single putative pathogen, *Albugo laibachii*, was present. However, Sanger sequencing of the same sample revealed *Peronospora violae* instead, along with an unidentified mite (*Acari*).

For the ONT2m run, the DNA of sample used for ONT2l was further purified using AMPure XP beads. In this run, flow cell loading resulted in a very low accumulation of sequences. Bioinformatics analysis revealed *C. sativus* (40.4%) and C. melo (34.6%) but no putative pathogens among the 104 quality-filtered sequences.

For the ONT2n run, a diseased *S. tuberosum* stem sample with visible *T. cucumeris* infection was powdered in liquid N_2, and DNA was extracted using $(NH_4)_2SO_4$ lysis, followed by double purification with FavorPrep and AMPure XP beads to rule out the possibility that the latter procedure inhibited the last run. On a new flow cell, sequences accumulated rapidly, after 191 min of analyses, revealing *L. esculentum* (75.4%), *Pseudomonas* sp. (16.7%), and the putative pathogen *T. cucumeris* (2.0%) that matched the symptoms previously defined as *T. cucumeris*.

6. DISCUSSION

6.1. Mycorrhizal symbiosis of potato

In this thesis, the patterns of potato root AM fungal colonization in response to fertilizing treatments in the conventional and organic farming systems and plant host to AM fungal compatibility are reported (**I**, **II**). In general, the findings demonstrate that although potato roots have greater AM fungal colonization levels under organic than conventional farming (**I**), the mycorrhizal potential dependence of potato is very low (**I**, **II**).

The present work showed that there is a sufficient amount of AM fungal inoculum both in the organic and conventionally fertilized field soil. However, the colonization of potato roots by AM fungi was extremely low in both management types. Compared with potato, roots of the trap plant narrowleaf plantain showed considerably higher AM fungal colonization, indicating that the field soils were not exhausted of AM fungi (**I**). In contrast to the present work, a study conducted in the Peruvian Andes (Senés-Guerrero et al. 2014) showed that potato fields harbor a high AM fungal diversity, which could be explained by the low-input management system. However, a study conducted in Italy (Cesaro et al. 2008) corroborated my work (**I**), indicating low proportion of potato root system colonized in the field.

In study **I**, AM fungal root colonization tended to be higher in the organic farming systems both in field-grown potato roots and in plantain roots. The lower AM fungal colonization in the conventional farming system can be explained by lower fresh organic matter input and higher phosphorus content. These data are broadly consistent with the major trends of AM fungal abundance decline in response to P fertilization (Liu et al. 2020; Treseder 2004). There is clear evidence that with increasing P availability, plants become less dependent on AM fungi and down-regulate their mycorrhiza formation (Smith and Read 2008).

Study **I** showed that potato roots are not prone to mycorrhization in high-input farming systems, which could be explained by evidence that newer plant cultivars tend to have lower AM fungal root colonization (García de León et al. 2020; but see Lehmann et al. 2012). To test this

further, study **II** was conducted to assess differences in fungal community structure, including AM fungi, both within roots and in soil, of 21 potato cultivars. The results of this study showed that of all sequences, AM fungi accounted for 0.6% and there were no significant differences among cultivars. The results of study **II** confirm that modern potato cultivars grown in high-input farming systems have low mycorrhizal dependence (Lehmann et al. 2012). The results are also in agreement with a crop rotation study by Soonvald et al. (2020), which showed that potato grown in plots with higher soil fertility had significantly lower AM fungal diversity compared to wheat and barley. Thus, potato crop differs from most cereal crops in responsiveness to AM fungi, possibly because potato has the greatest demand for fertilizers and pesticides compared with other agricultural crops. Study **II** also clearly showed that in commercial potato field, at the end of the growing season, the relative abundance of AM fungi is significantly higher compared to the beginning of the growing season. That can be explained by the adverse effect of multiple sequential agrotechnical operations carried out during the growing period. Also, the potato plant, a crop with a short life span from planting to maturity, may stop growing before AM fungi recover from soil disturbance. To date, commercialization of AM fungi has gained popularity, but before their extensive use in potato production, practitioners should consider the fact that under high P supply and higher levels of soil disturbances, both in organic and conventional farming, plants potentially depend and benefit little from AM symbiosis, and thus show low root colonization levels.

6.2. Diversity of soilborne phytopathogenic fungi

Seasonal and cultivar-driven changes of soilborne phytopathogenic fungal communities in conventional potato production, both within roots and in soil, were studied for the first time on a large number of potato cultivars using high-throughput sequencing (**II**). The cultivar influenced the root-pathogenic fungal richness and community composition more than the sampling time or sampling time and cultivar interaction. Additionally, cultivar had a significant effect on the relative abundance of root fungal pathogens. However, only two cultivars, Rosagold and Catania, differed from each other based on a post hoc test (**II**). The cultivar effect on pathogenic fungal richness and abundance can be explained by different susceptibility of crop cultivars to various diseases and their expression under certain environmental conditions. Contrary to the pattern of

root-pathogenic fungal richness, the highest proportion of variance in relative pathogen abundance in roots was explained by sampling time. The relative pathogen abundance in roots was higher in senescence stage compared to flowering stage (**II**). This is in line with studies indicating that aging affects immunity in plants (Sharabani et al. 2013). Generally, seedlings and senescent plants are more prone to pathogen infection, whereas in the middle of growth period, plants have a more developed root epidermis that does not allow the penetration of fungal hyphae into the root.

Soil pathogen richness, relative abundance, and community composition were explained by sampling time but not cultivar. Higher richness occurred in the senescent stage compared to the beginning of growth season or flowering stages (**II**). The higher pathogen abundance was evident again at the SSC stage (**II**). This can be explained by root exudates that can inhibit or stimulate the microbial growth (Baetz and Martinoia 2014; Lekota et al. 2020).

6.3. Factors affecting the abundance of dominant fungal pathogens

The factors affecting the abundance of dominant fungal pathogens, both within roots and in soil, were studied for the first time on a large number of potato cultivars using high-throughput sequencing (**II**). The present work shows that depending on the specific fungal pathogenic taxa, the pathogen abundance in potato roots can be affected by plant growth stage and/or cultivar. This is in line with studies indicating that potato cultivars differ in their ability to defend themselves against different soilborne pathogens (Sedláková et al. 2013; Yanar et al. 2005). However, this pattern revealed only two most abundant pathogen taxa, *P. cucumerina* and *Rhizoctonia* sp. (**II**), of which only the latter is a widespread pathogen taxa of potato (Tsror 2010). The high abundance of these taxa can be explained by their strategy of invading and colonizing the host, and subsequently shifting to the necrotrophic stage (Pétriacq et al. 2016; Zrenner et al. 2020). Similarly to overall pathogen abundance in root samples, sampling time had the strongest influence on the relative abundance of *P. cucumerina*, which peaked at the SSC stage. Again, this could be explained by its hemibiotrophic lifestyle. There was also a marginally greater abundance of *P. cucumerina* in the roots of the cultivars Rosagold

and Esmee compared to Fontane (**II**). Interestingly, this is the first report of high abundance and cultivar specificity of *Plectosphaerella cucumerina*, which is better known as a root pathogen of many other plant species (Carlucci et al. 2012).

Among the dominant soilborne pathogens, *Rhizoctonia* sp. abundance was strongly influenced by cultivar. The results of this study showed that *Rhizoctonia* sp. abundance was marginally higher in the roots of the cultivars Merlot, Concordia, Solist, Manitou, Karlena, and Glorietta. In these cultivars, the relative abundance of *Rhizoctonia* sp. increased with time. Conversely, Laudine, Rosagold, Excellency, Viviana, Bellefleur, Fontane, Antonia, Mariska, Madeleine, Champion, Esmee, Erika, and Arielle showed almost no occurrence of *Rhizoctonia* sp. (**II**). Interestingly, according to the information provided by the breeding company Norika (Norika GmbH), the cultivar Merlot has high resistance to *Rhizoctonia* sp. Nevertheless, this study found that Merlot showed marginally stronger abundance of *Rhizoctonia* sp. 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. This finding highlights the importance of breeding for disease resistance as an important strategy for reducing crop losses caused by crop diseases.

The analyses conducted in this study revealed that among most abundant pathogenic taxa in potato field soil, *Gibellulopsis nigrescens*, *P. cucumerina* and *Didymellaceae* sp. abundance were affected by sampling time, with lowest abundance in the beginning of the growing season (**II**). The present work showed that in the crop rotation context, *P. cucumerina* needs further attention as a root-invading pathogen of potato. The abundance of *Fusarium* sp. in soil was only marginally affected by cultivar (**II**). The results demonstrate the potential role of root exudates of different cultivars that may shape the composition of soil microbiome, including the suppression of soil pathogens.

In general, the findings of this study demonstrate that the occurrence of major pathogens may strongly vary among potato cultivars. Overall, the results demonstrate that in roots, cultivar can be the primary factor determining pathogen community composition.

6.4. Rapid molecular diagnostics of plant pathogens

Under increasing threat of plant disease outbreaks, the speed of detection of plant disease agents is essential to avoid large-scale crop losses. Therefore, the capacity of the third-generation sequencing device MinION for ultrarapid pathogen identification was tested with plant tissues (**III**). The present work showed that, although multiple quality issues were detected in MinION runs, accurate molecular identification from sample preparation to taxonomic interpretation may take as little as 2.5 h using nanopore sequencing in the metagenome mode (Table 7). Compared to a previous study by Schmidt et al. (2017), where identification of bacterial human pathogens was performed in 4 h, this work showed that the nanopore analysis work flow for plant tissues can be potentially reduced to < 2 h. Multiple other human pathogen studies report on running the full analysis work flow in 1 day (Charalampous et al. 2018; Quick et al. 2016; Votintseva et al. 2017). These diagnostics rates of MinION cannot be beaten by instruments of other HTS platforms that require several hours for library preparation and at least one day for sequencing (Reuter et al. 2015).

Still, care needs to be taken to account for multiple potential technical biases. First, it is required that the DNA is easily extractable in high quantity and purity (Tomlinson et al. 2010), amplification is performed by methods alternative to those of conventional slow polymerases (Notomi et al. 2015), nanopore library preparation follows methods for rapid library kits, and the sequencing process is limited to ca. 15 min after obtaining a critical number of reads (Votintseva et al. 2017). MinION diagnostics tool has great potential for rapid diagnostics of pathogens and potentially other organisms, but caution should be exercised to control or account for multiple potential technical biases such as low accumulation of sequences and high contamination risk from a previous run.

In metagenomics analyses, a key limitation of the MinION approach is poor availability of reference genomes, because species with available genomes have a much greater chance of accumulating hits than species with no available genomes. This is in line with previous studies (Ashikawa et al. 2018; Kilianski et al. 2015), which showed that MinION is well suited to diagnostics of human pathogens that are well-known and well-referenced in public sequence databases. Trials presented in study **III** revealed that shortened DNA extraction protocols may yield lower quality

and quantity of DNA, whereas culled incubation and centrifugation steps in library preparation may result in dilute and poorly indexed libraries overrepresented by short fragments. Although this study successfully identified microorganisms from *S. tuberosum* using a library 13-fold more dilute than recommended, it may be useful to add a certain amount of so-called carrier DNA to secure preparation of high-quality libraries (Mojarro et al. 2018).

7. CONCLUSIONS

Study **I** confirmed the first hypothesis according to which potato roots have greater AM fungal colonization levels under organic than conventional farming. The present work also showed that there was a sufficient amount of AM fungal inoculum both in the organic and conventional field soil. However, the colonization of potato roots by AM fungi was extremely low in both management types. Therefore, it can be assumed that the tested cultivar may be relatively little dependent and little benefitting from mycorrhizal symbiosis. There are several contradictory studies on this topic, and the current study explored only one potato cultivar. Thus, the results cannot be extrapolated to other cultivars.

The second hypothesis that AM fungal community composition, richness, and relative abundance in root and soil differ among plant growth stages and across the potato cultivars was partly proven in study **II**. Due to no detection of AM fungi in several samples, the analysis does not enable to determine AM fungal species richness and community composition of different potato cultivars. Nevertheless, it can be concluded that modern potato cultivars growing in the high-input farming system may be relatively little dependent and little benefitting from mycorrhizal symbiosis. In addition, the results showed that at the end of the growing season the relative abundance of AM fungi in the soil is significantly higher compared to the beginning of the growing season, which may reflect possible adverse effects of multiple sequential agrotechnical operations on AM fungi.

Of the other part of the second hypothesis that pathogenic community composition, richness, and relative abundance in root and soil differ among plant growth stages and across the potato cultivars was fully confirmed in study **II**. The relative abundance of pathogenic fungi was different between plant growth stages and cultivars in soil and roots. In roots, a pathogenic fungal richness and community composition were different between cultivars. In soil, the community composition of putative pathogenic fungi changed significantly over time.

The third aim, testing the MinION device for rapid plant fungal pathogen detection, was partly successful. As a scientific novelty, the author found that MinION can be utilized for rapid and accurate identification

of dominant pathogenic organisms from plant tissues. The study clearly demonstrated that the MinION device is well suited for rapid PCR-free diagnosis of fungal pathogens and other eukaryotic organisms, which may take as little as 150 min from sample preparation (including DNA extraction, library preparation, sequencing, and bioinformatics) to data interpretation (**III**). However, care should be taken of a number of possible limitations that might lead to misdiagnosis. Shortened DNA extraction protocols may yield lower quality and quantity of DNA, resulting in the pathogen remaining undetected. A major drawback of this approach is some contamination from the previous MinION run. In addition, care should be taken to secure profound reference sequence data to avoid misdiagnosis.

This is the first time that in depth information about the diversity, abundance, and community composition of putative pathogenic fungi and symbiotic AM fungi in potato fields in Estonia is presented. The findings are of direct practical relevance, demonstrating that AM fungi are present in the current conventional potato production systems, but potatoes exhibit low root colonization levels. In order to benefit from AM fungi in potato cultivation, a potato growing technology that involves less soil disturbances could be used. Also, the results of this thesis indicate the importance of plant breeding, concerning disease resistance. Moreover, the results of this study offer useful and novel implications for rapid pathogen diagnostics that may help to avoid large-scale crop losses caused by new emerging pathogens that are undetectable by symptoms.

REFERENCES

Adams, I. P., Glover, R. H., Monger, W. A., Mumford, R., Jackeviciene, E., Navalinskiene, M., et al. (2009). Next-generation sequencing and metagenomic analysis: A universal diagnostic tool in plant virology. Molecular Plant Pathology, 10(4), 537–545. https://doi.org/10.1111/j.1364-3703.2009.00545.x

Adolf, B., Andrade-Piedra, J., Bittara Molina, F., Przetakiewicz, J., Hausladen, H., Kromann, P., et al. (2020). Fungal, Oomycete, and Plasmodiophorid Diseases of Potato. In: Campos H., Ortiz O. (eds) The Potato Crop. Springer, Cham.

An, G. H., Kobayashi, S., Enoki, H., Sonobe, K., Muraki, M., Karasawa, T., & Ezawa, T. (2010). How does arbuscular mycorrhizal colonization vary with host plant genotype? An example based on maize (*Zea mays*) germplasms. Plant and Soil, 327(1), 441–453. https://doi.org/10.1007/s11104-009-0073-3

Anderson, M. J., Gorley, R. N., & Clarke, K. R. (2008). PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods; PRIMER-E: Plymouth, UK, 2008.

Andrivon, D., Ramage, K., Guérin, C., Lucas, J. M., & Jouan, B. (1997). Distribution and fungicide sensitivity of *Colletotrichum coccodes* in French potato-producing areas. Plant Pathology, 46(5), 722–728. https://doi.org/10.1046/j.1365-3059.1997.d01-60.x

Anslan, S., Bahram, M., Hiiesalu, I., & Tedersoo, L. (2017). PipeCraft: Flexible open-source toolkit for bioinformatics analysis of custom high-throughput amplicon sequencing data. Molecular Ecology Resources, 17(6), e234–e240. https://doi.org/10.1111/1755-0998.12692

Ashikawa, S., Tarumoto, N., Imai, K., Sakai, J., Kodana, M., Kawamura, T., et al. (2018). Rapid identification of pathogens from positive blood culture bottles with the MinION nanopore sequencer. Journal of Medical Microbiology, 67(11), 1589–1595. https://doi.org/10.1099/jmm.0.000855

Aslam, S., Tahir, A., Aslam, M. F., Alam, M. W., Shedayi, A. A., & Sadia, S. (2017). Recent advances in molecular techniques for the identification of phytopathogenic fungi – a mini review. Journal of Plant Interactions, 12(1), 493–504. https://doi.org/10.1080/17429145.2017.1397205

Baetz, U., & Martinoia, E. (2014). Root exudates: the hidden part of plant defense. Trends in Plant Science, 19(2), 90–98. https://doi.org/10.1016/J.TPLANTS.2013.11.006

Baldi, P., & La Porta, N. (2020). Molecular Approaches for Low-Cost Point-of-Care Pathogen Detection in Agriculture and Forestry. Frontiers in Plant Science, 11. https://doi.org/10.3389/fpls.2020.570862

Bálint, M., Bahram, M., Eren, A. M., Faust, K., Fuhrman, J. A., Lindahl, B., et al. (2016, September 1). Millions of reads, thousands of taxa: Microbial community structure and associations analyzed via marker genesa. FEMS Microbiology Reviews. Oxford University Press. https://doi.org/10.1093/femsre/fuw017

Banerjee, S., Walder, F., Büchi, L., Meyer, M., Held, A. Y., Gattinger, A., et al. (2019). Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in roots. ISME Journal, 13(7), 1722–1736. https://doi.org/10.1038/s41396-019-0383-2

Baon, J. B., Smith, S. E., & Alston, A. M. (1993). Mycorrhizal responses of barley cultivars differing in P efficiency. Plant and Soil, 157(1), 97–105. https://doi.org/10.1007/bf00038752

Barba, M., Czosnek, H., & Hadidi, A. (2014). Historical Perspective, Development and Applications of Next-Generation Sequencing in Plant Virology. Viruses, 6(1), 106–136. https://doi.org/10.3390/v6010106

Bebber, D. P., Holmes, T., & Gurr, S. J. (2014). The global spread of crop pests and pathogens. Global Ecology and Biogeography, 23(12), 1398–1407. https://doi.org/10.1111/geb.12214

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., et al. (2013). Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods in Ecology and Evolution, 4(10). https://doi.org/10.1111/2041-210X.12073

Benítez-Páez, A., Portune, K. J., & Sanz, Y. (2016). Species-level resolution of 16S rRNA gene amplicons sequenced through the MinION™ portable nanopore sequencer. GigaScience. https://doi.org/10.1186/s13742-016-0111-z

Benitez, M. S., Taheri, W. I., & Lehman, R. M. (2016). Selection of fungi by candidate cover crops. Applied Soil Ecology, 103, 72–82. https://doi.org/10.1016/j.apsoil.2016.03.016

Berg, G., & Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiology Ecology, 68(1), 1–13. https://doi.org/10.1111/j.1574-6941.2009.00654.x

Bhattarai, I. D., & Mishra, R. R. (1984). Study on the vesicular-arbuscular mycorrhiza of three cultivars of potato (*Solanum tuberosum* L.). Plant and Soil, 79(2), 299–303. https://doi.org/10.1007/BF02182354

Birch, P. R. J., Bryan, G., Fenton, B., Gilroy, E. M., Hein, I., Jones, J. T., et al. (2012). Crops that feed the world 8: Potato: Are the trends of increased global production sustainable? Food Security, 4(4), 477–508. https://doi.org/10.1007/s12571-012-0220-1

Blin, P., Robic, K., Khayi, S., Cigna, J., Munier, E., Dewaegeneire, P., et al. (2021). Pattern and causes of the establishment of the invasive bacterial potato pathogen *Dickeya solani* and of the maintenance of the resident pathogen *D. dianthicola*. Molecular Ecology, 30(2), 608–624. https://doi.org/10.1111/mec.15751

Bolton, M. D., Panella, L., Campbell, L., & Khan, M. F. R. (2010). Temperature, moisture, and fungicide effects in managing *Rhizoctonia* root and crown rot of sugar beet. Phytopathology, 100(7), 689–697. https://doi.org/10.1094/PHYTO-100-7-0689

Boonham, N., Kreuze, J., Winter, S., van der Vlugt, R., Bergervoet, J., Tomlinson, J., & Mumford, R. (2014). Methods in virus diagnostics: From ELISA to next generation sequencing. Virus Research, 186, 20–31. https://doi.org/10.1016/j.virusres.2013.12.007

Bowles, T. M., Jackson, L. E., Loeher, M., & Cavagnaro, T. R. (2017). Ecological intensification and arbuscular mycorrhizas: a meta-analysis of tillage and cover crop effects. Journal of Applied Ecology, 54(6), 1785–1793. https://doi.org/10.1111/1365-2664.12815

Brachaczek, A., Kaczmarek, J., & Jedryczka, M. (2016). Monitoring blackleg (*Leptosphaeria* spp.) ascospore release timing and quantity enables optimal fungicide application to improved oilseed rape yield and seed quality. European Journal of Plant Pathology, 145(3), 643–657. https://doi.org/10.1007/s10658-016-0922-x

Bradley, P., Gordon, N. C., Walker, T. M., Dunn, L., Heys, S., Huang, B., et al. (2015). Rapid antibiotic-resistance predictions from genome sequence data for *Staphylococcus aureus* and *Mycobacterium tuberculosis*. Nature Communications, 6(1), 10063. https://doi.org/10.1038/ncomms10063

Bronzato Badial, A., Sherman, D., Stone, A., Gopakumar, A., Wilson, V., Schneider, W., & King, J. (2018). Nanopore Sequencing as a Surveillance Tool for Plant Pathogens in Plant and Insect Tissues. Plant Disease, 102(8), 1648–1652. https://doi.org/10.1094/PDIS-04-17-0488-RE

Burton, W. G. (1989). The Potato. Third Edition. John Wiley & Son, Inc., New York.

Buysens, C., Alaux, P. L., César, V., Huret, S., Declerck, S., & Cranenbrouck, S. (2017). Tracing native and inoculated *Rhizophagus irregularis* in three potato cultivars (Charlotte, Nicola and Bintje) grown under field conditions. Applied Soil Ecology, 115, 1–9. https://doi.org/10.1016/j.apsoil.2017.03.007

Campion, C., Chatot, C., Perraton, B., & Andrivon, D. (2003). Anastomosis groups, pathogenicity and sensitivity to fungicides of *Rhizoctonia solani* isolates collected on potato crops in France. European Journal of Plant Pathology, 109(9), 983–992. https://doi.org/10.1023/B:EJPP.0000003829.83671.8f

Campos, H., & Ortiz, O. (2020). The Potato Crop. Springer, Cham. https://doi.org/https://doi.org/10.1007/978-3-030-28683-5

Carlucci, A., Raimondo, M. L., Santos, J., & Phillips, A. J. L. (2012). *Plectosphaerella* species associated with root and collar rots of horticultural crops in southern Italy. Persoonia - Molecular Phylogeny and Evolution of Fungi, 28(1), 34–48. https://doi.org/10.3767/003158512X638251

Cesaro, P., Van Tuinen, D., Copetta, A., Chatagnier, O., Berta, G., Gianinazzi, S., & Lingua, G. (2008). Preferential colonization of *Solanum tuberosum* L. Roots by the fungus *Glomus intraradices* in arable soil of a potato farming area. Applied and Environmental Microbiology, 74(18), 5776–5783. https://doi.org/10.1128/AEM.00719-08

Chalupowicz, L., Dombrovsky, A., Gaba, V., Luria, N., Reuven, M., Beerman, A., et al. (2019). Diagnosis of plant diseases using the Nanopore sequencing platform. Plant Pathology, 68(2), 229–238. https://doi.org/10.1111/ppa.12957

Charalampous, T., Richardson, H., Kay, G. L., Baldan, R., Jeanes, C., Rae, D., et al. (2018). Rapid Diagnosis of Lower Respiratory Infection using Nanopore-based Clinical Metagenomics. bioRxiv, 387548. https://doi.org/10.1101/387548

Charlermroj, R., Himananto, O., Seepiban, C., Kumpoosiri, M., Warin, N., Gajanandana, O., et al. (2014). Antibody array in a multiwell

plate format for the sensitive and multiplexed detection of important plant pathogens. Analytical Chemistry, 86(14), 7049–7056. https://doi.org/10.1021/ac501424k

Colin, J. . (1998). Potato. FAO/IPGRI Technical Guidelines for the Safe Movement of Germplasm n.19. https://hdl.handle.net/10568/100266. Accessed 19 March 2021

Das, S., Shah, F. A., Butler, R. C., Falloon, R. E., Stewart, A., Raikar, S., & Pitman, A. R. (2014). Genetic variability and pathogenicity of *Rhizoctonia solani* associated with black scurf of potato in New Zealand. Plant Pathology, 63(3), 651–666. https://doi.org/10.1111/ppa.12139

Davies, F. T., Calderón, C. M., & Huaman, Z. (2005). Influence of Arbuscular Mycorrhizae Indigenous to Peru and a Flavonoid on Growth, Yield, and Leaf Elemental Concentration of `Yungay' Potatoes. HortScience, 40. https://doi.org/https://doi.org/10.21273/HORTSCI.40.2.381

Dean, R., Van Kan, J. A. L., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., et al. (2012, May). The Top 10 fungal pathogens in molecular plant pathology. Molecular Plant Pathology. Wiley-Blackwell. https://doi.org/10.1111/j.1364-3703.2011.00783.x

Deb, D., Khan, A., & Dey, N. (2020). Phoma diseases: Epidemiology and control. Plant Pathology, 69(7), 1203–1217. https://doi.org/10.1111/ppa.13221

Delavaux, C. S., Smith-Ramesh, L. M., & Kuebbing, S. E. (2017). Beyond nutrients: a meta-analysis of the diverse effects of arbuscular mycorrhizal fungi on plants and soils. Ecology, 98(8), 2111–2119. https://doi.org/10.1002/ecy.1892

DeShields, J. B., Bomberger, R. A., Woodhall, J. W., Wheeler, D. L., Moroz, N., Johnson, D. A., & Tanaka, K. (2018). On-Site Molecular Detection of Soil-Borne Phytopathogens Using a Portable Real-Time PCR System. Journal of visualized experiments: JoVE, 132(132), 56891. https://doi.org/10.3791/56891

Detheridge, A. P., Brand, G., Fychan, R., Crotty, F. V., Sanderson, R., Griffith, G. W., & Marley, C. L. (2016). The legacy effect of cover crops on soil fungal populations in a cereal rotation. Agriculture, Ecosystems & Environment, 228, 49–61. https://doi.org/10.1016/J.AGEE.2016.04.022

Devaux, A., Goffart, J. P., Petsakos, A., Kromann, P., Gatto, M., Okello, J., et al. (2019). Global food security, contributions from sustain-

able potato agri-food systems. In The Potato Crop: Its Agricultural, Nutritional and Social Contribution to Humankind (pp. 3–35). Springer International Publishing. https://doi.org/10.1007/978-3-030-28683-5_1

Donoso, A., & Valenzuela, S. (2018). In-field molecular diagnosis of plant pathogens: recent trends and future perspectives. Plant Pathology, 67(7), 1451–1461. https://doi.org/10.1111/ppa.12859

Doonan, J., Denman, S., McDonald, J. E., & Golyshin, P. N. (2017). Shotgun metagenomic sequencing analysis of soft-rot enterobacteriaceae in polymicrobial communities. In Methods in Molecular Biology (Vol. 1539, pp. 85–97). Humana Press Inc. https://doi.org/10.1007/978-1-4939-6691-2_7

Douds, D. D., Nagahashi, G., Reider, C., & Hepperly, P. R. (2007). Inoculation with arbuscular mycorrhizal fungi increases the yield of potatoes in a high P soil. Biological Agriculture and Horticulture, 25(1), 67–78. https://doi.org/10.1080/01448765.2007.10823209

Drenkhan, R., Riit, T., Adamson, K., & Hanso, M. (2016). The earliest samples of *Hymenoscyphus albidus* vs. *H. fraxineus* in Estonian mycological herbaria. Mycological Progress, 15(8), 835–844. https://doi.org/10.1007/s11557-016-1209-5

Duan, Y., Ge, C., Zhang, X., Wang, J., & Zhou, M. (2014). A rapid detection method for the plant pathogen *Sclerotinia sclerotiorum* based on loop-mediated isothermal amplification (LAMP). Australasian Plant Pathology, 43(1), 61–66. https://doi.org/10.1007/s13313-013-0239-6

Duffy, E. M., & Cassells, A. C. (2000). The effect of inoculation of potato (*Solanum tuberosum* L.) microplants with arbuscular mycorrhizal fungi on tuber yield and tuber size distribution. Applied Soil Ecology, 15(2), 137–144. https://doi.org/10.1016/S0929-1393(00)00089-5

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. Bioinformatics, 26(19), 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Elmer, W. H. (2001). Seeds as vehicles for pathogen importation. Biological Invasions, 3(3), 263–271. https://doi.org/10.1023/A:1015217308477

Escuredo, O., Seijo-Rodríguez, A., Meno, L., Rodríguez-Flores, M. S., & Seijo, M. C. (2019). Seasonal Dynamics of Alternaria during the Potato Growing Cycle and the Influence of Weather on the Early Blight Disease in North-West Spain. American Journal of Potato

Research, 96(6), 532–540. https://doi.org/10.1007/s12230-019-09739-2

Esfahani, M. N. (2005). Susceptibility assessment of potato cultivars to *Fusarium* dry rot species. Potato Research, 48(3–4), 215–226. https://doi.org/10.1007/BF02742378

Fang, Y., & Ramasamy, R. P. (2015). Current and prospective methods for plant disease detection. Biosensors. MDPI AG. https://doi.org/10.3390/bios5030537

FAO. (2009). International Year of the Potato 2008 - New lighton a hidden treasure. Food and Agriculture Organization of the United Nations Rome, Italy. http://www.fao.org/3/i0500e/i0500e00.htm. Accessed 28 January 2021

Fellers, J. P., Webb, C., Fellers, M. C., Shoup Rupp, J., & De Wolf, E. (2019). Wheat Virus Identification Within Infected Tissue Using Nanopore Sequencing Technology. Plant Disease, 103(9), 2199–2203. https://doi.org/10.1094/PDIS-09-18-1700-RE

Fiers, M., Edel-Hermann, V., Chatot, C., Le Hingrat, Y., Alabouvette, C., & Steinberg, C. (2012). Potato soil-borne diseases. A review. Agronomy for Sustainable Development. Springer. https://doi.org/10.1007/s13593-011-0035-z

Frac, M., Hannula, S. E., Belka, M., & Jędryczka, M. (2018). Fungal boil healthiodiversity and their role in s. Frontiers in Microbiology. Frontiers Media S.A. https://doi.org/10.3389/fmicb.2018.00707

Frost, K. E., Groves, R. L., & Charkowski, A. O. (2013). Integrated control of potato pathogens through seed potato certification and provision of clean seed potatoes. Plant Disease, 97(10), 1268–1280. https://doi.org/10.1094/PDIS-05-13-0477-FE

Fu, L., Niu, B., Zhu, Z., Wu, S., & Li, W. (2012). CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics (Oxford, England), 28(23), 3150–2. https://doi.org/10.1093/bioinformatics/bts565

Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Retrieved December 10, 2020, from https://nt.ars-grin.gov/fungaldatabases/. https://nt.ars-grin.gov/fungaldatabases/

Gallou, A., Lucero Mosquera, H. P., Cranenbrouck, S., Suárez, J. P., & Declerck, S. (2011). Mycorrhiza induced resistance in potato plantlets challenged by *Phytophthora infestans*. Physiological and

Molecular Plant Pathology, 76(1), 20–26. https://doi.org/10.1016/j.pmpp.2011.06.005

García de León, D., Moora, M., Öpik, M., Neuenkamp, L., Gerz, M., Jairus, T., et al. (2016). Symbiont dynamics during ecosystem succession: co-occurring plant and arbuscular mycorrhizal fungal communities. FEMS Microbiology Ecology, 92(7), fiw097. https://doi.org/10.1093/femsec/fiw097

García de León, D., Vahter, T., Zobel, M., Koppel, M., Edesi, L., Davison, J., et al. (2020). Different wheat cultivars exhibit variable responses to inoculation with arbuscular mycorrhizal fungi from organic and conventional farms. PLOS ONE, 15(5), e0233878. https://doi.org/10.1371/journal.pone.0233878

Geisseler, D., & Scow, K. M. (2014). Long-term effects of mineral fertilizers on soil microorganisms - A review. Soil Biology and Biochemistry. https://doi.org/10.1016/j.soilbio.2014.03.023

Gianinazzi, S., Gollotte, A., Binet, M. N., van Tuinen, D., Redecker, D., & Wipf, D. (2010). Agroecology: The key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza, 20(8), 519–530. https://doi.org/10.1007/s00572-010-0333-3

Ginnan, N. A., Dang, T., Bodaghi, S., Ruegger, P. M., McCollum, G., England, G., et al. (2020). Disease-Induced Microbial Shifts in Citrus Indicate Microbiome-Derived Responses to Huanglongbing Across the Disease Severity Spectrum. Phytobiomes Journal, 4(4), 375–387. https://doi.org/10.1094/PBIOMES-04-20-0027-R

Gosling, P., Hodge, A., Goodlass, G., & Bending, G. D. (2006, April 1). Arbuscular mycorrhizal fungi and organic farming. Agriculture, Ecosystems and Environment. Elsevier. https://doi.org/10.1016/j.agee.2005.09.009

Gryndler, M., Larsen, J., Hršelová, H., Řezáčová, V., Gryndlerová, H., & Kubát, J. (2006). Organic and mineral fertilization, respectively, increase and decrease the development of external mycelium of arbuscular mycorrhizal fungi in a long-term field experiment. Mycorrhiza, 16(3), 159–166. https://doi.org/10.1007/s00572-005-0027-4

Hannula, S. E., de Boer, W., & van Veen, J. (2012). A 3-year study reveals that plant growth stage, season and field site affect soil fungal communities while cultivar and GM-trait have minor effects. PLoS ONE, 7(4). https://doi.org/10.1371/journal.pone.0033819

Hausladen, H., & Leiminger, J. (2007). Potato early blight in Germany (*Alternaria solani, Alternaria alternata*). In Special Report no.12. Proc.

10th Workshop of a European Network for Development of an Integrated Control Strategy of Potato Late Blight. (pp. 370:189–194).

Heltoft, P., Molteberg, E. L., Nærstad, R., & Hermansen, A. (2015). Effect of Maturity Level and Potato Cultivar on Development of *Fusarium* Dry Rot in Norway. Potato Research, 58(3), 205–219. https://doi.org/10.1007/s11540-015-9300-x

Hijri, M. (2016). Analysis of a large dataset of mycorrhiza inoculation field trials on potato shows highly significant increases in yield. Mycorrhiza, 26(3), 209–214. https://doi.org/10.1007/s00572-015-0661-4

Hills, K., Collins, H., Yorgey, G., McGuire, A., & Kruger, C. (2020). Improving Soil Health in Pacific Northwest Potato Production: a Review. American Journal of Potato Research. Springer. https://doi.org/10.1007/s12230-019-09742-7

Hooker, W. J. (1981). Compendium of Potato Diseases. The American Phytopathological Society.

Hu, Y., Green, G. S., Milgate, A. W., Stone, E. A., Rathjen, J. P., & Schwessinger, B. (2019). Pathogen Detection and Microbiome Analysis of Infected Wheat Using a Portable DNA Sequencer. Phytobiomes Journal, 3(2), 92–101. https://doi.org/10.1094/PBIOMES-01-19-0004-R

Hyde, K. D., Udayanga, D., Manamgoda, D. S., Tedersoo, L., Larsson, E., Abarenkov, K., et al. (2013). Incorporating molecular data in fungal systematics: a guide for aspiring researchers. http://arxiv.org/abs/1302.3244. Accessed 25 July 2019

Ismail, Y., Mccormick, S., & Hijri, M. (2013). The arbuscular mycorrhizal fungus, Glomus irregulare, controls the mycotoxin production of *Fusarium sambucinum* in the pathogenesis of potato. FEMS Microbiology Letters, 348(1), 46–51. https://doi.org/10.1111/1574-6968.12236

Jalli, M., Kaseva, J., Andersson, B., Ficke, A., Nistrup-Jørgensen, L., Ronis, A., et al. (2020). Yield increases due to fungicide control of leaf blotch diseases in wheat and barley as a basis for IPM decision-making in the Nordic-Baltic region. European Journal of Plant Pathology, 158(2), 315–333. https://doi.org/10.1007/s10658-020-02075-w

Jansa, J., Wiemken, a., & Frossard, E. (2006). The effects of agricultural practices on arbuscular mycorrhizal fungi. Geological Society, London, Special Publications, 266(1), 89–115. https://doi.org/10.1144/GSL.SP.2006.266.01.08

Jeger, M. J., Hide, G. A., Van Den Boogert, P. H. J. F., Termorshuizen, A. J., & Van Baarlen, P. (1996). Pathology and control of soil-borne fungal pathogens of potato. In Potato Research. Kluwer Academic Publishers. https://doi.org/10.1007/bf02357949

Jia, T., Yao, Y., Guo, T., Wang, R., & Chai, B. (2020). Effects of Plant and Soil Characteristics on Phyllosphere and Rhizosphere Fungal Communities During Plant Development in a Copper Tailings Dam. Frontiers in Microbiology, 11. https://doi.org/10.3389/fmicb.2020.556002

Kalia, A., & Gosal, S. K. (2011, September). Effect of pesticide application on soil microorganisms. Archives of Agronomy and Soil Science. Taylor & Francis . https://doi.org/10.1080/03650341003787582

Kerkhof, L. J., Dillon, K. P., Häggblom, M. M., & McGuinness, L. R. (2017). Profiling bacterial communities by MinION sequencing of ribosomal operons. Microbiome, 5(1), 116. https://doi.org/10.1186/s40168-017-0336-9

Kettles, G. J., & Luna, E. (2019). Food security in 2044: How do we control the fungal threat? Fungal Biology, 123(8), 558–564. https://doi.org/10.1016/j.funbio.2019.04.006

Kilianski, A., Haas, J. L., Corriveau, E. J., Liem, A. T., Willis, K. L., Kadavy, D. R., et al. (2015). Bacterial and viral identification and differentiation by amplicon sequencing on the MinION nanopore sequencer. GigaScience, 4(1), 12. https://doi.org/10.1186/s13742-015-0051-z

Köhl, L., Lukasiewicz, C. E., & Van der Heijden, M. G. A. (2016). Establishment and effectiveness of inoculated arbuscular mycorrhizal fungi in agricultural soils. Plant Cell and Environment, 39(1), 136–146. https://doi.org/10.1111/pce.12600

Kõljalg, U., Tedersoo, L., Nilsson, R. H., & Abarenkov, K. (2016). Digital identifiers for fungal species. Science (New York, N.Y.), 352(6290), 1182–3. https://doi.org/10.1126/science.aaf7115

Koske, R. E., & Gemma, J. N. (1989). A modified procedure for staining roots to detect VA mycorrhizas. Mycological Research, 92(4), 486–488. https://doi.org/10.1016/S0953-7562(89)80195-9

Lamichhane, J. R., Osdaghi, E., Behlau, F., Köhl, J., Jones, J. B., & Aubertot, J. N. (2018). Thirteen decades of antimicrobial copper compounds applied in agriculture. A review. Agronomy for Sustainable Development. Springer-Verlag France. https://doi.org/10.1007/s13593-018-0503-9

Landschoot, S., Carrette, J., Vandecasteele, M., De Baets, B., Höfte, M., Audenaert, K., & Haesaert, G. (2017). Boscalid-resistance in Alternaria alternata and Alternaria solani populations: An emerging problem in Europe. Crop Protection, 92, 49–59. https://doi.org/10.1016/j.cropro.2016.10.011

Le, D. T., & Vu, N. T. (2017). Progress of loop-mediated isothermal amplification technique in molecular diagnosis of plant diseases. Applied Biological Chemistry. Springer Netherlands. https://doi.org/10.1007/s13765-017-0267-y

Leff, J. W., Lynch, R. C., Kane, N. C., & Fierer, N. (2017). Plant domestication and the assembly of bacterial and fungal communities associated with strains of the common sunflower, Helianthus annuus. New Phytologist, 214, 412–423. https://doi.org/10.1111/nph.14323

Lehmann, A., Barto, E. K., Powell, J. R., & Rillig, M. C. (2012). Mycorrhizal responsiveness trends in annual crop plants and their wild relatives—a meta-analysis on studies from 1981 to 2010. Plant and Soil, 355(1). https://doi.org/10.1007/S11104-011-1095-1

Lehtonen, M. J., Ahvenniemi, P., Wilson, P. S., German-Kinnari, M., & Valkonen, J. P. T. (2008). Biological diversity of *Rhizoctonia solani* (AG-3) in a northern potato-cultivation environment in Finland. Plant Pathology, 57(1). https://doi.org/10.1111/j.1365-3059.2007.01694.x

Lekota, M., Modisane, K. J., Apostolides, Z., & van der Waals, J. E. (2020). Metabolomic Fingerprinting of Potato Cultivars Differing in Susceptibility to *Spongospora subterranea* f. sp. *subterranea* Root Infection. International Journal of Molecular Sciences, 21(11), 3788. https://doi.org/10.3390/ijms21113788

Liu, C., Ravnskov, S., Liu, F., Rubæk, G. H., & Andersen, M. N. (2018). Arbuscular mycorrhizal fungi alleviate abiotic stresses in potato plants caused by low phosphorus and deficit irrigation/partial root-zone drying. Journal of Agricultural Science, 156(1), 46–58. https://doi.org/10.1017/S0021859618000023

Liu, J., Zhang, J., Li, D., Xu, C., & Xiang, X. (2020). Differential responses of arbuscular mycorrhizal fungal communities to mineral and organic fertilization. MicrobiologyOpen, 9(1). https://doi.org/10.1002/mbo3.920

Liu, Y., Qi, A., Haque, M. E., Bhuiyan, M. Z. R., & Khan, M. F. R. (2021). Combining penthiopyrad with azoxystrobin is an effective alternative to control seedling damping-off caused by *Rhizoctonia*

solani on sugar beet. Crop Protection, 139, 105374. https://doi.org/10.1016/j.cropro.2020.105374

Loján, P., Senés-Guerrero, C., Suárez, J. P., Kromann, P., Schüßler, A., & Declerck, S. (2017). Potato field-inoculation in Ecuador with *Rhizophagus irregularis*: no impact on growth performance and associated arbuscular mycorrhizal fungal communities. Symbiosis, 73(1), 45–56. https://doi.org/10.1007/s13199-016-0471-2

Luchi, N., Capretti, P., Pazzagli, M., & Pinzani, P. (2016). Powerful qPCR assays for the early detection of latent invaders: interdisciplinary approaches in clinical cancer research and plant pathology. Appl Microbiol Biotechnol. https://doi.org/10.1007/s00253-016-7541-5

Luchi, N., Ioos, R., & Santini, A. (2020). Fast and reliable molecular methods to detect fungal pathogens in woody plants. Applied Microbiology and Biotechnology. Springer. https://doi.org/10.1007/s00253-020-10395-4

Mancini, V., Murolo, S., & Romanazzi, G. (2016). Diagnostic methods for detecting fungal pathogens on vegetable seeds. Plant Pathology. https://doi.org/10.1111/ppa.12515

Mao, L., Liu, Y., Shi, G., Jiang, S., Cheng, G., Li, X., et al. (2014). Wheat cultivars form distinctive communities of root-associated arbuscular mycorrhiza in a conventional agroecosystem. Plant and Soil, 374(1–2), 949–961. https://doi.org/10.1007/s11104-013-1943-2

Martin, F. N., & Tooley, P. W. (2004). Identification of *Phytophthora* isolates to species level using restriction fragment length polymorphism analysis of a polymerase chain reaction-amplified region of mitochondrial DNA. Phytopathology, 94(9), 983–991. https://doi.org/10.1094/PHYTO.2004.94.9.983

Martinelli, F., Scalenghe, R., Davino, S., Panno, S., Scuderi, G., Ruisi, P., et al. (2015). Advanced methods of plant disease detection. A review. Agronomy for Sustainable Development. Springer-Verlag France. https://doi.org/10.1007/s13593-014-0246-1

Masella, A. P., Bartram, A. K., Truszkowski, J. M., Brown, D. G., & Neufeld, J. D. (2012). PANDAseq: paired-end assembler for illumina sequences. BMC bioinformatics, 13, 31. https://doi.org/10.1186/1471-2105-13-31

McArthur, D., & Knowles, N. R. (1993). Influence of Vesicular-Arbuscular Mycorrhizal Fungi on the Response of Potato to Phosphorus

Deficiency. Plant physiology, 101, 147–160. https://doi.org/101/1/147 [pii]

McDonald, B. A., & Stukenbrock, E. H. (2016). Rapid emergence of pathogens in agro-ecosystems: global threats to agricultural sustainability and food security. Philosophical Transactions of the Royal Society B: Biological Sciences, 371(1709), 20160026. https://doi.org/10.1098/rstb.2016.0026

McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L., & Swan, J. A. (1990). A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytologist, 115, 495–501. https://doi.org/10.1111/j.1469-8137.1990.tb00476.x

Mojarro, A., Hachey, J., Ruvkun, G., Zuber, M. T., & Carr, C. E. (2018). CarrierSeq: a sequence analysis workflow for low-input nanopore sequencing. BMC Bioinformatics, 19(1), 108. https://doi.org/10.1186/s12859-018-2124-3

Moorman, T., & Reeves, B. F. (1979). The role of endomycorrhizae in revegetation practices in the semi-arid west. II. A bioassay to determine the effect of land disturbance on endomycorrhizal populations. American Journal of Botany. http://www.jstor.org/stable/2442619. Accessed 4 February 2016

Ngakou, A., Megueni, C., Nwaga, D., Mabong, M. R., Djamba, F. E., & Gandebe, M. (2006). *Solanum tuberosum* (L.) Responses to Soil Solarization and Arbuscular Mycorrhizal Fungi Inoculation under Field Conditions: Growth, Yield, Health Status of Plants and Tubers. Middle-East Journal of Scientific Research, 1(1), 23–30. https://doi.org/10.1021/jf0607359

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., et al. (2016). FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology, 20, 241–248. https://doi.org/10.1016/J.FUNECO.2015.06.006

Niessen, L., & Vogel, R. F. (2010). Detection of *Fusarium graminearum* DNA using a loop-mediated isothermal amplification (LAMP) assay. International Journal of Food Microbiology, 140(2–3), 183–191. https://doi.org/10.1016/j.ijfoodmicro.2010.03.036

Nilsson, R. H., Hyde, K. D., Pawłowska, J., Ryberg, M., Tedersoo, L., Aas, A. B., et al. (2014). Improving ITS sequence data for identification of plant pathogenic fungi. Fungal Diversity, 67(1), 11–19. https://doi.org/10.1007/s13225-014-0291-8

Nitzan, N., Cummings, T. F., & Johnson, D. A. (2008). Disease potential of soil- and tuberborne inocula of *Colletotrichum coccodes* and black dot severity on potato. Plant Disease, 92(11), 1497–1502. https://doi.org/10.1094/PDIS-92-11-1497

Oo, M. M., Lim, G. T., Jang, H. A., & Oh, S. K. (2017). Characterization and pathogenicity of new record of anthracnose on various chili varieties caused by *Colletotrichum scovillei* in Korea. Mycobiology, 45(3), 184–191. https://doi.org/10.5941/MYCO.2017.45.3.184

Paul, R., Ostermann, E., & Wei, Q. (2020). Advances in point-of-care nucleic acid extraction technologies for rapid diagnosis of human and plant diseases. Biosensors and Bioelectronics, 169, 112592. https://doi.org/10.1016/j.bios.2020.112592

Pétriacq, P., Stassen, J. H. M., & Ton, J. (2016). Spore density determines infection strategy by the plant pathogenic fungus *Plectosphaerella cucumerina*. Plant Physiology, 170(4), 2325–2339. https://doi.org/10.1104/pp.15.00551

Pozo, M. J., López-Ráez, J. A., Azcón-Aguilar, C., & García-Garrido, J. M. (2015). Phytohormones as integrators of environmental signals in the regulation of mycorrhizal symbioses. New Phytologist, 205(4), 1431–1436. https://doi.org/10.1111/nph.13252

Prigigallo, M. I., Abdelfattah, A., Cacciola, S. O., Faedda, R., Sanzani, S. M., Cooke, D. E. L., & Schena, L. (2016). Metabarcoding Analysis of *Phytophthora* Diversity Using Genus-Specific Primers and 454 Pyrosequencing. https://doi.org/10.1094/PHYTO-07-15-0167-R

Qi, Y., Li, Y., Xie, W., Lu, R., Mu, F., Bai, W., & Du, S. (2020). Temporal-spatial variations of fungal composition in PM2.5 and source tracking of airborne fungi in mountainous and urban regions. Science of the Total Environment, 708, 135027. https://doi.org/10.1016/j.scitotenv.2019.135027

Quick, J., Loman, N. J., Duraffour, S., Simpson, J. T., Severi, E., Cowley, L., et al. (2016). Real-time, portable genome sequencing for Ebola surveillance. Nature, 530(7589), 228–232. https://doi.org/10.1038/nature16996

Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C., & Moënne-Loccoz, Y. (2009). The rhizosphere: A playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant and Soil, 321(1–2), 341–361. https://doi.org/10.1007/s11104-008-9568-6

Ravnskov, S., Larsen, J., Olsson, P. A., & Jakobsen, I. (1999). Effects of various organic compounds on growth and phosphorus uptake of an arbuscular mycorrhizal fungus. New Phytologist, 141(3), 517–524. https://doi.org/10.1046/j.1469-8137.1999.00353.x

Ray, M., Ray, A., Dash, S., Mishra, A., Achary, K. G., Nayak, S., & Singh, S. (2017, January 15). Fungal disease detection in plants: Traditional assays, novel diagnostic techniques and biosensors. Biosensors and Bioelectronics. Elsevier Ltd. https://doi.org/10.1016/j.bios.2016.09.032

Reuter, J. A., Spacek, D. V., & Snyder, M. P. (2015). High-Throughput Sequencing Technologies. Molecular Cell, 58(4), 586–597. https://doi.org/10.1016/J.MOLCEL.2015.05.004

Rezki, S., Campion, C., Iacomi-Vasilescu, B., Preveaux, A., Toualbia, Y., Bonneau, S., et al. (2016). Differences in stability of seed-associated microbial assemblages in response to invasion by phytopathogenic microorganisms. PeerJ, 2016(4), e1923. https://doi.org/10.7717/peerj.1923

Ristaino, J. B., Saville, A. C., Paul, R., Cooper, D. C., & Wei, Q. (2020). Detection of phytophthora infestans by loop-mediated isothermal amplification, real-time LAMP, and droplet digital PCR. Plant Disease, 104(3), 708–716. https://doi.org/10.1094/PDIS-06-19-1186-RE

Ritchie, H., & Roser, M. (2019). Crop Yields. https://ourworldindata.org/crop-yields. Accessed 27 January 2021

Rúa, M. A., Antoninka, A., Antunes, P. M., Chaudhary, V. B., Gehring, C., Lamit, L. J., et al. (2016). Home-field advantage? evidence of local adaptation among plants, soil, and arbuscular mycorrhizal fungi through meta-analysis. BMC Evolutionary Biology, 16(1), 122. https://doi.org/10.1186/s12862-016-0698-9

Runno-Paurson, E., Fry, W. E., Remmel, T., Mänd, M., & Myers, K. L. (2010). Phenotypic and genotypic characterisation of Estonian isolates of *Phytophthora infestans* in 2004-2007. Journal of Plant Pathology, 92. https://www.jstor.org/stable/41998813

Salamone, A. L., & Okubara, P. A. (2020). Real-time PCR quantification of *Rhizoctonia solani* AG-3 from soil samples. Journal of Microbiological Methods, 172, 105914. https://doi.org/10.1016/j.mimet.2020.105914

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., & Nelson, A. (2019). The global burden of pathogens and pests on

major food crops. Nature Ecology & Evolution, 3, 430–439. https://doi.org/https://doi.org/10.1038/s41559-018-0793-y

Schaad, N. W., & Frederick, R. D. (2002). Real-time PCR and its application for rapid plant disease diagnostics Real-time PCR and its application for rapid plant disease diagnostics. Canadian Journal of Plant Pathology, 250–258. https://doi.org/10.1080/07060660209507006

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., et al. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and environmental microbiology, 75(23), 7537–41. https://doi.org/10.1128/AEM.01541-09

Schmidt, K., Mwaigwisya, S., Crossman, L. C., Doumith, M., Munroe, D., Pires, C., et al. (2017). Identification of bacterial pathogens and antimicrobial resistance directly from clinical urines by nanopore-based metagenomic sequencing. Journal of Antimicrobial Chemotherapy, 72(1), 104–114. https://doi.org/10.1093/jac/dkw397

Secor, G. A., & Gudmestad, N. C. (1999). Managing fungal diseases of potato. Canadian Journal of Plant Pathology, 21(3), 213–221. https://doi.org/10.1080/07060669909501184

Sedláková, V., Dejmalová, J., DoleŽal, P., Hausvater, E., Sedlák, P., & BaŠtová, P. (2013). Characterization of forty-four potato varieties for resistance to common scab, black scurf and silver scurf. Crop Protection, 48, 82–87. https://doi.org/10.1016/j.cropro.2013.02.014

Senés-Guerrero, C., Torres-Cortés, G., Pfeiffer, S., Rojas, M., & Schüßler, A. (2014). Potato-associated arbuscular mycorrhizal fungal communities in the Peruvian Andes. Mycorrhiza, 24(6), 405–417. https://doi.org/10.1007/s00572-013-0549-0

Sharabani, G., Shtienberg, D., Borenstein, M., Shulhani, R., Lofthouse, M., Sofer, M., et al. (2013). Effects of plant age on disease development and virulence of *Clavibacter michiganensis* subsp. *michiganensis* on tomato. Plant Pathology, 62(5), 1114–1122. https://doi.org/10.1111/ppa.12013

Sharma-Poudyal, D., Schlatter, D., Yin, C., Hulbert, S., & Paulitz, T. (2017). Long-term no-till: A major driver of fungal communities in dryland wheat cropping systems. PLOS ONE, 12(9), e0184611. https://doi.org/10.1371/journal.pone.0184611

Smith, A. F., & Smith, S. E. (2011). What is the significance of the arbuscular mycorrhizal colonisation of many economically important crop plants? Plant Soil, 348, 63–79. https://doi.org/10.1007/s11104-011-0865-0

Smith, S. E., & Read, D. J. (2008). Mycorrhizal Symbiosis. Third ed. Academic Press, Amsterdam, The Netherlands. Elsevier. https://doi.org/10.1016/B978-012370526-6.50002-7

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. https://doi.org/10.1016/j.apsoil.2019.05.003

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(1). https://doi.org/10.1038/s41598-020-74952-7

Tedersoo, L., Anslan, S., Bahram, M., Drenkhan, R., Pritsch, K., Buegger, F., et al. (2020a). Regional-Scale In-Depth Analysis of Soil Fungal Diversity Reveals Strong pH and Plant Species Effects in Northern Europe. Frontiers in Microbiology, 11. https://doi.org/10.3389/fmicb.2020.01953

Tedersoo, L., Anslan, S., Bahram, M., Kõljalg, U., & Abarenkov, K. (2020b). Identifying the 'unidentified' fungi: a global-scale long-read third-generation sequencing approach. Fungal Diversity, 103(1), 273–293. https://doi.org/10.1007/s13225-020-00456-4

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N. S., Wijesundera, R., et al. (2014). Global diversity and geography of soil fungi. Science, 346(6213). https://doi.org/10.1126/science.1256688

Tedersoo, L., Drenkhan, R., Anslan, S., Morales-Rodriguez, C., & Cleary, M. (2018). High-throughput identification and diagnostics of pathogens and pests: Overview and practical recommendations. Molecular Ecology Resources. Blackwell Publishing Ltd. https://doi.org/10.1111/1755-0998.12959

Thirkell, T. J., Charters, M. D., Elliott, A. J., Sait, S. M., & Field, K. J. (2017). Are mycorrhizal fungi our sustainable saviours? Considerations for achieving food security. Journal of Ecology, 105(4), 921–929. https://doi.org/10.1111/1365-2745.12788

Tian, Z., Wu, Y., Guo, H., Gong, X., & Zhang, H. (2019). First Report of Potato Black Dot Caused by *Colletotrichum coccodes* in Hebei

Province of China . Plant Disease, 103(10), 2669–2669. https://doi.org/10.1094/pdis-02-19-0357-pdn

Tiwari, R. K., Kumar, R., Sharma, S., Naga, K. C., Subhash, S., & Sagar, V. (2020). Continuous and emerging challenges of silver scurf disease in potato. International Journal of Pest Management, 1–13. https://doi.org/10.1080/09670874.2020.1795302

Tkacz, A., Bestion, E., Bo, Z., Hortala, M., & Poole, P. S. (2020). Influence of plant fraction, soil, and plant species on microbiota: A multikingdom comparison. mBio, 11(1). https://doi.org/10.1128/mBio.02785-19

Tomlinson, J. A., Dickinson, M. J., & Boonham, N. (2010). Rapid Detection of *Phytophthora ramorum* and *P. kernoviae* by Two-Minute DNA Extraction Followed by Isothermal Amplification and Amplicon Detection by Generic Lateral Flow Device. Phytopathology, 100(2), 143–149. https://doi.org/10.1094/PHYTO-100-2-0143

Tordoni, E., Ametrano, C. G., Banchi, E., Ongaro, S., Pallavicini, A., Bacaro, G., & Muggia, L. (2021). Integrated eDNA metabarcoding and morphological analyses assess spatio-temporal patterns of airborne fungal spores. Ecological Indicators, 121, 107032. https://doi.org/10.1016/j.ecolind.2020.107032

Treseder, K. K. (2004). A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. New Phytologist, 164(2), 347–355. https://doi.org/10.1111/j.1469-8137.2004.01159.x

Trivedi, P., Delgado-Baquerizo, M., Trivedi, C., Hamonts, K., Anderson, I. C., & Singh, B. K. (2017). Keystone microbial taxa regulate the invasion of a fungal pathogen in agro-ecosystems. Soil Biology and Biochemistry, 111, 10–14. https://doi.org/10.1016/j.soilbio.2017.03.013

Tsror, L. (2010). Biology, Epidemiology and Management of Rhizoctonia solani on Potato. Journal of Phytopathology, 158(10), 649–658. https://doi.org/10.1111/j.1439-0434.2010.01671.x

Uibopuu, A., Moora, M., Öpik, M., & Zobel, M. (2012). Temperate forest understorey species performance is altered by local arbuscular mycorrhizal fungal communities from stands of different successional stages. Plant and Soil, 356(1–2), 331–339. https://doi.org/10.1007/s11104-011-1116-0

Van Dijk, E., Auger, H., Jaszczyszyn, Y., & Thermes, C. (2014). Ten years of next-generation sequencing technology. Trends in genetics: TIG. Elsevier Current Trends. https://doi.org/10.1016/j.tig.2014.07.001

Verbruggen, E., & Toby Kiers, E. (2010). Evolutionary ecology of mycorrhizal functional diversity in agricultural systems. Evolutionary Applications, 3(5–6), 547–560. https://doi.org/10.1111/j.1752-4571.2010.00145.x

Verbruggen, E., van der Heijden, M. G. A., Rillig, M. C., & Kiers, T. E. (2013). Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. New Phytologist, 197(4), 1104–1109. https://doi.org/10.1111/j.1469-8137.2012.04348.x

Votintseva, A. A., Bradley, P., Pankhurst, L., Del Ojo Elias, C., Loose, M., Nilgiriwala, K., et al. (2017). Same-day diagnostic and surveillance data for tuberculosis via whole-genome sequencing of direct respiratory samples. Journal of Clinical Microbiology, 55(5), 1285–1298. https://doi.org/10.1128/JCM.02483-16

West, J. S. (2014). Plant Pathogen Dispersal. In eLS. Chichester, UK: John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470015902.a0021272

Whipps, J. M. (2004). Prospects and limitations for mycorrhizas in biocontrol of root pathogens. Canadian Journal of Botany, 82(8), 1198–1227. https://doi.org/10.1139/b04-082

Wright, P. J., Falloon, R. E., & Hedderley, D. (2017). A long-term vegetable crop rotation study to determine effects on soil microbial communities and soilborne diseases of potato and onion. New Zealand Journal of Crop and Horticultural Science, 45(1), 29–54. https://doi.org/10.1080/01140671.2016.1229345

Wu, F., Wang, W., Ma, Y., Liu, Y., Ma, X., An, L., & Feng, H. (2013). Prospect of beneficial microorganisms applied in potato cultivation for sustainable agriculture. African Journal of Microbiology Research, 7, 2150–2158. https://doi.org/10.5897/AJMRx12.005

Yanar, Y., Yilmaz, G., Cesmeli, I., & Coskun, S. (2005). Characterization of *Rhizoctonia solani* isolates from potatoes in Turkey and screening potato cultivars for resistance to AG-3 isolates. Phytoparasitica, 33(4), 370–376. https://doi.org/10.1007/BF02981304

Yao, M. K., Tweddell, R. J., & Désilets, H. (2002). Effect of two vesicular-arbuscular mycorrhizal fungi on the growth of micropropagated potato plantlets and on the extent of disease caused by *Rhizoctonia*

solani. Mycorrhiza, 12, 235–242. https://doi.org/10.1007/s00572-002-0176-7

Yu, L., Nicolaisen, M., Larsen, J., & Ravnskov, S. (2013). Organic fertilization alters the community composition of root associated fungi in *Pisum sativum*. Soil Biology and Biochemistry, 58, 36–41. https://doi.org/10.1016/j.soilbio.2012.11.004

Zimudzi, J., van der Waals, J. E., Coutinho, T. A., Cowan, D. A., & Valverde, A. (2018). Temporal shifts of fungal communities in the rhizosphere and on tubers in potato fields. Fungal Biology, 122(9), 928–934. https://doi.org/10.1016/J.FUNBIO.2018.05.008

Zrenner, R., Genzel, F., Verwaaijen, B., Wibberg, D., & Grosch, R. (2020). Necrotrophic lifestyle of *Rhizoctonia solani* AG3-PT during interaction with its host plant potato as revealed by transcriptome analysis. Scientific Reports, 10(1), 12574. https://doi.org/10.1038/s41598-020-68728-2

SUMMARY IN ESTONIAN

SÜMBIONTSED JA PATOGEENSED SEENED EESTI KARTULIKASVATUSES

Muld on elupaigaks miljonitele mikroorganismidele, kes võivad taimede kasvu mõjutada nii positiivselt kui ka negatiivselt. Taimedele kasulikud mikroorganismid pakuvad olulisi ökosüsteemiteenuseid, mida saaks edukalt ära kasutada säästvas taimekaitses. Teadmised kahjulike mikroorganismide kohta aitavad leida viise, kuidas vähendada nende ulatuslikku levikut ja seeläbi ka majanduslikku kahju. Ent uute jätkusuutlike strateegiate edendamiseks ja sealhulgas mikroobikoosluse edukaks kasutamiseks põllumajanduses, tuleb rohkem teada põllu mullaelustiku kohta.

Kartul (*Solanum tuberosum* L.) on teiste põllukultuuridega võrreldes haigustele väga vastuvõtlik. Seetõttu kujutavad taimehaigused kogu maailma kartulitootmisele tõsist ohtu. Lisaks on kliimamuutuste ja vaba turumajanduse tingimustes muutunud üsna tavaliseks paljude erinevate patogeenide levimine uutele aladele ja kohanemine uute peremeestaimedega. Sel põhjusel on põllukultuurid, sealhulgas kartul, muutuvates oludes uutest taimekahjustajatest eriti ohustatud. Nende muutuste taustal on teadlastele väga oluline teada saada, kuidas kaitsta põllukultuure, sealhulgas kartulit, taimekahjustajate eest, sealjuures loodust kunstlike sisenditega üle koormamata.

Enamiku maismaataimede juured moodustavad mullas elavate krohmseentega arbuskulaarset mükoriisat (AM). AM-seened parandavad taimedel toitainete omastamist, soodustavad taimede kasvu ja pakuvad kaitset nii biootilise kui ka abiootilise stressi eest. Hoolimata neist teada olevatest kasulikest omadusest, puuduvad siiski põhjalikud teadmised, kuidas AM-seeni keskkonda säästvas kartulikasvatustehnoloogias edukalt kasutada. Varasemalt on katsetööd keskendunud peamiselt *in vitro* uuringutele, mille tulemused ei pruugi peegeldada paljude muude mõjutustega põllutingimusi. Kuna traditsiooniline kartulikasvatustehnoloogia sisaldab kasvuhooajal mitmeid järjestikuseid võimalikke häiringuid põhjustavaid mullaharimisvõtteid ja pestitsiiditöötlusi, siis on tarvidus neis tingimustes täpsemalt uurida seenekoosluse muutusi erinevatel taime kasvuetappidel, leidmaks optimaalsemaid lahendusi säästva, ent majanduslikult jätkusuutliku kartulikasvatustehnoloogia loomisel. Lisaks on teada, et

kartulisordid on haiguskindluse poolest erinevad, kuid kartuli juurtega sümbioosi moodustavate AM-seente võimaliku sordispetsiifilisuse kohta on vähe andmeid. Aretusprogrammides on järjest olulisemaks muutunud haiguskindlate sortide aretus, kuid teadaolevalt ei ole aretusprogrammidesse kaasatud mükoriisaseeni.

Mikroobikoosluse kohta täpsema teabe saamiseks on esmatähtis töötada välja kiired ja täpsed patogeenide taimekoest tuvastamise meetodid. Eriti oluline on taimepatogeenide tuvastamine presümptomaatilises staadiumis, kuna see aitaks vältida taimehaiguste epideemiaid. Haigustekitajate tuvastamine asümptomaatilistest materjalidest, näiteks kartulimugulatest, on aga äärmiselt keeruline, sest alguses puuduvad haigusele iseloomulikud sümptomid ning patogeeni hulk proovis võib olla väga madal. Polümeraasi ahelreaktsioonil (PCR), näiteks tavapärasel PCR-il ja kvantitatiivsel PCR-il (qPCR) põhinevate taimepatogeenide määramise meetodite kasutuselevõtt on parandanud taimehaiguse täpsema identifitseerimise võimet ka siis, kui proovis on patogeeni hulk väike. Siiski on PCR-tehnoloogia üsna aeganõudev ning meetodit ei saa kasutada laboriväliseks kiireks diagnoosimiseks. Samuti ei suudeta nende meetoditega tihtipeale tuvastada taimehaiguste kompleksset esinemist. Seetõttu on kaasaaegse põllumajanduse üks suuremaid väljakutseid just selliste tehnoloogiate arendamine, mis võimaldaksid taimehaiguseid määrata koheselt põllul.

Käesolevas töös analüüsitakse Eesti traditsioonilise kasvatustehnoloogiaga kartulipõldudel kartulijuuri ja mulda asustavate patogeensete seente ja taimedega sümbioosis kasvavate AM-seente mitmekesisuse näitajaid ning nende seoseid taime kasvufaasi ja sordiga. Töö raames määrati kindlaks kartulijuurte AM-seentega koloniseerituse ulatus ning võrreldi AM-seente inokulatsiooni ulatust tava- ja mahepõllumajanduse viljelussüsteemis. Doktoritöö teises osas käsitletakse töö raames välja töötatud ülikiireid protokolle taimekoest patogeenide tuvastamiseks.

Töö eesmärgid:

1. Selgitada viljelusviisi (tava- ja mahepõllumajandus) mõju AM-seente kolonisatsioonile taimejuurtes (**I**).
2. Hinnata kartulisordi mõju AM-seente ja patogeenide liigirikkusele, suhtelisele ohtrusele ja liigilisele koosseisule nii juurtes kui ka mullas (**II**).
3. Testida taskusekvenaatori MinION sobivust ülikiireks taimepatogeenide diagnostikaks (**III**).

Töö hüpoteesid:

1. Tavaviljelusega võrreldes on maheviljeluses AM-seentega koloniseerituse ulatus kõrgem.
2. AM-seente ja patogeenide liigirikkus, suhteline ohtrus ning liigiline koosseis kartulijuurtes ja mullas sõltub taime kasvufaasist ja kartulisordist.

Materjal ja metoodika

Selleks, et selgitada, kas ja kuidas mõjutavad erinevad viljelussüsteemid (tava ja mahe) kartuli juurte AM-seente kolonisatsiooni, koguti 2010. aasta kasvuhooajal Eesti Maaülikooli tava-mahe süsteemi võrdluskatsest kartuli juureproovid (**I**). Põldkatse mullas leiduvate AM-seente inokulumpotentsiaali hindamiseks rajati nõukatse süstlehise teelehega (*Plantago lanceolata* L.). Kartuli ja süstlehise teelehe juureproovidest määrati mikroskoobiga AM-seente kolonisatsiooni ulatus.

Kartulijuurtes ja mullas leiduvate patogeensete ja sümbiontsete mükoriisaseente mitmekesisusnäitajate hindamiseks ning kartulisordi ja juuri asustavate seente võimaliku spetsiifilisuse selgitamiseks rajati 21 kartulisordiga põldkatse, kust koguti 2014. aasta (**II**) kasvuhooajal juure- ja mullaproovid, mida analüüsiti kasutades järgmise põlvkonna sekveneerimistehnoloogiat.

Uudse taskusekvenaatori MinION abil taimekoest ultrakiireks patogeenide tuvastamiseks (**III**) koguti 2018–2019 aasta kasvuhooajal nähtavate taimehaiguste sümptomitega taimi ning seejärel analüüsiti neid muutes tööetappe.

Tulemused

Töö tulemused (**I**) kinnitasid esimest tööhüpoteesi – AM-seente kolonisatsioonitase oli maheviljeluses kõrgem kui tavaviljeluses. Samas selgus, et mõlemas süsteemis oli siiski piisaval hulgal AM seeneleviseid selleks, et moodustada sümbioosi mudeltaimeks olnud süstlehise teelehega (*Plantago lanceolata* L.). Kuigi AM-seente kolonisatsioonitase oli antud katsetes mõnevõrra kõrgem maheviljeluse põldudel, oli kartulijuurte kolonisatsioon mõlema viljelusviisi puhul siiski väga madal. Sellest võib järeldada, et testitud kartulisort Reet võib AM sümbioosist vähe sõltuda.

Teine tööhüpotees leidis uuringu tulemusena vaid osalise tõestuse, sest kartulijuurte ja põllumulla DNA sekveneerimistulemusi analüüsides selgus, et paljudes proovides ei leidunud AM-seente DNA järjestusi. Seetõttu ei olnud võimalik täpsemalt analüüsida ka AM-seente liigirikkuse ja koosluse mustreid. Saadud tulemustest järeldub, et kõrge sisendiga põllumajandussüsteemis kasvavatel tänapäevastel kartulisortidel on madal AM-seentega koloniseerituse ulatus (**II**). Lisaks selgus, et kasvuperioodi lõpus on põllumulla AM-seente suhteline ohtrus võrreldes kasvuperioodi algusega oluliselt kõrgem. Selle põhjuseks võib olla kasvuperioodi alguses läbiviidavate mitmete järjestikuste agrotehniliste võtete selline võimalik negatiivne mõju, kus mullas looduslikult eksisteerivate AM seente ja lühikese kasvuajaga kartulitaime vahel ei saa sümbioos moodustuda.

Hüpoteesi teine osa, kus väideti, et patogeenide liigirikkus, suhteline ohtrus ning liigiline koosseis kartulijuurtes ja mullas sõltub kasvufaasidest ja kartulisortidest, leidis katsete käigus kinnitust (**II**). Sekveneerimisel tuvastatud patogeensete seente suhteline ohtrus juurtes ja mullas sõltus nii taime kasvufaasist kui ka kartulisordist. Lisaks selgus, et kui juurte patogeensete seente liigirikkust ja liigilist koosseisu mõjutas kartulisort, siis mulla patogeensete seeneliikide koosseisu mõjutas taime kasvufaas. Seega lubavad antud töö tulemused järeldada, et taimesortide geneetiline mitmekesisus põllul võib aidata luua põllukultuure ohustavatele patogeenidele vähem soodsa keskkonna ja seeläbi aidata luua jätkusuutlikuma agroökosüsteemi.

Taskusekvenaatori MinION testimine taimepatogeenide kiireks tuvastamiseks osutus osaliselt edukaks. Antud töö tulemusena leiti teadusele uudsena, et MinION-i saab kasutada taimekoes domineerivate patogeenide kiireks ja täpseks tuvastamiseks. Uuringu tulemused näitasid selgelt, et MinION sobib hästi nii seenhaiguste kui ka teiste eukarüootsete patogeenide kiireks PCR-vabaks diagnoosimiseks (**III**). Siiski tuleb olla ettevaatlik mitmete võimalike analüüsimisel ette tulevate kõrvalekallete suhtes. Näiteks võivad lühendatud DNA eraldusprotokollid anda madalama DNA kvaliteedi ja koguse, mistõttu võib patogeen üleüldse määramata jääda. Lisaks võib MiniON-i puuduseks olla proovide ristsaastumine. Nimelt leiti, et MiniON-i reaktsiooni käigus kanti paljud sekventsid eelmistelt reaktsioonidelt üle. Leiti ka, et valediagnooside vältimiseks tuleb võrdlusjärjestuste andmebaase põhjalikumaks muuta.

Järeldused

Antud doktoritöö raames uuriti kartulipõldudel patogeenide ja sümbiontsete AM-seente koosluse struktuuri. Uuringute tulemused on kartulikasvatuses suure praktilise väärtusega ning kinnitavad, et seniste traditsiooniliste kasvatustehnoloogiate juures ei ole AM-seentega inokuleerimine otstarbekas. Seetõttu tuleb säästlikus kartulikasvatuses panustada rohkem sortide resistentsusaretusele ning juba aretusprogrammides võtta arvesse taim-mikroorganism vastastikuseid toimeid ja mõjusid. Selle uuringu olulise tulemusena on võimalik parandada patogeenide kiiret diagnostikat, mis omakorda aitab kaasa taimepatogeenidest tingitud ulatuslike saagikadude vähendamisele.

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I

ORIGINAL PUBLICATIONS

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The indigenous arbuscular mycorrhizal fungal colonisation potential in potato roots is affected by agricultural treatments

K. Loit[1,2,*], L. Soonvald[1], M. Kukk[1], A. Astover[2], E. Runno-Paurson[3], T. Kaart[4] and M. Öpik[5]

[1]Estonian University of Life Sciences, Chair of Plant Health, Kreutzwaldi 1, EE51014 Tartu, Estonia
[2]Estonian University of Life Sciences, Chair of Soil Science, Kreutzwaldi 1, EE51014 Tartu, Estonia
[3]Estonian University of Life Sciences, Chair of Crop Science and Plant Biology, Kreutzwaldi 1, EE51014 Tartu, Estonia
[4]Estonian University of Life Sciences, Chair of Animal Breeding and Biotechnology, Kreutzwaldi 1, EE51014 Tartu, Estonia
[5]University of Tartu, Department of Botany, 40 Lai St., EE51005 Tartu, Estonia
[*]Correspondence: kaire.loit@emu.ee

Abstract. There is an urgent need to develop novel approaches to enhance sustainable agriculture while not reducing crop yields. Arbuscular mycorrhizal (AM) fungi establish symbiotic associations with most crop plants improving plant performance and soil health. This study investigated the extent of colonisation of potato roots by indigenous AM fungi in the arable soil under conventional and organic farming systems. Potato roots had greater AM fungal colonisation levels under organic than conventional farming, though in general, root colonisation levels were extremely low in both farming systems. Potato root AM fungal colonisation was lower with higher soil P content and higher with higher annual C input. Trap plant root AM fungal colonisation was considerably higher than in field potato roots and showed that soil mycorrhizal inoculum potential was higher in organic than in conventional farming. Thus, the positive impact of manure application in organic fields to the potato AM fungal colonisation can be explained by previous higher total annual C fresh organic matter input and lower soil P content under treatment. Furthermore, the natural AM fungal abundance in the soil was sufficient to colonise trap plant roots, suggesting a low mycorrhizal dependence of the studied potato cultivar.

Key words: Cropping systems, Glomeromycota, management practices, *Solanum tuberosum* L.

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are the most widespread symbionts of plant roots in terrestrial habitats. AM fungi provide a major contribution to plant nutrient uptake in most ecosystems and thus improve plant growth (Smith & Read, 2008; Smith & Smith, 2011). In addition to nutritional benefits, AM fungi protect host plants against root and shoot pathogens, including nematodes, other fungi and viruses, and enhance host plant resistance to various abiotic stresses such as drought, salinity and high heavy

metal concentrations in soil (Augé et al., 2015; Pozo et al., 2015). The ecosystem services provided by AM fungi in agroecosystems make them an important group of soil biota to be managed for crop production both in conventional and sustainable systems (Gianinazzi et al., 2010; Mahmood & Rizvi, 2010; Bender et al., 2016).

Diversity and functioning of AM fungi in agroecosystems are affected by differences in management regimes. Intensive agricultural management approaches are characterised by high N and P inputs (Verbruggen & Kiers, 2010). Environmental conditions with high nutrient input cause a decrease in host plant resource allocation to AM fungi and therefore have a negative impact on AM fungal biodiversity and species richness (Mäder et al., 2000; Verbruggen et al., 2010). The low-input systems, on the other hand, promote AM fungal colonisation, because plants benefit from the AM fungi by increased soil nutrient uptake when these are available at low concentrations (Mäder et al., 2000).

AM fungi are native to agricultural soils and form a mutualistic symbiosis with the majority of crop plants (Douds et al., 2007). Potato is a non-grain crop of global importance but has one of the heaviest production demands for fertilizer and pesticide inputs of all vegetable crops (Wu et al., 2013). For plants such as potato, which have a low root density in soil, the AM symbiosis may be of particular significance in coping with P-deficiency stress in natural ecosystems (McArthur & Knowles, 1993). Previous inoculation studies have reported high levels of AM fungal colonisation in potato roots (Davies et al., 2005; Douds et al., 2007), but the knowledge about AM fungi colonising potato roots in field conditions under common agricultural practices is scarce.

Therefore, it is necessary to determine how the natural AM fungal colonisation in potato roots is affected by different agricultural management regimes.

We specifically asked: (1) How do the conventional and organic farming systems influence potato root AM fungal colonisation? (2) How do the conventional management treatments with different mineral fertilization levels and different organic treatments with cover crop and manure amendment influence potato root AM fungal colonisation? We characterized AM fungal colonisation of potato roots in field grown potato plants and in addition we determined AM fungal inoculum potential (IP) using a trap plant bioassay.

MATERIALS AND METHODS

Field experiment

The field site was located in Tartu, Estonia (58°22′ N, 26°40′ E). This field was established in 2008 as a part of the 5-year crop rotation experiment with two organic (OFS) and four conventional farming systems (CFS). In the crop rotation experiment, red clover (*Trifolium pratense* L.), winter wheat (*Triticum aestivum* L.), pea (*Pisum sativum* L.), potato (*Solanum tuberosum* L.) and barley (*Hordeum vulgare* L.) were grown in succession. In both organic management systems, a winter cover crop (CC) for green manure was used. Winter oilseed rape (*Brassica napus* L.) seeds as CC were sown at the rate of 6 kg ha^{-1} before the potato cropping in September 2009 and ploughed under in April the year 2010. No cattle manure was added to one treatment (CC), whereas composted cattle manure (M) was added to the second organic treatment (CC+M). No fungicides, herbicides or insecticides were applied under organic systems. Weeds were removed mechanically. In a CFS, four mineral fertilizer treatments were used: $N_0P_0K_0$,

$N_{50}P_{25}K_{95}$, $N_{100}P_{25}K_{95}$, and $N_{150}P_{25}K_{95}$. Conventional systems were treated with several synthetic pesticides. Field operations and their timings are shown in Table S1.

Table S1. Field operations and their timings during year 2010 in the study site

Field operation	Conventional farming system	Organic farming system
Planting date	May 6	May 6
Planting rate	3 t ha 57,000 tubers ha^{-1}	3 t ha 57,000 tubers ha^{-1}
Harvest date	August 30	August 30
Fertilization	$N_0P_0K_0$ – not fertilized	April 20 Winter cover crop
	$N_{50}P_{25}K_{95}$ 1) May 4th $N_{20}P_{25}K_{95}$ (Kemira Grow How Power 5:14:28 400 kg ha^{-1}) 2) June 7th $N_{30}P_0K_0$ (AN* 34:0:0)	
	$N_{100}P_{25}K_{95}$ 1) May 4th $N_{20}P_{25}K_{95}$ (Kemira Grow How Power 5:14:28 400 kg ha^{-1}) 2) June 7th $N_{60}P_0K_0$ (AN* 34:0:0) 3) June 16th $N_{20}P_0K_0$ (AN* 34:0:0) $N_{150}P_{25}K_{95}$ 1) May 4th N20P25K95 (Kemira Grow How Power 5:14:28 400 kg ha^{-1}) 2) June 7th N90P0K0 (AN* 34:0:0) 3) June 16th N40P0K0 (AN* 34:0:0)	April 20 Winter cover crop + composted cattle manure 40 t ha^{-1}
Herbicide application	June 7 Titus 25 DF (50 g ha) (containing 12.5 g ha^{-1} rimsulfuron)	No herbicides applied
Insecticide application	July 22 Fastac 50 (0.3 L ha) (containing 15 g ha^{-1} alpha–cypermethrin) August 6th Decis 2.5 EC (0.2 L ha^{-1}) (containing 5 g ha^{-1} deltamethrin)	No insecticides applied
Fungicide application	June 25 Shirlan 500 SC (0.4 L ha) (containing 200 g ha^{-1} fluazinam) July 8th and 22nd Ridomil Gold MZ 68 (2.5 kg ha^{-1}) (containing 100 g ha^{-1} metalaxyl-M + 1.6 kg ha^{-1} mancozeb) August 6th Ranman 400 SC (0.15 L ha^{-1}) (containing 60 g ha^{-1} cyazofamid)	No fungicides applied

*AN – ammonium salpeter.

Treatments were arranged in a systematic block design with each plot in four replications. The size of each test plot was 60 m^2. Organic and conventional plots were separated by an 18 m long section of mixed grasses to avoid contamination with synthetic pesticides and mineral fertilizers. The distance between seed tubers was 27 cm, and the distance between rows was 70 cm. The soil of the trial field was *Stagnic Luvisol* (LVj) with sandy loam texture with a humus layer of 20–30 cm. No irrigation was used. The data of total annual carbon inputs from cover crops, straw, roots of pea, weeds and cattle manure (kg C ha^{-1} y^{-1}) to the soil before potato were obtained from earlier publications about this field trial (Kauer et al., 2015; Madsen et al., 2016).

Potato root samples were collected in July 2010 from plants of the locally bred potato cultivar 'Reet' (Tsahkna & Tähtjärv, 2008). Three potato plants were sampled randomly from each of the four replicate plots of all treatments of both farming systems (a total of 72 samples). Roots were sampled at the potato flowering (BBCH60) stage (Hack et al., 2001). Root samples were dried with silica gel and preserved airtight at room temperature as described by Uibopuu et al. (2012).

Trap plant bioassay

Narrowleaf plantain (*Plantago lanceolata* L.), as an AM host plant commonly used in experiments, was used to evaluate the mycorrhizal inoculum potential (IP) of field soil. Three soil samples were collected randomly from each of the four replicate plots of all treatments before soil tillage in spring 2011, and stored in darkness at 10 °C until use. Before the use in experiment, the three soil samples per plot were pooled and handled as one composite sample. Each composite sample was thoroughly mixed with autoclaved sand in a 1:1 v/v ratio to improve drainage and aeration of the soil mixture. *P. lanceolata* seeds were germinated in Petri dishes on moist filter paper, following Uibopuu et al. (2012). Three seedlings per pot were planted on January 2011 in plastic pots (13 x 15 cm, depth x diameter). One seedling was retained per pot after four weeks of growth. Plants were kept in a greenhouse under controlled conditions, watered as needed with tap water and grown for 3 months. At harvest, shoots and roots were separated and handled like described previously. The infectivity bioassay of Moorman & Reeves (1979) was used to quantify the relative density of colonising propagules of AM fungi.

Root staining and assessment of AM fungal root colonisation

Root samples from both field and pot experiment were stained with Trypan blue according to Koske & Gemma (1989). Briefly, roots were cleared in 10% KOH, acidified with 1% HCl and stained with 0.01% trypan blue in lactoglycerol. Root colonisation by AM fungi was estimated using the magnified grid-line intersections method (McGonigle et al., 1990), by scoring 120 fields of view per sample under the compound microscope at 400x magnification, as described in Uibopuu et al. (2012). Total root length colonised was estimated.

Soil chemical analysis

In mid-April 2010, before tillage, soil samples were collected from the bulk soil at a depth of 0–25 cm. Eight sub-samples were taken from each plot and mixed to obtain a composite sample for each plot. Soils were air-dried and sieved through a 2 mm sieve. Soil pH was determined in a 1 M KCl solution (1:2.5). Soil organic carbon (C_{org}) was measured using the Tjurin method (Vorobeva, 1998), and total nitrogen (N_{tot}) concentration was measured using the Kjeldahl method (van Reeuwijk, 1995). The concentrations of plant-available nutrients in the soil (P, K, Ca and Mg) were determined by the ammonium lactate (AL) method (Egnér et al., 1960).

Statistical data analysis

Statistical analysis was performed using R 3.22 (R Core Team 2015) within R-Studio environment (0.99.484; RStudio 2015). First, normal distribution (*Shapiro-Wilk* test) and equality of variances in treatment groups (*Levene* test) of data were explored. Then, the non-parametric *Kruskal-Wallis rank sum* test was conducted to

determine the differences in among treatments followed by the *Dunn´s* test for pairwise comparison. For the *Dunn´s* test, the *P*-values were adjusted according to the *Bonferroni correction*. When comparing only two groups, the non-parametric *Wilcoxon signed-rank* test was used. To study the relationship between soil parameters and AM fungal colonisation *Pearson´s correlation* analysis was carried out.

RESULTS AND DISCUSSION

Potato root AM fungal colonisation

AM fungal colonisation level in roots of the potato cultivar 'Reet' was higher in OFS than CFS, though colonisation levels were very low in both systems (median 0%, maximum 3.3% in CFS and 5.0% in OFS; *Wilcoxon signed-rank* test, $W = 415.5$, $p = 0.007$; Fig. 1, A). Individual treatments influenced root AM fungal colonisation levels differently. AM fungal colonisation was higher in treatment CC+M than treatments N50 and N150 (*Kruskal-Wallis* test, $\chi^2 = 11.85$, $df = 5$, $p = 0.037$; Fig. 2, A). There were no statistically significant differences among other treatments.

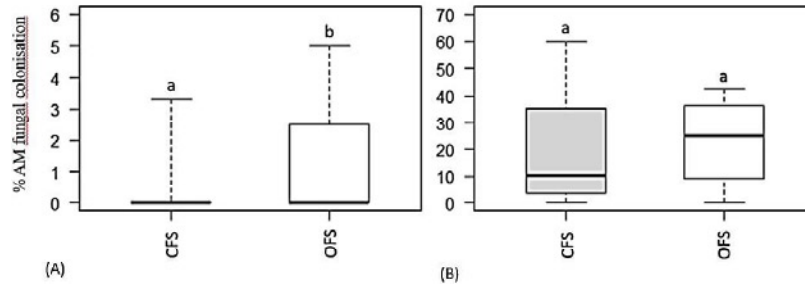
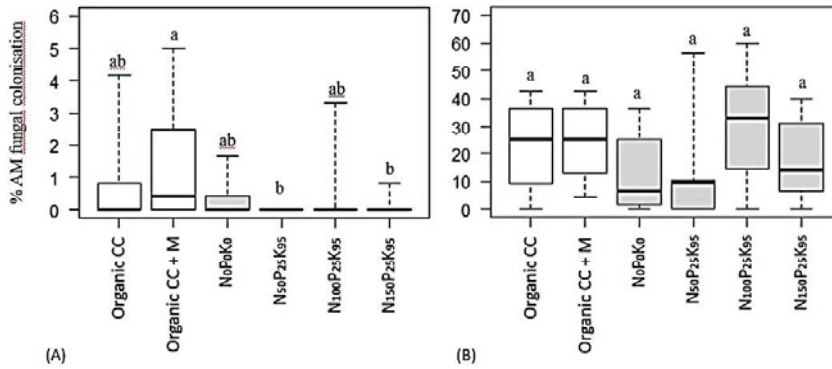


Figure 1. Median values (± min, max) of percent of arbuscular mycorrhizal (AM) fungal colonisation of potato roots in conventional (CFS) and organic farming systems (OFS) in the field trial (A) and narrowleaf plantain roots grown in pots in sand inoculated with soil from the same conventional and organic farming systems (B). Box plots indicate median (bold horizontal line), interquartile ranges (box) and minimum and maximum values (whiskers). Different letters above the boxes indicate statistically significant differences at $P < 0.05$ among treatments (*Wilcoxon rank sum* test). Grey boxes – CFS, open boxes – OFS.

Potato root AM fungal colonisation level was negatively correlated with soil phosphorus content ($r = -0.50$, $p = 0.012$, Fig. 3, A; Table S2) and positively with total annual C input to the soil ($r = 0.46$, $p = 0.023$; Fig. 3, B; Table S2). When exploring these relationships separately by treatment groups, the relationship between soil phosphorus content and potato root AM fungal colonisation level remained for OFS ($r = -0.78$, $p = 0.022$; Table S3), but not for CFS ($r = -0.11$, $p = 0.67$; Table S4). In the case of relationship between total annual C input to soil and potato root AM fungal colonisation level, there were no significant relationships neither for OFS ($r = 0.26$, $p = 0.52$; Table S3) nor CFS ($r = -0.35$, $p = 0.17$; Table S4). Exceptionally, in OFS, potato root AM fungal colonisation level was negatively correlated with C_{org} ($r = -0.76$,

$p = 0.03$; Table S3). There were no significant relationships between soil pH, C_{org}, K, Ca, Mg and total N content, and potato AM fungal colonisation (Table S2, Table S5).



Figuer 2. Median values (± min, max) of percent of AM fungal colonisation of potato roots in different agricultural treatments in the field trial (A) and narrowleaf plantain roots in greenhouse trial inoculated with soils from the same agricultural treatments in the field (B). Box plots indicate median (bold horizontal line), interquartile ranges (box) and minimum and maximum values (whiskers). Different letters above the boxes indicate statistically significant differences at $P < 0.05$ among treatments (*Kruskal-Wallis rank sum* test, as post hoc test, was used *Dunn* test with *Bonferroni* correction). Open boxes – organic farming systems, grey boxes – conventional farming systems. CC – farming system with cover crops, CC + M – farming system with cover crops and composted cattle manure, $N_0P_0K_0$ – control system with no additional fertilizers used, $N_{50}P_{25}K_{95}$, $N_{100}P_{25}K_{95}$, and $N_{150}P_{25}K_{95}$ – systems with different N rates used.

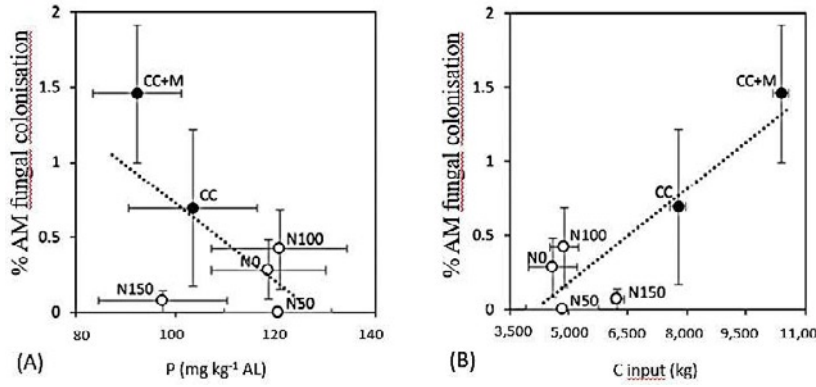


Figure 3. Relationships between AM fungal colonisation of potato roots and (A) soil phosphorus content and (B) total annual carbon input in the field trial. The points with whiskers indicate the mean (± standard error) of four replicate samples per treatment; the dotted lines present the linear relationship. Closed circles – organic farming systems, open circles – conventional farming systems. See Fig. 2 legend for treatment coding.

Table S2. Linear correlation coefficients between soil characteristics and AMF colonisation in potato roots

	pH KCl	C_{org} (%)	P (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})	Ca (mg kg^{-1})	Total N (%)	Total C input (kg ha^{-1} y^{-1})	(%)
pH KCl	1								
C_{org} (%)	**0.57***	1							
P (mg kg)	**0.63***	0.39	1						
K (mg kg)	0.27	0.33	**0.48***	1					
Mg (mg kg)	**0.78***	**0.45***	**0.56***	0.38	1				
Ca (mg kg)	**0.86***	**0.67***	**0.59***	0.4	**0.85***	1			
Total N (%)	**0.55***	**0.74***	**0.41***	**0.54***	**0.44***	**0.62***	1		
Total C input (kg ha^{-1} y^{-1})	0.35	**0.50***	-0.24	0.12	0.25	**0.43***	**0.52***	1	
AM fungal c olonisation (%)	-0.04	0.09	**-0.50***	-0.18	-0.09	-0.00	0.09	**0.46***	1

Statistically significant relationships ($p < 0.05$) are indicated with asterisks.

Table S3. Linear correlation coefficients between soil characteristics and AMF colonisation of potato in OFS

	pH KCl	C_{org} (%)	P (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})	Ca (mg kg^{-1})	Total N (%)	Total C input (kg ha^{-1} y^{-1})	AM fungal (%)
pH KCl	1								
Corg (%)	0.09	1							
P (mg kg)	0.67	0.59	1						
K (mg kg)	0.53	0.35	0.32	1					
Mg (mg kg)	**0.90***	-0.09	0.51	0.34	1				
Ca (mg kg)	**0.96***	0.25	**0.79***	0.53	**0.87***	1			
Total N (%)	0.37	0.56	0.44	**0.81***	0.10	0.46	1		
Total C input (kg ha^{-1} y^{-1})	0.28	0.07	-0.10	**0.74***	0.25	0.31	0.67	1	
AM fungal colonisation (%)	-0.31	**-0.76***	**-0.78***	0.00	-0.31	-0.47	-0.13	0.26	1

Statistically significant relationships ($p < 0.05$) are indicated with asterisks.

Table S4. Linear correlation coefficients between soil characteristics and AMF colonisation of potato in CFS

	pH KCl	C_{org} (%)	P (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})	Ca (mg kg^{-1})	Total N (%)	Total C input (kg ha^{-1} y^{-1})	AM fungal colonisation (%)
pH KCl	1								
Corg (%)	**0.63***	1							
P (mg kg)	**0.82***	**0.67***	1						
K (mg kg)	0.26	**0.62***	**0.53***	1					
Mg (mg kg)	**0.76***	**0.68***	**0.87***	**0.57***	1				
Ca (mg kg)	**0.81***	**0.71***	**0.85***	**0.57***	**0.88***	1			
Total N (%)	**0.61***	**0.81***	**0.72***	**0.57***	**0.72***	**0.62***	1		
Total C input (kg ha^{-1} y^{-1})	0.23	0.30	0.11	0.39	0.02	0.17	0.25	1	
AM fungal colonisation (%)	-0.26	0.04	-0.11	-0.18	-0.20	-0.15	-0.22	-0.35	1

Statistically significant relationships ($p < 0.05$) are indicated with asterisks.

Table S5. Average (±standard error) soil characteristics by treatments

	Treatment					
	N0	N50	N100	N150	CC	CC±M
pH KCl	5.95 (± 0.21)	5.83 (± 0.22)	5.73 (± 0.19)	5.72 (± 0.11)	5.94 (± 0.10)	6.03 (± 0.19)
C_{org} (%)	1.22 (± 0.12)	1.17 (± 0.08)	1.30 (± 0.12)	1.34 (± 0.10)	1.46 (± 0.06)	1.43 (± 0.07)
P (mg kg)	118.6 (± 11.4)	120.6 (± 10.7)	120.8 (± 13.5)	97.5 (± 12.9)	103.4 (± 12.9)	92.3 (± 9.0)
K (mg kg)	146.4 (± 5.3)	170.8 (± 6.8)	167.4 (± 8.8)	165.0 (± 10.7)	139.1 (± 9.7)	167.1 (± 11.3)
Mg (mg kg)	127.8 (± 15.1)	147.0 (± 12.6)	147.3 (± 14.6)	120.7 (± 15.0)	143.1 (± 11.3)	161.3 (± 33.4)
Ca (mg kg)	1,156 (± 143.0)	1,310 (± 133.4)	1,247 (± 84.0)	1,185 (± 105.0)	1377 (± 90.0)	1,452 (± 137.8)
Total N (%)	0.122 (± 0.007)	0.126 (± 0.002)	0.127 (± 0.010)	0.127 (± 0.008)	0.129 (± 0.011)	0.145 (± 0.005)
Total C input (kg $ha^{-1}y^{-1}$)	4,604 (± 640.1)	4,841 (± 955.4)	4,900 (± 615.1)	6,241 (± 445.0)	7,778 (± 195.1)	1,0394 (± 195.1)

Means with different letters are statistically significantly different among treatments ($p < 0.05$, *Tukey* test). See Fig. 2 legend for treatment coding.

AM fungal inoculum potential of arable soil

Root AM fungal colonisation of narrowleaf plantain in greenhouse trial was significantly greater ($p < 0.001$) than that of field-grown potato roots in both farming systems (data not shown). The median AM fungal colonisation levels in the narrowleaf plantain roots were 10.6% (range 0–60%) in CFS and 25.0% (range 0–42.5%) in OFS. The AM fungal colonisation of narrowleaf plantain showed an insignificant tendency to be higher in the OFS than in the CFS (*Wilcoxon signed-rank* test, $W = 451.5$, $p = 0.14$; Fig. 1B). AM fungal root colonisation values in soils from individual field treatments varied considerably, but with no significant differences between treatments.

DISCUSSION

By combining a field trial and trap plant greenhouse assay we show that AM fungal colonisation in potato roots in the field conditions was very low, regardless of sufficient amount of AM fungal inoculum in the field soils to support moderate root colonisation levels of trap plant narrowleaf plantain inoculated with these field soils. AM fungal root colonisation tended to be higher in the organic farming systems both in the field grown potato roots and greenhouse-grown plantain roots. The individual fertilizing treatments in the conventional and organic farming systems did not show regular differences in root AM fungal colonisation levels neither in the field potato roots, nor trap plant roots, with the exception of positive effect of manure amendment in organic farming system as compared to inorganic fertilizer addition in the conventional farming system. We found that high soil P content decreased root AM fungal colonisation and higher annual C input to the soil increased root AM fungal colonisation. Therefore, the positive impact of organic farming on the potato AM fungal colonisation in our study system can be explained by previous higher fresh organic matter input and lower soil P content under manure amendment treatment. These results suggest that the potato cultivar studied by us could be a relatively poor AM host, and that AM fungal abundance and functioning is further decreased by higher soil fertility, but can be improved by organic farming practices such as use of manure as fertiliser.

In the present study root mycorrhizal colonisation rate was measured for a locally bred potato cultivar 'Reet'. This relatively new cultivar is described by breeders Tsahkna & Tähtjärv (2008). Breeding programs are generally conducted in experimental stations under high nutrient levels (Philippot et al., 2013). This has resulted in several crops showing lower root mycorrhizal fungal colonisation and lower mycorrhizal growth response than their wild progenitors, though large variations exist (Martin-Robles et al., 2018). It is therefore conceivable that modern potato varieties may have lower mycorrhizal dependence caused by selective breeding under conditions where plants receive little benefit from mycorrhizal symbiosis. This possibility is supported by evidence that newer cultivated plant varieties tend to have lower AM fungal root colonisation (Lehmann et al., 2012). Similar to our results, very low root colonisations have also been reported in earlier field surveys of potato (Cesaro et al., 2008). Furthermore, potato root AM fungal colonisation can vary to a large degree across plant growth phases (Buysens et al., 2017).

Additionally, soil in our field experiment had high to very high plant-available P levels (Schick et al., 2013), which could be one of the reasons for low observed AM fungal root colonisation. Negative relationship between root AM fungal colonisation and

soil P level as observed in this study, is frequently reported in other agricultural and natural systems (Verbruggen et al., 2013). As P availability increases, plants in return become less dependent on AM fungi and down-regulate their mycorrhiza formation (Smith & Read, 2008). Still, not only phosphorus fertilization negatively affects AM fungi, but previous studies have shown that high-input conventional farming as a whole chemical-dependent system negatively affects AM fungi (Kabir, 2005; Verbruggen et al., 2010; Prosser et al., 2015).

However, our root colonisation data verified the previous findings (Jansa et al., 2006) that manure amendment has beneficial effect on plant colonisation by AM fungi compared to the application of inorganic fertilizers. This is in accordance with findings by Gryndler et al. (2006), who showed that in manured soil the concentration of AM fungal spores and mycelial growth increased with mineral fertilization. Furthermore AM fungal colonisation rate in plant roots might also be influenced by other factors related to farming practices like cropping history. A meta-analysis conducted by Lekberg & Koide (2005) showed that avoiding non-mycorrhizal plants in crop rotation has a positive effect on subsequent mycorrhizal colonisation. The cover crop used in the current study was winter oilseed rape, which is a non-mycorrhizal crop plant. Therefore, low AM fungal colonisation rate in potato roots of our study could in part result from usage of non-mycorrhizal crops as a cover crop, which possibly decreased the positive effect of AM fungi for the following crop.

In comparison to the field crop, potato, the trap plant narrowleaf plantain roots showed higher AM fungal colonisation, indicating that the field soils were not exhausted of AM fungi. It is noteworthy that the higher root colonisation of plantains was obtained on the field soil diluted with sand, effectively reducing the amount of available AM fungal propagules for the host plants compared to that available for potato plants in the field. Narrowleaf plantain is a widely used plant species because of its commonly high mycorrhizal colonisation and responsiveness, as well as its broad range of AM fungal partners (Schnoor et al., 2011; Davison et al., 2015). Our comparison of potato and plantain also confirms that host plant–AM fungal relationships depend on both symbiosis partners (e.g., Bever, 2002), whereby the same fungal inoculum may result in very different plant root colonisation levels, fungal community compositions and plant growth in the case of different host plant species. Host-AM fungal compatibility may also influence potato yield, as shown earlier in inoculation trials of micropropagated potatoes (Duffy & Cassells, 2000). Whether a different AM fungal community would result in a different root colonisation (and ultimately, yield) of the potato cultivar studied by us, requires further testing.

CONCLUSIONS

Our study demonstrated extremely low AM fungal colonisation rate in the roots of potato in field conditions. At the same time, AM fungal colonisation was higher in organic than in conventional farming system, and was related to higher fresh organic matter input and lower soil phosphorus content. Interestingly, both soils from the conventional and organic field had relatively high AM fungal inoculum potential as detected by trap plant assay with narrowleaf plantain. These results suggest that plant species or cultivar can have a strong influence on AM fungal colonisation levels. Further research is needed to clarify whether other varieties of potato show similarly low levels

of AM fungal colonisation as the cultivar used in this study, and to which degree plant growth phase affects this measure. Furthermore, it is necessary to explore how AM fungal species diversity in field potato roots relates to the root colonisation levels and potato yield of different cultivars under the regionally used potato cropping systems. This would provide guidelines for the most efficient management of AM fungi in these cropping systems with regionally used cultivars, fertilization levels, cover crop and pesticide usages considering optimal potato production at the regional scale.

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REFERENCES

Augé, R.M., Toler, H.D. & Saxton, A.M. 2015. Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. *Mycorrhiza* **25**, 13–24. doi.org/10.1007/s00572-014-0585-4

Bender, S.F., Wagg, C. & van der Heijden, MGA. 2016. An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. *Trends in Ecology & Evolution* **31**, 440–452.

Bever, J.D. 2002. Host-specificity of AM fungal population growth rates can generate feedback on plant growth. *Plant Soil* **244**, 281–290.

Buysens, C., Alaux, P.L., Cesar, V., Huret, S, Declerck, S. & Cranenbrouck, S. 2017. Tracing native and inoculated Rhizophagus irregularis in three potato cultivars (Charlotte, Nicola and Bintje) grown under field conditions. *Applied Soil Ecology* **115**, 1–9.

Cesaro, P., van Tuinen, D., Copetta, A., Chatagnier, O., Berta, G., Gianinazzi, S. & Lingua, G. 2008. Preferential colonization of Solanum tuberosum L. roots by the fungus Glomus intraradices in arable soil of a potato farming area. Applied and Environmental Microbiology **74**(18), 5776–5783

Davies, Jr, F.T., Calderón, C.M. & Huaman, Z. 2005. Influence of Arbuscular Mycorrhizae Indigenous to Peru and a Flavonoid on Growth, Yield, and Leaf Elemental Concentration of `Yungay' Potatoes. *HortScience* **40**, 381–385.

Davison, J., Moora, M., Öpik, M., Adholeya, A., Ainsaar, L., Ba, A., Burla, S., Diedhiou, A.G., Hiiesalu, I., Jairus, T., Johnson, NC., Kane, A., Koorem, K., Kochar, M., Ndiaye, C., Pärtel, M., Reier, Ü., Saks, Ü., Singh, R., Vasar, M., Zobel, M. 2015. Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. *Science* **349**, 970–973.

Douds, D.D., Jr., Nagahashi, G., Reider, C. & Hepperly, P.R. 2007. Inoculation with Arbuscular Mycorrhizal Fungi Increases the Yield of Potatoes in a High P Soil. http://handle.nal.usda.gov/10113/21020

Duffy, E.M. & Cassells, A.C. 2000. The effect of inoculation of potato (Solanum tuberosum L.) microplants with arbuscular mycorrhizal fungi on tuber yield and tuber size distribution. *Appl. Soil Ecol.* 1**5**, 137–144.

Egnér, H., Riehm, H. & Domingo, W. 1960. *Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Böden. II. Chemische Extraktionsmethoden zur Phosphor- und Kaliumbestimmung*. (Studies on the chemical soil analysis as a basis for the assessment of the nutrient status of soils. II. Chemical extraction methods for phosphorus and potassium determination). Kungliga Lantbrukshögskolans Annaler, 26, 199–215 (in German).

Gianinazzi, S., Gollotte, A., Binet, M.N., van Tuinen, D., Redecker, D. & Wipf, D. 2010. Agroecology: The key role of arbuscular mycorrhizas in ecosystem services. *Mycorrhiza* **20**(8), 519–530. doi.org/10.1007/s00572-010-0333-3

Gryndler, M., Larsen, J., Hršelová, H., Řezáčová, V., Gryndlerová, H. & Kubát, J. 2006. Organic and mineral fertilization, respectively, increase and decrease the development of external mycelium of arbuscular mycorrhizal fungi in a long-term field experiment. *Mycorrhiza* **16**, 159–166. doi 10.1007/s00572-005-0027-4

Hack, H., Gal, H., Klemke, T., Klose, R., Meier, U., Strauss, R., Witzenberger, A. & Witzenberger, A. 2001. The BBCH scale for phenological growth stages of potato (*Solanum tuberosum* L.). In: Meier U, ed. Growth stages of mono- and dicotyledonous plants, BBCH Monograph. Berlin, Braunschweig, Germany: Federal Biological Research Centre for Agriculture and Forestry, 7–16.

Jansa, J., Wiemken, A. & Frossard, E. 2006. The effects of agricultural practices on arbuscular mycorrhizal fungi. Geological Society, London, Special Publications, **266**(1), 89–115. doi.org/10.1144/GSL.SP.2006.266.01.08

Kabir, Z. 2005. Tillage or no-tillage: Impact on mycorrhizae. *Canadian Journal of Plant Science* **85**(1), 23–29. doi.org/10.4141/P03-160

Kauer, K., Tein, B., Sanchez de Cima, D., Talgre, L., Eremeev, V., Loit, E. & Luik, A. 2015. Soil carbon dynamics estimation and dependence on farming system in a temperate climate. *Soil and Tillage Research* **154**, 53–63. doi.org/10.1016/j.still.2015.06.010

Koske, R.E. & Gemma, J.N. 1989. A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research* **92**(4), 486–488. doi.org/10.1016/S0953-7562(89)80195-9

Lehmann, A., Barto, E.K., Powell, J.R. & Rillig, M.C. 2012. Mycorrhizal responsiveness trends in annual crop plants and their wild relatives–a meta-analysis on studies from 1981 to 2010. *Plant and Soil* **355**, 231–250.

Lekberg, Y. & Koide, R.T. 2005. Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. *New Phytologist* **168**, 189–204. doi: 10.1111/j.1469-8137.2005.01490.x

Mäder, P., Edenhofer, S., Boller, T., Wiemken, A. & Niggli U. 2000. Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic, biological) and high-input (conventional) farming systems in a crop rotation. *Biology and Fertility of Soils* **31**, 150–156.

Madsen, H., Talgre, L., Eremeev, V., Alaru, M., Kauer, K. & Luik, A. 2016. Do green manures as winter cover crops impact the weediness and crop yield in an organic crop rotation? *Biological Agriculture & Horticulture* **32**, 182–191. doi. 10.1080/01448765.2016.1138141

Mahmood, I. & Rizvi, R. 2010. Mycorrhiza and organic farming. Asian Journal of Plant Sciences, 9, 241–248. doi.org/10.3923/ajps.2010.241.248

Martín-Robles, N., Lehmann, A., Seco, E., Aroca, R., Rillig, M.C. & Milla, R. 2018. Impacts of domestication on the arbuscular mycorrhizal symbiosis of 27 crop species. *New Phytologist*, doi: 10.1111/nph.14962

McArthur, D.A.J., Knowles, N.R. 1993. Influence of species of vesicular-arbuscular mycorrhizal fungi and phosphorus nutrition on growth, development, and mineral nutrition of potato (*Solanum tuberosum* L.). *Plant Physiology* **102**, 771–782. doi.org/10.1104/pp.102.3.771

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L. & Swan, J.A. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* **115**, 495–501. doi.org/10.1111/j.1469-8137.1990.tb00476.x

Moorman, T. & Reeves, B.F. 1979. The role of endomycorrhizae in revegetation practices in the semi-arid west. II. A bioassay to determine the effect of land disturbance on endomycorrhizal populations. *American Journal of Botany* **66**, 14–18. http://www.jstor.org/stable/2442619. Accessed 20 June 2017

Philippot, L., Raaijmakers, J.M., Lemanceau, P. & van der Putten, W.H. 2013. Going back to the roots: the microbial ecology of the rhizosphere. Nature Reviews. *Microbiology* 1**1**(11), 789–799. doi.org/10.1038/nrmicro3109

Pozo, M.J., Lopez-Raez, J.A., Azcon-Aguilar, C. & Garcia-Garrido, J.M. 2015. Phytohormones as integrators of environmental signals in the regulation of mycorrhizal symbioses. *New Phytologist* **205**, 1431–1436.

Prosser, R., Lissemore, L., Shahmohamadloo, R. & Sibley, P. 2015. Effect of biosolids-derived triclosan and triclocarban on the colonization of plant roots by arbuscular mycorrhizal fungi. *Science of the Total Environment* **508**, 427–434. doi.org/10.1016/j.scitotenv.2014.12.014

Schick, B.J., Haneklaus, S., Rückamp, D., Astover, A., Busch, S., Kuligowski, K., Rossner, H., Sarvi, M., Ylivainio, K., Ziółkowski, M. & Schnug, E. 2013. Report on P status in agricultural soils of relevant areas of the Baltic Sea Region Report on P status in agricultural soils of relevant areas of the Baltic Sea Region. http://www.balticmanure.eu/download/Reports/bm_report_on_p_status_in_agricultural_soils_web.pdf. Accessed 15 June 2017

Schnoor, T.K. Lekberg, Y., Rosendahl, S. & Olsson, P.A. 2011. Mechanical soil disturbance as a determinant of arbuscular mycorrhizal fungal communities in semi-natural grassland. *Mycorrhiza* **21**, 211–220.

Smith, A.F. & Smith, S.E. 2011. What is the significance of the arbuscular mycorrhizal colonization of many economically important crop plants? *Plant Soil* **348**, 63–79. doi.org/10.1007/s11104-011-0865-0

Smith, S.E. & Read, D.J. 2008. Mycorrhizal Symbiosis. Amsterdam: Academic Press.

Tsahkna A., Tähtjärv, T. 2008. The new potato variety 'Reet'. *Latvian Journal of Agronomy* **11**, 159–164.

Uibopuu, A., Moora, M., Öpik, M. & Zobel, M. 2012. Temperate forest understorey species performance is altered by local arbuscular mycorrhizal fungal communities from stands of different successional stages. *Plant and Soil* **356**, 331–339.

Van Reeuwijk, L.P. 1995. Procedures for soil analysis, fifth ed. ISRIC Technical Paper 9, Wageningen, Netherlands.

Verbruggen, E., Röling, W.F., Gamper, H.A., Kowalchuk, G.A., Verhoef, H.A. & van der Heijden, M.G.A. 2010. Positive effects of organic farming on below-ground mutualists : large-scale comparison of mycorrhizal fungal communities in agricultural soils. *New Phytologist* **186**, 968–979.

Verbruggen, E., van der Heijden, M.G.A., Rillig, M.C. & Kiers, T.E. 2013. Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. *New Phytologist* **197**(4), 1104–1109. doi.org/10.1111/j.1469-8137.2012.04348.x

Verbruggen, E. & Kiers, E.T., 2010. Evolutionary ecology of mycorrhizal functional diversity in agricultural systems. *Evol. Appl.* **3**, 547–560.

Vorobeva, L.A. 1998. Chemical analysis of soils. Moscow University Press, Moscow, 272 pp. (in Russian).

Wu, F., Wang, W., Ma, Y., Liu, Y., Ma, X., An, L. & Feng, H. 2013. Prospect of beneficial microorganisms applied in potato cultivation for sustainable agriculture. *African Journal of Microbiology Research* **7**, 2150–2158. doi.org/10.5897/AJMRx12.005

II

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

Kaire Loit [1,*], Liina Soonvald [1], Alar Astover [2], Eve Runno-Paurson [1,3], Maarja Öpik [4] and Leho Tedersoo [5]

1 Chair of Plant Health, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Fr. R. Kreutzwaldi 1, 51006 Tartu, Estonia; liina.soonvald@emu.ee (L.S.); eve.runno-paurson@emu.ee (E.R.-P.)
2 Chair of Soil Science, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Fr. R. Kreutzwaldi 1, 51006 Tartu, Estonia; alar.astover@emu.ee
3 Chair of Crop Science and Plant Biology, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Fr. R. Kreutzwaldi 1, 51006 Tartu, Estonia
4 Department of Botany, University of Tartu, 40 Lai St., 51005 Tartu, Estonia; maarja.opik@ut.ee
5 Department of Microbiology, University of Tartu, Ravila 14a, 50411 Tartu, Estonia; leho.tedersoo@ut.ee
* Correspondence: kaire.loit@emu.ee

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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).

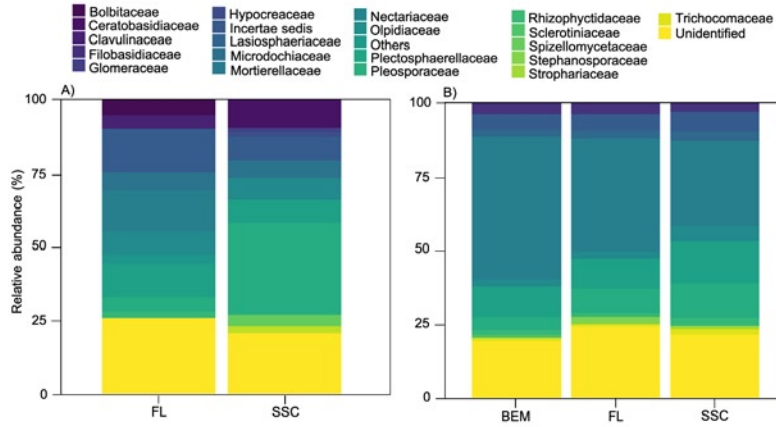

Figure 1. Taxonomic composition of root (**A**) and soil (**B**) fungal communities in different sampling times across all cultivars and replication blocks. BEM, before emergence; FL, flowering stage; SSC, senescence stage.

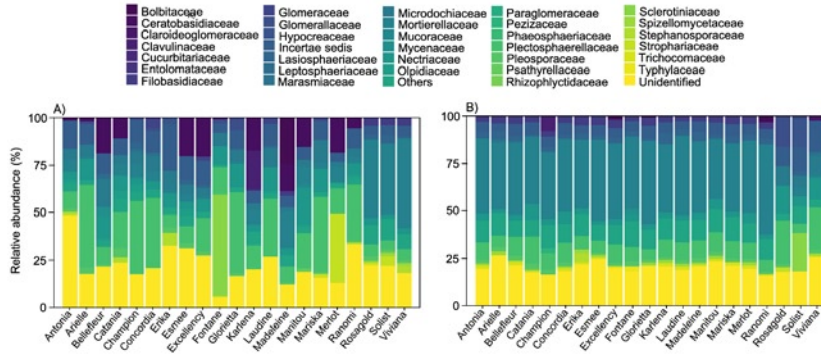

Figure 2. Taxonomic composition of root (**A**) and soil (**B**) fungal communities in different potato cultivars across all sampling times and replication blocks.

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^2_{adj}	*Pseudo F*	*p*	R^2_{adj}	*Pseudo F*	*p*	R^2_{adj}	*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^2_{adj}, 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).

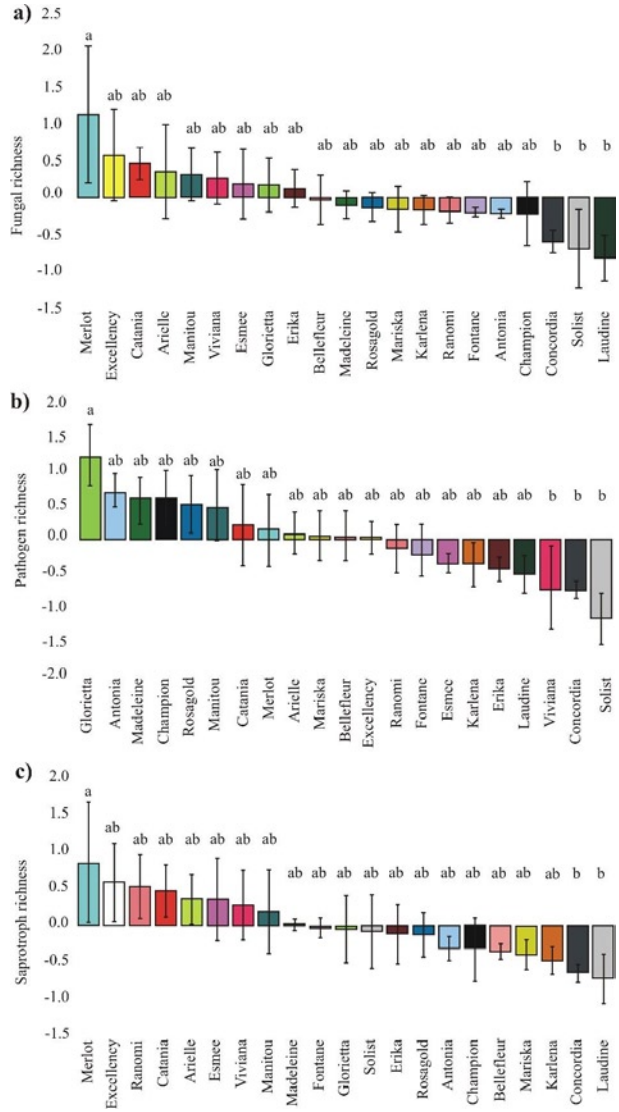

Figure 3. Results of a general linear model estimating the effect of cultivar for root (**a**) overall fungal, (**b**) pathogen, (**c**) saprotroph richness collected across all sampling times and replication blocks. Colours represent the cultivars. Different letters indicate statistically significant 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. Similarily 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		
			R^2adj	*Pseudo F*	*p*	R^2adj	*Pseudo F*	*p*	R^2adj	*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^2_{adj}, adjusted R^2; *Pseudo-F*, pseudo-F statistic; *p*, calculated probability. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

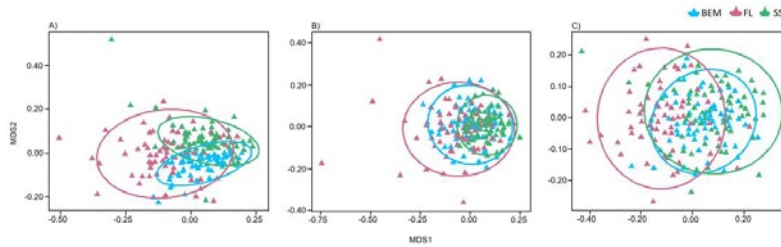

Figure 4. Non-metric multi-dimensional scaling, to model the effect of time on soil (**A**) overall fungal, (**B**) pathogen and (**C**) saprotroph community composition collected at different sampling points across all cultivars and replication blocks. BEM, before emergence; FL, flowering stage; SSC, 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.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/10/1535/s1, Figure S1: Results of a general linear model estimating the species richness and relative abundance of pathogen and saprotroph communities in root (A,C) and soil (B,D). Table S1: The layout of potato field, and the list of varieties, Table S2: characteristics of the study site. Main field operations and their timings, Table S3: Soil properties on field site, Table S4: OTU table, Table S5: Tukey post hoc test of significant variables for the overall fungal richness in roots and soil, Table S6: Tukey post hoc test of significant variables for the pathogen fungal richness in roots and soil, Table S7: Tukey post hoc test of significant variables for the saprotroph fungal richness in roots and soil, Table S8: Tukey post hoc test of significant variables for the pathogen relative abundance in roots and soil, Table S9: Tukey post hoc test of significant variables for the saprotroph relative abundance in roots and soil, Table S10: Relative abundance (%) of the four most dominant OTUs from potato roots, and rhizosphere sampled in different time points, Table S11: Tukey post hoc test of significant variables for the most abundant pathogens in roots, Table S12: Tukey post hoc test of significant variables for the most abundant pathogens in soil.

Author Contributions: Conceptualization, M.Ö., E.R.-P., L.T. and K.L.; methodology, L.T., L.S. and K.L.; formal analysis, L.T., A.A., L.S. and K.L.; data curation, L.S. and K.L.; writing—original draft preparation, L.S., M.Ö., L.T., A.A. and K.L.; writing—review and editing, L.T., M.Ö., A.A., E.R.-P., L.S., K.L.; visualisation, L.S., K.L.; funding acquisition, L.T., E.R.-P., A.A., K.L. All authors have read and agreed to the published version of the manuscript.

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References

1. Ramankutty, N.; Mehrabi, Z.; Waha, K.; Jarvis, L.; Kremen, C.; Herrero, M.; Rieseberg, L.H. Trends in Global Agricultural Land Use: Implications for Environmental Health and Food Security. *Annu. Rev. Plant Biol.* **2018**, *69*, 789–815. [CrossRef] [PubMed]
2. Tilman, D.; Balzer, C.; Hill, J.; Befort, B.L. Global food demand and the sustainable intensification of agriculture. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 20260–20264. [CrossRef] [PubMed]
3. Lakshmanan, V.; Selvaraj, G.; Bais, H.P. Functional Soil Microbiome: Belowground Solutions to an Aboveground Problem. *Plant Physiol.* **2014**, *166*, 689–700. [CrossRef] [PubMed]
4. De Vries, F.T.; Thébault, E.; Liiri, M.; Birkhofer, K.; Tsiafouli, M.A.; Bjørnlund, L.; Jørgensen, H.B.; Brady, M.V.; Christensen, S.; De Ruiter, P.C.; et al. Soil food web properties explain ecosystem services across European land use systems. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 14296–14301. [CrossRef]
5. Banerjee, S.; Walder, F.; Büchi, L.; Meyer, M.; Held, A.Y.; Gattinger, A.; Keller, T.; Charles, R.; Van Der Heijden, M.G.A. Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in roots. *ISME J.* **2019**, *13*, 1722–1736. [CrossRef]
6. Hartmann, M.; Frey, B.; Mayer, J.; Mäder, P.; Widmer, F. Distinct soil microbial diversity under long-term organic and conventional farming. *ISME J.* **2014**, *9*, 1177–1194. [CrossRef]
7. Bardgett, R.D.; Van Der Putten, W.H. Belowground biodiversity and ecosystem functioning. *Nature* **2014**, *515*, 505–511. [CrossRef]
8. Philippot, L.; Raaijmakers, J.M.; Lemanceau, P.; Van Der Putten, W.H. Going back to the roots: The microbial ecology of the rhizosphere. *Nat. Rev. Microbiol.* **2013**, *11*, 789–799. [CrossRef]
9. Van Der Heijden, M.G.A.; Bardgett, R.D.; Van Straalen, N.M. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.* **2008**, *11*, 296–310. [CrossRef]
10. Detheridge, A.P.; Brand, G.; Fychan, R.; Crotty, F.V.; Sanderson, R.; Griffith, G.W.; Marley, C.L. The legacy effect of cover crops on soil fungal populations in a cereal rotation. *Agric. Ecosyst. Environ.* **2016**, *228*, 49–61. [CrossRef]
11. Francioli, D.; Schulz, E.; Lentendu, G.; Wubet, T.; Buscot, F.; Reitz, T. Mineral vs. Organic Amendments: Microbial Community Structure, Activity and Abundance of Agriculturally Relevant Microbes Are Driven by Long-Term Fertilization Strategies. *Front. Microbiol.* **2016**, *7*, 1446. [CrossRef]
12. Säle, V.; Aguilera, P.; Laczko, E.; Mäder, P.; Berner, A.; Zihlmann, U.; Van Der Heijden, M.G.A.; Oehl, F. Impact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi. *Soil Biol. Biochem.* **2015**, *84*, 38–52. [CrossRef]
13. Schmidt, R.; Mitchell, J.; Scow, K. Cover cropping and no-till increase diversity and symbiotroph:saprotroph ratios of soil fungal communities. *Soil Biol. Biochem.* **2019**, *129*, 99–109. [CrossRef]
14. Soonvald, L.; Loit, K.; Runno-Paurson, E.; Astover, A.; Tedersoo, L. The role of long-term mineral and organic fertilisation treatment in changing pathogen and symbiont community composition in soil. *Appl. Soil Ecol.* **2019**, *141*, 45–53. [CrossRef]
15. Brader, G.; Compant, S.; Vescio, K.; Mitter, B.; Trognitz, F.; Ma, L.-J.; Sessitsch, A. Ecology and Genomic Insights into Plant-Pathogenic and Plant-Nonpathogenic Endophytes. *Annu. Rev. Phytopathol.* **2017**, *55*, 61–83. [CrossRef] [PubMed]
16. Grayston, S.J.; Wang, S.; Campbell, C.D.; Edwards, A.C. Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biol. Biochem.* **1998**, *30*, 369–378. [CrossRef]

17. Zimudzi, J.; Van Der Waals, J.E.; Coutinho, T.A.; Cowan, D.A.; Valverde, A. Temporal shifts of fungal communities in the rhizosphere and on tubers in potato fields. *Fungal Biol.* **2018**, *122*, 928–934. [CrossRef] [PubMed]
18. Badri, D.V.; Vivanco, J.M. Regulation and function of root exudates. *Plant Cell Environ.* **2009**, *32*, 666–681. [CrossRef]
19. Canarini, A.; Kaiser, C.; Merchant, A.; Richter, A.; Wanek, W. Root Exudation of Primary Metabolites: Mechanisms and Their Roles in Plant Responses to Environmental Stimuli. *Front. Plant Sci.* **2019**, *10*, 157. [CrossRef]
20. Wissuwa, M.; Mazzola, M.; Picard, C. Novel approaches in plant breeding for rhizosphere-related traits. *Plant Soil* **2009**, *321*, 409–430. [CrossRef]
21. Food and Agriculture Organization of the United Nations. *FAOSTAT Database*; FAO: Rome, Italy, 2020; Available online: http://www.fao.org/faostat/en/#data/TP (accessed on 3 June 2020).
22. Fry, W.E.; Goodwin, S.B. Re-emergence of Potato and Tomato Late Blight in the United States. *Plant Dis.* **1997**, *81*, 1349–1357. [CrossRef]
23. Toth, I.K.; Van Der Wolf, J.M.; Saddler, G.; Lojkowska, E.; Hélias, V.; Pirhonen, M.; Tsoror (Lahkim), T.; Elphinstone, J.G. Dickeya species: An emerging problem for potato production in Europe. *Plant Pathol.* **2011**, *60*, 385–399. [CrossRef]
24. Patil, V.U.; Girimalla, V.; Sagar, V.; Bhardwaj, V.; Chakrabarti, S.K. Draft Genome Sequencing of Rhizoctonia solani Anastomosis Group 3 (AG3- PT) Causing Stem Canker and Black Scurf of Potato. *Am. J. Potato Res.* **2018**, *95*, 87–91. [CrossRef]
25. Atallah, Z.K.; Bae, J.; Jansky, S.H.; Rouse, D.I.; Stevenson, W.R. Multiplex Real-Time Quantitative PCR to Detect and Quantify *Verticillium dahliae* Colonization in Potato Lines that Differ in Response to Verticillium Wilt. *Phytopathology* **2007**, *97*, 865–872. [CrossRef] [PubMed]
26. Stefańczyk, E.; Sobkowiak, S.; Brylińska, M.; Śliwka, J. Diversity of Fusarium spp. associated with dry rot of potato tubers in Poland. *Eur. J. Plant Pathol.* **2016**, *145*, 871–884. [CrossRef]
27. Fry, W. Phytophthora infestans: The plant (and R gene) destroyer. *Mol. Plant Pathol.* **2008**, *9*, 385–402. [CrossRef]
28. Bakker, M.G.; Manter, D.K.; Sheflin, A.M.; Weir, T.L.; Vivanco, J.M. Harnessing the rhizosphere microbiome through plant breeding and agricultural management. *Plant Soil* **2012**, *360*, 1–13. [CrossRef]
29. Estonian Weather Service. Meteorological Yearbook of Estonia 2014. Available online: http://www.ilmateenistus.ee/wp-content/uploads/2013/01/aastaraamat_2014.pdf (accessed on 10 June 2020).
30. Egnér, H.; Riehm, H.; Domingo, W.R. Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Böden. II. Chemische Extraktionsmethoden zur Phosphor- und Kaliumbestimmung. *K. Lantbr. Ann.* **1960**, *26*, 199–215.
31. Hack, H.; Gall, H.; Klemke, T.H.; Klose, R.; Meier, U.; Stauss, R.; Witzenberger, A. The BBCH scale for phonological growth stages of potato (*Solanum tuberosum* L.). In *Growth Stages of Mono- and Dicotyledonous Plants, BBCH Monograph*; Meier, U., Ed.; Federal Biological Research Centre for Agriculture and Forestry: Berlin, Germany, 2001; pp. 7–16.
32. De León, D.G.; Moora, M.; Öpik, M.; Neuenkamp, L.; Gerz, M.; Jairus, T.; Vasar, M.; Bueno, C.G.; Davison, J.; Zobel, M. Symbiont dynamics during ecosystem succession: Co-occurring plant and arbuscular mycorrhizal fungal communities. *FEMS Microbiol. Ecol.* **2016**, *92*, fiw097. [CrossRef]
33. Tedersoo, L.; Bahram, M.; Põlme, S.; Kõljalg, U.; Yorou, N.S.; Wijesundera, R.; Ruiz, L.V.; Vasco-Palacios, A.M.; Thu, P.Q.; Suija, A.; et al. Global diversity and geography of soil fungi. *Science* **2014**, *346*, 1256688. [CrossRef]
34. Schloss, P.D.; Westcott, S.L.; Ryabin, T.; Hall, J.R.; Hartmann, M.; Hollister, E.B.; Lesniewski, R.A.; Oakley, B.B.; Parks, D.H.; Robinson, C.J.; et al. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Appl. Environ. Microbiol.* **2009**, *75*, 7537–7541. [CrossRef]
35. Masella, A.P.; Bartram, A.K.; Truszkowski, J.M.; Brown, D.G.; Neufeld, J.D. PANDAseq: Paired-end assembler for illumina sequences. *BMC Bioinform.* **2012**, *13*, 31. [CrossRef] [PubMed]
36. Edgar, R.C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **2010**, *26*, 2460–2461. [CrossRef] [PubMed]

37. Anslan, S.; Bahram, M.; Hiiesalu, I.; Tedersoo, L.; Ashikawa, S.; Tarumoto, N.; Imai, K.; Sakai, J.; Kodana, M.; Kawamura, T.; et al. PipeCraft: Flexible open-source toolkit for bioinformatics analysis of custom high-throughput amplicon sequencing data. *Mol. Ecol. Resour.* **2017**, *17*, e234–e240. [CrossRef] [PubMed]
38. Bengtsson-Palme, J.; Ryberg, M.; Hartmann, M.; Branco, S.; Wang, Z.; Godhe, A.; De Wit, P.; Sánchez-García, M.; Ebersberger, I.; De Sousa, F.; et al. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods Ecol. Evol.* **2013**, *4*, 914–919. [CrossRef]
39. Fu, L.; Niu, B.; Zhu, Z.; Wu, S.; Li, W. CD-HIT: Accelerated for clustering the next-generation sequencing data. *Bioinformatics* **2012**, *28*, 3150–3152. [CrossRef] [PubMed]
40. Kõljalg, U.; Tedersoo, L.; Nilsson, R.H.; Abarenkov, K.; Nilsson, R.H. Digital identifiers for fungal species. *Science* **2016**, *352*, 1182–1183. [CrossRef]
41. Nguyen, N.H.; Song, Z.; Bates, S.T.; Branco, S.; Tedersoo, L.; Menke, J.; Schilling, J.S.; Kennedy, P.G. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol.* **2016**, *20*, 241–248. [CrossRef]
42. Anderson, M.J.; Gorley, R.N.; Clarke, K.R. *PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods*; PRIMER-E: Plymouth, UK, 2008.
43. Guyonnet, J.P.; Cantarel, A.A.M.; Simon, L.; Haichar, F.E.Z. Root exudation rate as functional trait involved in plant nutrient-use strategy classification. *Ecol. Evol.* **2018**, *8*, 8573–8581. [CrossRef]
44. Haichar, F.E.Z.; Santaella, C.; Heulin, T.; Achouak, W. Root exudates mediated interactions belowground. *Soil Biol. Biochem.* **2014**, *77*, 69–80. [CrossRef]
45. Agrios, G.N. *Plant Pathology*, 5th ed.; Academic Press: Cambridge, MA, USA, 2005; ISBN 9780120445653.
46. Ma, A.; Zhuang, X.; Wu, J.; Cui, M.; Lv, D.; Liu, C.; Zhuang, G. Ascomycota Members Dominate Fungal Communities during Straw Residue Decomposition in Arable Soil. *PLoS ONE* **2013**, *8*, e66146. [CrossRef] [PubMed]
47. Osorio, N.W.; Habte, M. Synergistic Influence of an Arbuscular Mycorrhizal Fungus and a P Solubilizing Fungus on Growth and P Uptake ofLeucaena leucocephalain an Oxisol. *Arid. Land Res. Manag.* **2001**, *15*, 263–274. [CrossRef]
48. Li, F.; Chen, L.; Redmile-Gordon, M.; Zhang, J.; Zhang, C.; Ning, Q.; Li, W. Mortierella elongata 's roles in organic agriculture and crop growth promotion in a mineral soil. *Land Degrad. Dev.* **2018**, *29*, 1642–1651. [CrossRef]
49. Carlucci, A.; Raimondo, M.; Santos, J.M.; Phillips, A.J.L. Plectosphaerella species associated with root and collar rots of horticultural crops in southern Italy. *Persoonia Mol. Phylogeny Evol. Fungi* **2012**, *28*, 34–48. [CrossRef]
50. Raimondo, M.L.; Carlucci, A. Characterization and pathogenicity assessment ofPlectosphaerellaspecies associated with stunting disease on tomato and pepper crops in Italy. *Plant Pathol.* **2018**, *67*, 626–641. [CrossRef]
51. Gao, J.; Zhang, Y.; Zhao, X.J.; Wang, K.; Zhao, J. First Report of Potato Wilt Caused by Plectosphaerella cucumerina in Inner Mongolia, China. *Plant Dis.* **2016**, *100*, 2523. [CrossRef]
52. Atkins, S.D.; Clark, I.M.; Sosnowska, D.; Hirsch, P.R.; Kerry, B.R. Detection and Quantification of Plectosphaerella cucumerina, a Potential Biological Control Agent of Potato Cyst Nematodes, by Using Conventional PCR, Real-Time PCR, Selective Media, and Baiting. *Appl. Environ. Microbiol.* **2003**, *69*, 4788–4793. [CrossRef] [PubMed]
53. Tsror (Lahkim), L.T. Biology, Epidemiology and Management of Rhizoctonia solani on Potato. *J. Phytopathol.* **2010**, *158*, 649–658. [CrossRef]
54. Leplat, J.; Friberg, H.; Abid, M.; Steinberg, C. Survival of Fusarium graminearum, the causal agent of Fusarium head blight. A review. *Agron. Sustain. Dev.* **2012**, *33*, 97–111. [CrossRef]
55. Oberwinkler, F.; Riess, K.; Bauer, R.; Kirschner, R.; Garnica, S. Taxonomic re-evaluation of the Ceratobasidium-Rhizoctonia complex and Rhizoctonia butinii, a new species attacking spruce. *Mycol. Prog.* **2013**, *12*, 763–776. [CrossRef]
56. Veldre, V.; Abarenkov, K.; Bahram, M.; Martos, F.; Selosse, M.-A.; Tamm, H.; Kõljalg, U.; Tedersoo, L. Evolution of nutritional modes of Ceratobasidiaceae (Cantharellales, Basidiomycota) as revealed from publicly available ITS sequences. *Fungal Ecol.* **2013**, *6*, 256–268. [CrossRef]

57. Aoki, T.; O'Donnell, K.; Geiser, D.M. Systematics of key phytopathogenic Fusarium species: Current status and future challenges. *J. Gen. Plant Pathol.* **2014**, *80*, 189–201. [CrossRef]
58. Walder, F.; Schlaeppi, K.; Wittwer, R.; Held, A.Y.; Vogelgsang, S.; Van Der Heijden, M.G.A. Community Profiling of Fusarium in Combination with Other Plant-Associated Fungi in Different Crop Species Using SMRT Sequencing. *Front. Plant Sci.* **2017**, *8*, 2019. [CrossRef] [PubMed]
59. O'Donnell, K.; Ward, T.J.; Robert, V.A.R.G.; Crous, P.W.; Geiser, D.M.; Kang, S. DNA sequence-based identification of Fusarium: Current status and future directions. *Phytoparasitica* **2015**, *43*, 583–595. [CrossRef]
60. Inceoğlu, O.; Salles, J.F.; Van Overbeek, L.; Van Elsas, J.D. Effects of Plant Genotype and Growth Stage on the Betaproteobacterial Communities Associated with Different Potato Cultivars in Two Fields. *Appl. Environ. Microbiol.* **2010**, *76*, 3675–3684. [CrossRef]
61. Schlemper, T.R.; Leite, M.F.A.; Lucheta, A.R.; Shimels, M.; Bouwmeester, H.J.; Van Veen, J.A.; Kuramae, E.E. Rhizobacterial community structure differences among sorghum cultivars in different growth stages and soils. *FEMS Microbiol. Ecol.* **2017**, *93*. [CrossRef]
62. Gao, C.; Montoya, L.; Xu, L.; Madera, M.; Hollingsworth, J.; Purdom, E.; Hutmacher, R.B.; Dahlberg, J.A.; Coleman-Derr, D.; Lemaux, P.G.; et al. Strong succession in arbuscular mycorrhizal fungal communities. *ISME J.* **2019**, *13*, 214–226. [CrossRef]
63. Chaparro, J.M.; Badri, D.V.; Vivanco, J.M. Rhizosphere microbiome assemblage is affected by plant development. *ISME J.* **2014**, *8*, 790–803. [CrossRef]
64. Nguyen, C. Rhizodeposition of organic C by plants: Mechanisms and controls. *Agron. EDP Sci.* **2003**, *23*, 375–396. [CrossRef]
65. Chaparro, J.M.; Badri, D.V.; Bakker, M.G.; Sugiyama, A.; Manter, D.K.; Vivanco, J.M. Root Exudation of Phytochemicals in Arabidopsis Follows Specific Patterns That Are Developmentally Programmed and Correlate with Soil Microbial Functions. *PLoS ONE* **2013**, *8*, e55731. [CrossRef]
66. Kaiser, C.; Kilburn, M.R.; Clode, P.L.; Fuchslueger, L.; Koranda, M.; Cliff, J.B.; Solaiman, Z.M.; Murphy, D.V. Exploring the transfer of recent plant photosynthates to soil microbes: Mycorrhizal pathway vs direct root exudation. *New Phytol.* **2015**, *205*, 1537–1551. [CrossRef] [PubMed]
67. Lemanczyk, G.; Sadowski, C.K. Fungal communities and health status of roots of winter wheat cultivated after oats and oats mixed with other crops. *BioControl* **2002**, *47*, 349–361. [CrossRef]
68. Pérez-Piqueres, A.; Edel-Hermann, V.; Alabouvette, C.; Steinberg, C. Response of soil microbial communities to compost amendments. *Soil Biol. Biochem.* **2006**, *38*, 460–470. [CrossRef]
69. Bains, P.S.; Bennypaul, H.S.; Lynch, D.R.; Kawchuk, L.M.; Schaupmeyer, C.A. Rhizoctonia disease of potatoes (*Rhizoctonia solani*): Fungicidal efficacy and cultivar susceptibility. *Am. J. Potato Res.* **2002**, *79*, 99–106. [CrossRef]
70. Lundberg, D.S.; Lebeis, S.L.; Paredes, S.H.; Yourstone, S.; Gehring, J.; Malfatti, S.; Tremblay, J.; Engelbrektson, A.; Kunin, V.; Del Rio, T.G.; et al. Defining the core Arabidopsis thaliana root microbiome. *Nature* **2012**, *488*, 86–90. [CrossRef] [PubMed]
71. De León, D.G.; Vahter, T.; Zobel, M.; Koppel, M.; Edesi, L.; Davison, J.; Al-Quraishy, S.; Hozzein, W.N.; Moora, M.; Oja, J.; et al. Different wheat cultivars exhibit variable responses to inoculation with arbuscular mycorrhizal fungi from organic and conventional farms. *PLoS ONE* **2020**, *15*, e0233878. [CrossRef]
72. Bokulich, N.A.; Thorngate, J.H.; Richardson, P.M.; A Mills, D. PNAS Plus: From the Cover: Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *Proc. Natl. Acad. Sci. USA* **2013**, *111*, E139–E148. [CrossRef] [PubMed]

III

III **Loit, K.**; Adamson, K.; Bahram, M.; Puusepp, R.; Anslan, S.; Kiiker, R.; Drenkhan, R.; Tedersoo, L. (2019). Relative Performance of MinION (Oxford Nanopore Technologies) versus Sequel (Pacific Biosciences) Third-Generation Sequencing Instruments in Identification of Agricultural and Forest Fungal Pathogens. Applied and Environmental Microbiology, 85(21), ARTN e01368-19. DOI: 10.1128/AEM.01368-19.

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METHODS

Relative Performance of MinION (Oxford Nanopore Technologies) versus Sequel (Pacific Biosciences) Third-Generation Sequencing Instruments in Identification of Agricultural and Forest Fungal Pathogens

Kaire Loit,[a] Kalev Adamson,[b] Mohammad Bahram,[c] Rasmus Puusepp,[d] Sten Anslan,[e] Riinu Kiiker,[a] Rein Drenkhan,[b] Leho Tedersoo[d,f]

[a]Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu, Estonia
[b]Institute of Forestry and Rural Engineering, Estonian University of Life Sciences, Tartu, Estonia
[c]Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden
[d]Institute of Ecology and Earth Sciences, University of Tartu, Tartu, Estonia
[e]Zoological Institute, Technische Universität Braunschweig, Braunschweig, Germany
[f]Natural History Museum, University of Tartu, Tartu, Estonia

ABSTRACT Culture-based molecular identification methods have revolutionized detection of pathogens, yet these methods are slow and may yield inconclusive results from environmental materials. The second-generation sequencing tools have much-improved precision and sensitivity of detection, but these analyses are costly and may take several days to months. Of the third-generation sequencing techniques, the portable MinION device (Oxford Nanopore Technologies) has received much attention because of its small size and possibility of rapid analysis at reasonable cost. Here, we compare the relative performances of two third-generation sequencing instruments, MinION and Sequel (Pacific Biosciences), in identification and diagnostics of fungal and oomycete pathogens from conifer (Pinaceae) needles and potato (*Solanum tuberosum*) leaves and tubers. We demonstrate that the Sequel instrument is efficient for metabarcoding of complex samples, whereas MinION is not suited for this purpose due to a high error rate and multiple biases. However, we find that MinION can be utilized for rapid and accurate identification of dominant pathogenic organisms and other associated organisms from plant tissues following both amplicon-based and PCR-free metagenomics approaches. Using the metagenomics approach with shortened DNA extraction and incubation times, we performed the entire MinION workflow, from sample preparation through DNA extraction, sequencing, bioinformatics, and interpretation, in 2.5 h. We advocate the use of MinION for rapid diagnostics of pathogens and potentially other organisms, but care needs to be taken to control or account for multiple potential technical biases.

IMPORTANCE Microbial pathogens cause enormous losses to agriculture and forestry, but current combined culturing- and molecular identification-based detection methods are too slow for rapid identification and application of countermeasures. Here, we develop new and rapid protocols for Oxford Nanopore MinION-based third-generation diagnostics of plant pathogens that greatly improve the speed of diagnostics. However, due to high error rate and technical biases in MinION, the Pacific BioSciences Sequel platform is more useful for in-depth amplicon-based biodiversity monitoring (metabarcoding) from complex environmental samples.

KEYWORDS PacBio Sequel, molecular diagnostics, metabarcoding, metagenomics, fungal plant pathogens, needle pathogens, oomycetes, potato, *Solanum tuberosum*, Oxford Nanopore MinION, plant pathogens

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Address correspondence to Leho Tedersoo, leho.tedersoo@ut.ee.

K.L., K.A., M.B., and R.P. contributed equally to this work.

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Fungal and oomycete pathogens and hexapod pests cause enormous losses in agriculture and forestry. Rapid and precise identification of these antagonists enables efficient countermeasures and reduces the costs of biocides and losses to disease (1). Direct morphology-based and culture-based diagnoses are often too slow to prevent the spread of disease. Molecular methods such as PCR-based detection with specific oligonucleotide primers, DNA hybridization-based techniques, and DNA sequence analysis are more accurate and can be rapidly applied to infected tissues and environmental materials (2). However, methods using specific oligonucleotide primers or probes lack the capacity to detect species or strains other than those intended or, worse, yield false-positive signals (3). Although the DNA sequences from selected marker genes may provide high taxonomic resolution, Sanger sequencing of PCR products takes 1 to 3 days depending on access to a sequencing laboratory, and it may fail when DNA of several species or polymorphic alleles are amplified (4).

These disadvantages can be overcome by using a metabarcoding approach. Second- and third-generation high-throughput sequencing (HTS) platforms read hundreds of thousands to billions of DNA molecules simultaneously, recovering the targeted taxa when present at low proportions (5, 6). However, library preparation and running of HTS instruments typically take several days, and there may be queues of weeks to months at commercial service providers. Furthermore, a single sequencing run is costly, which renders it unfeasible for rapid identification of pathogens (7). In spite of millions of output reads, the second-generation SOLiD, Roche 454, Illumina, and Ion Torrent platforms suffer from short read length, which is suboptimal for accurate identification of microorganisms because of low taxonomic resolution of 100- to 500-bp marker gene fragments (8, 9). Third-generation sequencing platforms of Pacific Biosciences (PacBio; RSII and Sequel instruments) and Oxford Nanopore Technologies (ONT; MinION, GridION, and PromethION instruments) enable average sequence lengths of >20,000 bases, but this comes at a 5 to 20% error rate (7, 10–12). In PacBio instruments, the built-in circular consensus sequencing generates multiple copies of the same fragment with a highly accurate consensus (13, 14). Therefore, long consensus molecules have been readily used in *de novo* assembly of complex genomes (15) and DNA barcoding (16). PacBio-based metabarcoding analyses provide greater resolution than short-read second-generation HTS tools in bacteria (9, 17, 18) and fungi (12), including plant pathogens (19).

Compared with other HTS platforms that are represented by large and quite expensive machines, the MinION device is the size of a cell phone and has the cost of a computer, making it affordable to governmental institutions, research laboratories, and small companies (20, 21). Its small size and low power consumption enable carrying the device, a basic analysis toolkit, batteries, and a computer virtually anywhere, as demonstrated by *in situ* sequencing runs in a tropical rain forest (22), Antarctic desert (23), and space station (24). MinION has the capacity to produce >1,000,000 sequences per day, with average read lengths of around 20,000 bases and maximum read lengths approaching 1,000,000 bases (11). Because of low sequence quality, MinION has been used mostly in whole-genome sequencing analyses to resolve long repeats and bridge contigs or to resequence genomes (11, 25). The error rate of reads can be reduced from 10 to 15% to 1 to 5% by sequencing of the complementary strand ($1D^2$ method) or preparing tandem repeat molecules (concatemers), but these solutions are laborious and enable low sequencing depth and, hence, are seldom used (26–29). MinION has been used to generate long DNA barcodes from consensus sequences (30) and to detect specific human pathogens that are easily distinguishable and well represented in reference sequence databases (31, 32). Although multiple reports claim achieving species-level taxonomic resolution in complex environmental samples (33–35), the high error rate renders nanopore sequencing poorly suited for exploratory metabarcoding analyses of natural communities. Conversely, the metagenomics approach has gained popularity for identification of human pathogens to skip the entire PCR step and avoid associated biases (22, 36, 37). Recently, Bronzato Badial et al. (38) demonstrated that plant-pathogenic bacteria and viruses can be

TABLE 1 Detailed information about MinION and Sequel sequencing runs

Run ID	Sample (*n*)	Primer	MinION chemistry	Sequencing cell	DNA quantity (ng)	Sequencing time (min)	No. of reads obtained (% qualified)
ONT1	Pinaceae needles (36)	ITS1catta + ITS4ngsUni	SQK-LSK109	Flow cell 1 (new)	1,165	1,440	1,053,693 (17.7)
Sequel1	Pinaceae needles (36)	ITS1catta + ITS4ngsUni	NA[a]	SMRT cell 1	1,000	600	167,864 (72.6)
ONT2	*Solanum tuberosum* leaves and tubers (35)	ITS1catta + ITS4ngsUni; ITS1Oo + ITS4ngsUni	SQK-LSK109	Flow cell 1 (2nd use)	2,002	343	1,194,242 (3.1)
Sequel2	*S. tuberosum* leaves and tubers (35)	ITS1catta + ITS4ngsUni; ITS1Oo + ITS4ngsUni	NA	SMRT cell 1	1,000	600	177,635 (42.5)
ONT2a	*S. tuberosum* leaves (8)	ITS1catta + ITS4ngsUni	SQK-LSK109	Flow cell 1 (3rd use)	1,076	260	130,130 (10.9)
ONT2b	*S. tuberosum* leaves (8)	ITS1catta + ITS4ngsUni	SQK-LSK109	Flow cell 2 (new)	926	NA	Failed
ONT2f	*S. tuberosum* leaves (8)	ITS1catta + LR14	SQK-LSK308	Flow cell 3 (new)	473	75	5433 (4.9)
ONT2g	*S. tuberosum* tuber (1)	Metagenome	SQK-RAD004	Flow cell 2 (2nd use)	69	251	466,488 (14.2)
ONT2h	*S. tuberosum* tuber (1)	ITS1catta + LR11	SQK-LSK109	Flow cell 2 (3rd use)	448	165	767,611 (44.7)
ONT2i	*S. tuberosum* tuber (1)	Metagenome	SQK-RAD004	Flow cell 4 (new)	31	50	1142 (38.2)
ONT2j	*S. tuberosum* leaf (1)	Metagenome	SQK-RAD004	Flow cell 5 (new)	428	105	107,613 (48.8)
ONT2k	*Cucumis sativa* roots (1)	Metagenome	SQK-RAD004	Flow cell 5 (2nd use)	53	125	5044 (80.2)
ONT2l	*C. sativa* leaf (1)	Metagenome	SQK-RAD004	Flow cell 5 (3rd use)	80	95	23,093 (57.1)
ONT2m	*C. sativa* leaf (1)	Metagenome	SQK-RAD004	Flow cell 5 (4th use)	48	90	1074 (20.8)
ONT2n	*S. tuberosum* stem (1)	Metagenome	SQK-RAD004	Flow cell 6 (new)	56	74	51,175 (47.7)

[a]NA, not applicable.

detected using MinION, whereas Hu et al. (39) extended this to fungal pathogens of cereals.

The main objective of this study was to develop protocols for metabarcoding-based and metagenomics-based detection of fungal and oomycete plant pathogens using third-generation sequencing tools. In particular, we aimed to (i) test the relative biases and shortfalls of MinION-based and PacBio Sequel-based identification and evaluate the perspectives of these methods and (ii) test MinION protocols for ultrarapid pathogen identification. We performed several HTS runs using MinION and Sequel instruments and compared these results to Sanger sequencing, species-specific oligonucleotide-based PCR, and morphology-based identification where relevant. We tested the third-generation HTS methods in two plant pathosystems, conifer (Pinaceae) needles and potato (*Solanum tuberosum* L.) leaves and tubers.

RESULTS

Technical features of MinION and Sequel runs. We compared two MinION runs (ONT1 and ONT2) and two corresponding Sequel runs (Sequel1 and Sequel2) from the same pools of amplicon samples (needles of Pinaceae spp. and various tissues of *S. tuberosum* for both methods) in their technical performance and ability to recover the diversity of Fungi and Oomycota. Compared with Sequel, MinION had severalfold-greater initial sequencing depth, which further depended on the loaded DNA content and sequencing time (considering all MinION runs) (Table 1). The high sequencing depth of MinION was reduced severalfold during the quality filtering and demultiplexing, reaching a level comparable to that of Sequel. Among individual samples within libraries, variation in sequencing depth was slightly greater in MinION (coefficient of variation [CV], 67.8% to 93.4%) than in Sequel (62.8% to 64.5%). The Pearson correlation coefficient of sequencing depth (quality-filtered reads) of samples in MinION and Sequel ranged from 0.585 in the *S. tuberosum* data set ($n = 35$) to 0.853 in the Pinaceae species data set ($n = 36$), suggesting a substantial library preparation or sequencing bias in the former amplicon pool.

For the MinION data sets, chimeras (i.e., artificial reads originating from >1 parent or concatemers of the same read) were detected using the reference-based method of UCHIME but not the *de novo* method of the same program. Putatively chimeric molecules contributed 1.5 to 1.8% to the mapped reads, but nearly half of these were false positives based on manual checking. Interestingly, nearly half of the true chimeras (0.4% of all reads) included parents from different samples, indicating some chimera formation during the library preparation or sequencing process in addition to PCR.

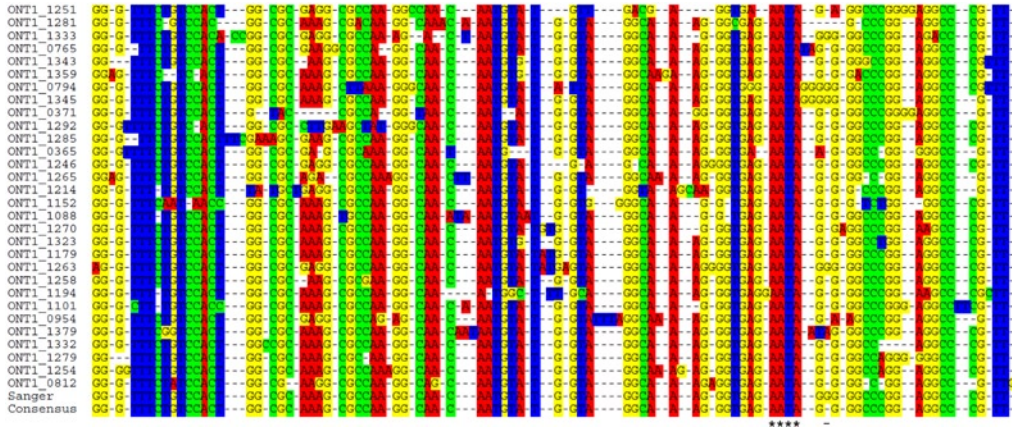

FIG 1 Screenshot example of multiple-sequence alignment of MinION reads mapped to the corresponding Sanger sequence of *Lophodermium pinastri*. Difference from the consensus is indicated with a dash; the accurately sequenced tetramer is indicated with asterisks.

Further manual inspection of demultiplexed sequences revealed that 5 to 8% of all chimeras are self-chimeric, i.e., 1.5-fold to 6-fold concatemeric repeats of itself. In the Sequel data sets, chimeras accounted for 1.9 to 3.7% of reads (including 1.5 to 2.4% detected *de novo*), with no self-chimeric reads remaining in the quality-filtered data.

The index switching (i.e., post-PCR chimeras, where sample molecular identifiers are attached to another molecule in the DNA library) rate was much greater in MinION (3.6% of reads in ONT2 run) than in Sequel (0.14% in Sequel2 run). Based on positive-control samples, we estimated that the error rate in Sequel runs is 0.1% (corresponds to polymerase errors) but 11 to 16% (depending on species) for the conventional 1D method and 11% for the $1D^2$ method (run ONT2f) of MinION. Alignments of hundreds of positive-control and other common sequence types revealed that errors are non-randomly distributed, i.e., occasionally there were no errors across 3 or 4 bases of the alignment, whereas homopolymeric sites exhibited large amounts of combined indels and substitutions (Fig. 1). Because of these nonrandom errors, we were able to construct consensus at 98.5 to 99.5% accuracy (only deletions remaining) with 100 or more reads using MinION. Conversely, most of the individual sequences of Sequel were identical to the Sanger sequences.

All MinION runs using one batch of R9.4 flow cells (1 and 2, accommodating runs ONT1, ONT2, ONT2a, ONT2g, and ONT2h; Table 1) were contaminated by a *Coniothyrium* sp. (INSD accession number JX320132), but this ascomycete was not observed in negative-control samples, other batches or flow cell types, or Sequel. At least partly because of this, the dominant fungal taxa recovered in samples differed in the MinION and Sequel runs (Tables 2 and 3).

Metabarcoding analyses of MinION and Sequel. The MinION run ONT1 included diseased and asymptomatic needle samples of Pinaceae spp. and pure cultures of fungal pathogens. Of the 792,748 "passed" reads, 189,150 (23.9%) were demultiplexed and 183,343 (23.1%) were mapped to reference sequence databases based on the quality criteria (E value of $<e^{-40}$ and sequence similarity of >75%). The ITS1catta-ITS4ngsUni primer pair amplified mostly fungal DNA (99.9% of identified reads). Best hits were distributed across 2,483 fungal molecular operational taxonomic units (MOTUs), with the well-known conifer pathogens yielding hits to 1 or 2 different accession numbers. On average, samples hosted 203.4 ± 130.5 (mean ± standard deviation [SD]) MOTUs. Best hits to the contaminant *Coniothyrium* sp. (Ascomycota) contributed 26.3% of all sequences on average. Of the expected Pinaceae-associated taxa, *Hormonema*

TABLE 2 Identification of fungi in Pinaceae species needle samples

Sample ID	Species-specific primer(s)	Reads (%) per sample[a] (dominant taxa)	
		Sequel	MinION
115	Negative	LoPi, 35; CoTu, 27; RhySp, 3	CoSp 44; CoTu, 21; HeJu, 2
117	Negative	LoCo, 49; DiSp, 25; ViVi, 9	LoCo, 42 DiSp, 13; CoSp, 12
118	LeAc	NeGe, 36; DiSp, 10; HoMa, 9	NeGe, 32; CoSp, 9; DiSp, 6
119	Negative	LoPi, 34; RhySp, 16; CySp, 11	CoSp, 49; ChaeSp, 9; PhEu, 3
123	Negative	DiSp, 49; PlOs, 19; HoMa, 19	DiSp, 25; PlOs, 14; HoMa, 14
125	Negative	HoMa, 56; ViVi, 8; SpRu, 7	HoMa, 49; CoSp, 13; MyTa, 5
127	DoSe	LaCa, 68; HoMa, 25; InSp, 6	LaCa, 67; HoMa, 21; CoSp, 2
139	Negative	MyTa, 22; AuPu, 12; LaCa, 8	MyTa, 20; CoSp, 18; AuPu, 11
141	Negative	DiSp, 21; HoMa, 21; SpRu, 16	HoMa, 17; DiSp, 12; CoSp, 10
142	Negative	LoPi, 40; NeGe, 28; DoSe, 9	CoSp, 43; NeGe, 12; DoSe, 7
148	Negative	MyTa, 40; HeJu, 35; SpRu, 15	HeJu, 37; MyTa, 35; SpRu, 9
154	Negative	ViVi, 18; LoCo, 13; HeJu, 12	CoSp, 31; HeJu, 12; LoCo, 10
2404	NA	DoPi, 100	DoPi, 76; CoSp, 9; PsOp, 2
3904	NA	LeAc, 52; AlAl, 23; HaOr, 23	LeAc, 44; CoSp, 24; AlIr, 19
3906	NA	DoPi, 100	DoSe, 70; CoSp, 7; DoPi, 4
4154	DoPi, DoSe, LeAc	LoPi, 94; AnCo, 3; NeGe, 1	CoSp, 86; AnCo, 3; HoMa, 1
4162	Negative	LoPi, 62; DoSe, 9; CeFe, 6	CoSp, 57; CeFe, 10; DoSe, 9
4180	DoSe	DoSe, 32; LoPi, 22; MyTa, 18	DoSe, 27; CoSp, 26; MyTa, 21
4181	Negative	LoPi, 63; LoSp, 6; LoCo, 5	CoSp, 62; LoSp, 6; LoCo, 5
4192	DoSe	LoPi, 32; PhLa, 18; CyMi, 13	CoSp, 33; PhLa, 13; CyMi, 10
4194	LeAc	TrSp, 44; NeGe, 15; RhiSp, 11	TrSp, 40; NeGe, 8; ScSp, 7
4195	LeAc	HoMa, 52; LoPi, 11; TrSp, 10	HoMa, 44; CoSp, 15; TrSp, 10
4197	DoSe	LoPi, 46; DoSe, 25; RhySp, 8	CoSp, 49; DoSe, 19; LoPi, 14
4220	Negative	LoPi, 58; AnSp, 32; PhLa, 5	CoSp, 56; AnSp, 28; PhLa, 3
4221	DoSe	LoPi, 47; MyTa, 43; AuPu, 3	CoSp, 47; MyTa, 37; DoSe, 4
4222	DoSe	LoPi, 48; DoSe, 7; EuSp, 6	CoSp, 49; DoSe, 7; PhSp, 5
4223	DoSe, LeAc	HoMa, 25; DiSp, 10; DoSe, 8	HoMa, 20; CoSp, 8; DoSe, 8
5136	Negative	AsSy, 73; DiVi, 27	AsSy, 36; DiSp, 13; CoSp, 11
5137	Negative	HeAn, 56; DoSe, 32; AsSo 3	HeAn, 45; DoSe, 31; CoSp, 3
5146	Negative	GiTr, 17; DiSp, 17; CeSp, 14	CeSp, 14; PhLa, 11; GiTr, 10
5148	Negative	DiSp, 45; ArSp 11; GiTr, 11	DiSp, 29; DiVi 10; GiTr, 7
5151	Negative	CyMi, 22; MyTa, 11; ClSp, 9	CyMi, 18; CoSp, 15; MyTa, 11
5186	Negative	MyTa, 70; ViVi, 8; DiSp, 5	MyTa, 66; CoSp, 15; AuPu, 4
5194	Negative	DiVi, 26; HelSp, 20; MaOb, 11	DiVi, 17; MyTa, 10; DoSp, 10
5195	Negative	RaHy, 57; ZyVe, 19; ExSp, 18	RaHy, 41; CaSp, 12; ZyVe, 11
5297	Negative	ZyVe, 18; RhiSp, 16; RhMu 5	ZyVe, 17; CoSp, 10; ScSp, 10
5307	Negative	ZyVe, 20; ExSp, 12; MyTa, 10	ZyVe, 13; CoSp, 11; MyTa, 10
14374	ND[b]	LeAc, 38; DoSp, 13; TeSp, 10	LeAc, 31; PeIn, 10; CoSp, 7
14378	ND	HoMa, 46; NeSp, 23; ChSp, 6	HoMa, 42; PlSt, 16; CoSp, 8

[a]Percentages in Sequel and MinION columns indicate the percentage of sequences assigned to particular MOTUs. Abbreviations for species corresponding to dominant MOTUs: AlAl, *Alternaria alternata*; AlIr, *Alternaria iridiaustralis*; AnCo, *Anthostomella conorum*; AnSp, *Anthostomella* sp.; ArSp, *Articulospora* sp.; AsSo, *Ascocoryne solitaria*; AsSy, *Aspergillus sydowii*; AuPu, *Aureobasidium pullulans*; CaSp, Capnodiales sp.; CeFe, *Cenangium ferruginosum*; CeSp, Ceratobasidiaceae sp.; ChSp, *Chalara* sp.; ChaeSp, Chaetothyriales sp.; ClSp, *Cladosporium* sp.; CoSp, *Coniothyrium* sp.; CoTu, *Coleosporium tussilaginis*; CyMi, *Cyclaneusma minus*; CySp, *Cyphellophora* sp.; DiSp, Didymellaceae sp.; DiVi, *Didymella viburnicola*; DoPi, *Dothistroma pini*; DoSe, *Dothistroma septosporum*; DoSp, Dothideomycetes sp.; EuSp, Eurotiomycetes sp.; ExSp, *Extremus* sp.; GiTr, *Gibberella tricincta*; HaOr, *Hannaella oryzae*; HeAn, *Heterobasidion annosum*; HeJu, *Herpotrichia juniperi*; HelSp, Helotiales sp.; HoMa, *Hormonema macrosporum*; InSp, *Insecta* sp.; LaCa, *Lachnellula calyciformis*; LeAc, *Lecanosticta acicola*; LoCo, *Lophodermium conigenum*; LoPi, *Lophodermium pinastri*; LoSp, *Lophodermium* sp.; MaOb, *Malassezia obtusa*; MyTa, *Mycosphaerella tassiana*; NeGe, *Neocatenulostroma germanicum*; NeSp, *Nectria* sp.; PeIn, *Perusta inaequalis*; PhEu, *Phaeococcomyces eucalypti*; PhLa, *Phacidium lacerum*; PhSp, *Phaeomoniella* sp.; PlOs, *Pleurophoma ossicola*; PlSt, *Pleonectria strobi*; RaHy, *Ramularia hydrangeae-macrophyllae*; RhiSp, *Rhizosphaera* sp.; RhMu, *Rhodotorula mucilaginosa*.; RhySp, Rhytismataceae sp.; ScSp, *Scleroconidioma sphagnicola*; SpRu, *Sporobolomyces ruberrimus*; ZyVe, *Zymoseptoria verkleyi*; TeSp, Teratosphaeriaceae sp.; TrSp, *Truncatella spadicea*; ViVi, *Vishniacozyma victoriae*.

[b]ND, not determined.

macrosporum (6.2%), *Lophodermium conigenum* (5.0%), and a Didymellaceae sp. (4.3%) (all Ascomycota) yielded the greatest number of hits (Fig. 2A). All of these taxa occurred in 94 to 100% of samples.

The corresponding Sequel run Sequel1 revealed 121,965 demultiplexed reads that were clustered into 535 MOTUs, all above the quality threshold. Samples harbored 51.5 ± 41.6 MOTUs on average, nearly four times less than that in the MinION data set.

TABLE 3 Identification of fungi in *Solanum tuberosum* tissue samples

		Reads (%) per sample[a]	
Sample	**Sanger**	**Sequel**	**MinION**
KL001	Failed	MyTa, 10; BuCr, 8; SpRo, 7	CoSp, 24; CerSp, 16; LeAc, 11
KL002	Failed	KoCh, 27; PeEx, 13; SpRo, 8	DoSe, 27; KoCh, 9; CoSp, 8
KL003	Failed	BuCr, 21; FiSt, 14; ClSp, 12	BuCr, 17; ClSp, 12; CoSp, 11
KL004	Failed	EpNi, 12; MyTa, 10; SpRo, 10	NeGe, 13; CoSp, 11; NeMi, 9
KL005	FiWi	FiWi, 41; DioSp, 5; BuAu, 5	FiWi, 26; MyTa, 13; CoSp, 10
KL006	Failed	SpRo, 17; LeSp, 8; ViVi, 7	CoSp, 15; HoMa, 11; MyTa, 8
KL007	Failed	FiSt, 26; SpSp, 11; MyTa, 7	CoSp, 15; FiSt, 13; SpSp, 7
KL008	Failed	PaLa, 49; SpRo, 9; MyTa, 6	PaLa, 36; CoSp, 14; MyTa, 8
KL009	Failed	DiSp, 15; DiPo, 12; MyTa, 11	DiSp, 12; CoSp, 11; MyTa, 8
KL010	BoEx	BoSp, 47; FiSt, 12; SpRo, 5	BoSp, 26; CoSp, 18; FiSt, 5
KL011	BoEx	BoSp, 71; ExEq, 9; MyTa, 4	BoSp, 52; ExEq, 8; MyTa, 4
KL012	BoEx	BoSp, 96; ViTe, 2; MySp, 1	BoSp, 50; CoSp, 14; HeJu, 3
KL013	DioSp	MyTa, 17; BuCr, 9; ViVi, 8	CoSp, 24; MyTa, 13; HyaSp, 4
KL014	Failed	CyMa, 14; ViVi, 10; LeSp, 7	CoSp, 30; ViVi, 10; CoTu, 6
KL015	BoEx	BoSp, 92; BuCr, 1; FiSt, 1	BoSp, 37; LoCo, 19; CoSp, 8
KL016	Failed	ViVi, 18; PaLa, 15; LeSp, 12	CoSp, 29; PaLa, 10; HoMa, 7
KL017	BoEx	BoSp, 65; FiSt, 8; ViVi, 4	BoSp, 44; CoSp, 15; TrSp, 7
KL018	Failed	MyTa, 35; PlSp, 13; FiSt, 13	CoSp, 39; MyTa, 11; DoSe, 9
KL019	Failed	AuPu, 13; BuCr, 11; ViTe, 11	CoSp, 32; AuPu, 13; BoSp, 7
KL020	BoEx	BoSp, 49; SpSp, 9; FiSt, 9	CoSp, 29; BoSp, 17; SpSp, 5
KL021	Failed	MyTa, 35; ViVi, 15; ClSp, 10	MyTa, 30; CoSp, 20; AuPu, 9
KL022	ClSp	MyTa, 42; BuCr, 13; AuPu, 9	RhMu, 33; MyTa, 23; CoSp, 11
KL023	Failed	AuPu, 42; MyTa, 33; AlAl, 8	CoSp, 46; AnSp, 24; AuPu, 7
KL024	ClSp	MyTa, 51; SpRo, 9; DiBu, 6	MyTa, 29; CoSp, 20; DoSe, 10
KL025	Failed	ViVi, 18; DiSp, 18; ClSp, 13	CoSp, 30; DiSp, 12; ClSp, 8
KL026	ClSp	MyTa, 16; SuGr, 15; BuCr, 12	CoSp, 42; MyTa, 11; AtSp, 5
KL027	DioSp	BuCr, 65; ViVi, 9; MyTa, 7	CoSp, 75; CeFe, 4; BlGr, 3
KL028	Failed	DiSp, 97; HaVe, 3; CuMo, 0	DiSp, 57; PhBu, 6; PlSp, 6
KL029	Failed	DiSp, 54; BoSp, 46; PlCu, 0	DiSp, 32; BoSp, 28; PlSp, 6
KL030	Failed	PlCu, 100	PlCu, 17; ZyVe, 11; ScSp, 8
KL031	Failed	DiSp, 93; PeBi, 4; BoSp, 1	DiSp, 50; PlSp, 6; PhBu, 5
KL032	Failed	PlCu, 43; PsSp, 22; CuMo, 17	PlCu, 23; PlOr, 11; GeAs, 10
KL033	Failed	DeSp, 50; PlCu, 38; NeSp, 11	PlCu, 41; PlSp, 36; NeSp, 8
KL034	Failed	PenSp, 80; PlCu, 11; CuMo, 2	CeSp, 13; PhLa, 12; GiTr, 8
KL035	Failed	PeBi, 41; PenSp, 38; PeBr, 10	PeBi, 42; PeAe, 23; PeBr, 11

[a]Percentages in Sequel and MinION columns indicate the percentages of sequences assigned to particular MOTUs. Abbreviations for species corresponding to dominant MOTUs: AlAl, *Alternaria alternata*; AnSp, *Anthostomella* sp.; AtSp, Atheliaceae sp.; AuPu, *Aureobasidium pullulans*; BlGr, *Blumeria graminis*; BoEx, *Boeremia exigua*; BoSp, *Boeremia* sp.; BuAu, *Buckleyzyma aurantiaca*; BuCr, *Bullera crocea*; CeFe, *Cenangium ferruginosum*; CerSp, *Cercozoa* sp.; CeSp, Ceratobasidiaceae sp.; ClSp, *Cladosporium* sp.; CoSp, *Coniothyrium* sp.; CoTu, *Coleosporium tussilaginis*; CuMo, *Cutaneotrichosporon moniliiforme*; CyMa, *Cystofilobasidium macerans*; DeSp, *Dendryphion* sp.; DiBu, *Dioszegia butyracea*; DioSp, *Dioszegia* sp.; DiPo, *Didymella pomorum*; DiSp, Didymellaceae sp.; CeSp, *Celosporium* sp.; EpNi, *Epicoccum nigrum*; ExEq, *Exophiala equina*; ExPi, *Exobasidium pieridis-ovalifoliae*; FiSt, *Filobasidium stepposum*; FiWi, *Filobasidium wieringae*; GeAs, *Geomyces asperulatus*; GiTr, *Gibberella tricincta*; HaVe, *Harzia velata*; HeJu, *Herpotrichia juniperi*; HoMa, *Hormonema macrosporum*; HyaSp, *Hyaloscyphaceae* sp.; KoCh, *Kondoa changbaiensis*; LaCa, *Lachnellula calyciformis*; LeAc, *Lecanosticta acicola*; LeSp, *Leucosporidium* sp.; LoCo, *Lophodermium conigenum*; MySp, *Mycosphaerellaceae* sp.; MyTa, *Mycosphaerella tassiana*; NeGe, *Neocatenulostroma germanica*; NeMi, *Neocatenulostroma microsporum*; NeSp, *Nectria* sp.; PaLa, *Papiliotrema laurentii*; PeAe, *Penicillium aethiopicum*; PeBi, *Penicillium bialowiezense*; PeBr, *Penicillium brevicompactum*; PeEx, *Penicillium expansum*; PenSp, *Penicillium* sp.; PhBu, *Phoma bulgarica*; PhLa, *Phacidium lacerum*; PlCu, *Plectosphaerella cucumerina*; PlOr, *Plectosphaerella oratosquillae*; PlSp, Pleosporales sp.; PsSp, *Pseudogymnoascus* sp.; RaHy, *Ramularia hydrangeae-macrophyllae*; RhiSp, *Rhizosphaera* sp.; RhMu, *Rhodotorula mucilaginosa*; ScSp, *Scleroconidioma sphagnicola*; SpRo, *Sporobolomyces roseus*; SpSp, *Sporobolomyces* sp.; SuGr, *Suillus granulatus*; ZyVe, *Zymoseptoria verkleyi*; TrSp, *Truncatella spadicea*; ViTe, *Vishniacozyma tephrensis*; ViVi, *Vishniacozyma victoriae*.

Altogether, 99.9% reads were ascribed to fungi, with *L. pinastri* (17.8%), *Dothiostroma septosporum* (8.9%), and *Hormonema macrosporum* (7.0%) (all Ascomycota) dominating across the entire data set (Fig. 2B). These dominant taxa occurred in 43 to 65% of samples.

The ONT MinION run ONT2 recovered 255,137 passed sequences, of which 16.2% were demultiplexed and 14.4% were mapped to reference database reads. Based on the occurrence of Pinaceae-specific pathogens in potato samples, we estimated that

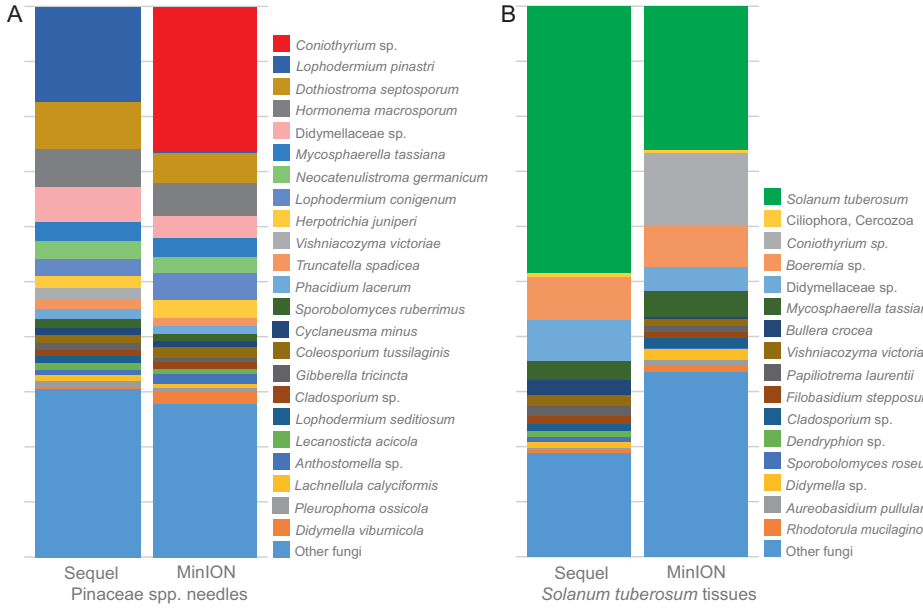

FIG 2 Column diagrams demonstrating relative abundance of MOTUs in Pinaceae species needles (A) and *Solanum tuberosum* leaves and tubers (B) based on the ITS1catta + ITS4ngsUni amplicons as revealed by Sequel and MinION instruments.

13.4% of the reads were carried over from the previous ONT1 run, in which we used the same primer and tag combinations. In the ONT2 run, these Pinaceae-specific MOTUs had proportionally similar relative abundances when compared across the same index combinations. The ITS1catta-ITS4ngsUni forward primer amplified mostly fungi (74.2% of identified reads; Fig. 2B) and plants (26.1% reads corresponding to nine MOTUs of *S. tuberosum*). Of fungi, MOTUs corresponding to *Coniothyrium* sp. (13.2% of reads), *Boeremia* sp. (7.5%), *Mycosphaerella tassiana* (4.6%), and Didymellaceae sp. (4.4%) (all Ascomycota) dominated. The average taxonomic richness was 81.7 ± 43.3 MOTUs per sample. The ITS1Oo-ITS4ngsUni primer pair revealed Oomycota (47.7%), other Heterokonta (19.2%), Fungi (23.6%), and Viridiplantae (9.5%). In each sample, 0 to 3 Oomycota taxa were found, and all of these occurred only once or twice (Table 4). The majority of samples produced no visible amplicon on gel with these primers, and correspondingly, no Oomycota taxa were recovered from these samples based on MinION sequencing.

The Sequel run Sequel2 revealed 75,573 demultiplexed reads that were separated into 308 MOTUs, all matching to reference sequences. On average, 39.6 ± 20.3 MOTUs were recovered per sample. In the ITS1catta-ITS4ngsUni amplicons, Fungi, Viridiplantae, Alveolata, and Rhizaria contributed to 51.0%, 48.4%, 0.5%, and 0.1% of reads, respectively. All plant reads were distributed across 25 MOTUs that were all assigned to *S. tuberosum*. Six of the MOTUs probably represent naturally high variation among the rRNA internal transcribed spacer (ITS) 1 and 2 sequences of *S. tuberosum* (based on INSD entries), whereas others represent pseudogenes or nonfunctional copies. These were rare to common (up to 3% of all variants) and sometimes exceeded the abundance of regular variants in individual samples. Of fungi, the largest number of reads belonged to *Boeremia* sp. (8.0%), Hysteriaceae sp. (7.4%), and *Mycosphaerella tassiana* (3.3%) (all Ascomycota) (Fig. 2B). The ITS1Oo-ITS4ngsUni reads were mostly assigned to Oomycota (62.9%), other Heterokonta (33.9%), Viridiplantae (3.0%), and Alveolata (0.2%). This

TABLE 4 Identification of Oomycota and other Heterokonta in *Solanum tuberosum* tissue samples based on the ITS1Oo + ITS4ngsUni amplicons

	Reads (%) per sample[a]	
Sample	**Sequel**	**MinION**
KL003	*Phytophthora infestans*, 90; *Peronospora radii*, 10	*P. infestans*, 84; *P. radii*, 11
KL004	Xanthophyceae sp., 100	Heterokonta sp., 86
KL005	*Peronospora agrestis*, 73; Xanthophyceae sp., 27	*P. agrestis*, 83; Heterokonta sp., 17
KL006	*Peronospora* sp., 85; *Eustigmatos* sp., 15	*Peronospora* sp., 79; Eustigmataceae sp., 13
KL007	Chromulinaceae sp., 84; *Hyaloperonospora parasitica*, 16	Chromulinaceae sp., 66; *H. parasitica*, 9
KL008	*Peronospora violae*, 100	*P. violae*, 84
KL010	Chromulinaceae sp., 100	Chromulinaceae sp., 93
KL013	*H. parasitica*, 100	*H. parasitica*, 100
KL014	Xanthophyceae sp., 100	Heterokonta sp., 50
KL021	*Peronospora variabilis*, 100	
KL022	*P. variabilis*, 100	
KL024	*P. variabilis*, 100	

[a]Percentages in Sequel and MinION columns indicate the percentages of sequences assigned to particular MOTUs. Samples with no PCR product and no sequences are excluded. Notably, plant and fungal sequences contributed on average 10% to MinION data (probably index switch artifacts from the fungal data set; not shown).

data subset yielded 0 to 2 MOTUs of Oomycota or other Heterokonta per sample (Table 4).

Taking these results together, Sequel and MinION recovered the same dominant fungal species (excluding the contaminant) in 60% and 63% of the Pinaceae spp. (Table 2) and *S. tuberosum* (Table 3) samples, respectively. These values increased to 78% and 83%, respectively, when considering the overlap in the three best-matching taxa. Inspection of the discordant samples revealed that contamination from the previous ONT1 run blurred the results of the *S. tuberosum* samples and MinION produced 1 to 2 orders of magnitude less high-quality reads matching to multiple species, such as *Vishniacozyma victoriae* and a *Cystobasidium* sp. (both Basidiomycota) and *L. pinastri* and a *Dendryphion* sp. (both Ascomycota), than Sequel (Fig. 2). These species had a relatively high proportion of homopolymers (>3-mers) per base compared with dominant but equally shared taxa ($F_{1,8} = 5.79$; $P = 0.088$), which may have reduced their relative abundances after quality filtering of nanopore sequences. The ITS1Oo-ITS4ngsUni data subsets were in stronger agreement in Sequel and MinION, apart from the lack of *Peronospora variabilis* among MinION reads, hence its unsuccessful diagnosis from three *S. tuberosum* leaf samples (Table 4).

The ONT2a MinION run was designed to test whether long indexes reduce index switching. The ONT2a run revealed an index switch rate of 3.8%. *S. tuberosum* (14 MOTUs) contributed to 18.7% of reads, whereas the contaminant *Coniothyrium* sp. accounted for 16.7% of reads, prevailing in half of the eight samples. Of other fungal species, *Papiliotrema laurentii* (11.5%) and *Filobasidium stepposum* (7.0%) (both Basidiomycota) and *Mycosphaerella tassiana* (5.6%) dominated. These species were less common in the same eight samples as recovered in the ONT2 run with regular indexes (3.8%, 2.7%, and 4.6%, respectively). In spite of severalfold differences in relative abundance of MOTUs, the ONT2a and ONT2 runs recovered the same dominant MOTUs in 75% of the samples.

The ONT2f run was intended to test suitability of the $1D^2$ method relative to the conventional 1D method based on ONT2 run. Because the $1D^2$ protocol requires DNA fragments of >3 kb, we sequenced a 3.2-kb amplicon (see Materials and Methods). The ONT2f run recovered only 3,241 $1D^2$ reads, of which 29.7% fell within a 10% interval of the expected read length of ca. 3,200 bases. The median read length was 954 bases. As the positive-control sample revealed no reads, the index switch rate could not be calculated. Of all sequences, *S. tuberosum* (17 MOTUs) accounted for 54.2% of them. Of fungi (39.8%), *Taphrina populina* (6.0%), *Parastagonospora* sp. (3.8%), and *Glarea lozoyensis* (3.0%) (all Ascomycota) dominated. These species were much less common in the ONT2 library (0.1%, <0.1%, and <0.1%, respectively). The same species were among the dominants in only 25% of samples based on the ONT2 and ONT2f runs. It remains

unknown whether these biases are related to sequencing of long amplicons or the 1D^2 method.

Metabarcoding versus metagenomics approach and sequence quality bins. We designed two MinION runs to compare the relative performance of the metagenomics approach (ONT2g) and metabarcoding approach (ONT2h) using a single diseased *S. tuberosum* tuber sample, KL036 (Table 1). The ONT2g metagenomics run yielded 66,133 and 400,355 passed and failed reads, respectively. The 5,000 randomly selected sequences from each of these bins revealed 1,325 passed reads and 1 failed read that met our quality standards (see Materials and Methods). Altogether, 37.4% of the passed reads represented sequences carried over from a previous run. After removal of these reads, the metagenomics data set was dominated by plant and bacterial reads. Best hits to *Lycopersicon esculentum* (tomato, 29.0% of reads) and seven species of *Solanum* (altogether, 22.6%) collectively represented *S. tuberosum*. Of bacteria, hits to *Agrobacterium tumefaciens* (10.5%), *Variovorax paradoxus* (9.7%), and *Sphingopyxis alaskensis* (3.8%) dominated. Fungal hits were less common; those to *Thanatephorus cucumeris* (1.6%; Basidiomycota) and *Boeremia exigua* (1.0%) prevailed. Of these best-matching taxa, the bacterial species *A. tumefaciens* and *V. paradoxus* are probably present given their strongest hits of 93% and 92% and average hits of 87% and 85% sequence similarity, respectively, to reference strains. Conversely, the particular species *S. alaskensis*, *B. exigua*, and *T. cucumeris* are probably absent, because their best hits reached 84%, 88%, and 86%, and all hits averaged 79%, 80%, and 80% similarity to reference sequences, respectively, suggesting that these MOTUs correspond to other species in these genera that have no genomic information available.

The ONT2h run represented a long amplicon of the same sample, recovering 342,923 passed reads and 423,688 failed reads. Of the randomly selected 5,000 sequences for each bin, 1,876 passed reads and 1,068 failed reads met the quality threshold (see Materials and Methods). The positive control used in the next-to-previous run accounted for 0.2% of all sequences, mostly in the failed bin. Out of the 18 most abundant MOTUs, the proportion of 11 MOTUs differed significantly ($P < 0.001$) among the passed and failed bins, indicating that reads of certain taxa are much more likely to be recorded as failed. Of the passed reads, matches to *Lignincola laevis* (64.3%) and *Verticillium biguttatum* (5.0%) (both Ascomycota) and *T. cucumeris* (3.0%) dominated. In the failed bin, *V. biguttatum* (19.9%), *L. laevis* (15.7%), and *Plectosphaerella cucumerina* (7.8%) (all Ascomycota) prevailed, followed by *T. cucumeris* (6.0%). Of the dominant taxa recovered, only *V. biguttatum*, *T. cucumeris*, and *P. cucumerina* were identified to the species level given their high maximum (>90%) and mean (>85%) sequence similarity to the reference. We tested whether the taxa relatively more abundant in the failed bin possess more homopolymers than those in the passed bin, but there was no significant relationship between the relative abundance of taxa in these bins and the proportion of 4-mers per base ($F_{1,9} = 2.23$; adjusted $R^2 = 0.110$; $P = 0.169$).

In the ONT2g metabarcoding and ONT2h metagenomics data sets derived from the same *S. tuberosum* sample, *T. cucumeris* was the only shared taxon. Other fungal species common in the metabarcoding data set were absent from the metagenomics data set, probably because their genomes are unavailable. Several of these ascomycetes may have best matches to *B. exigua*, the genome of which has been sequenced. This situation highlights limitations of the metagenomics approach when insufficient reference data are available.

Rapid pathogen diagnostics. We aimed to minimize the time from sample preparation to diagnosis based on MinION sequencing of metagenomes using multiple samples (see Materials and Methods). In particular, we reduced the lysis and centrifugation time in DNA extraction and purification protocols and limited sequencing time (Fig. 3).

For the ONT2i run, we used bead beating combined with Phire lysis and FavorPrep purification to obtain DNA from a single diseased *S. tuberosum* tuber sample. MinION

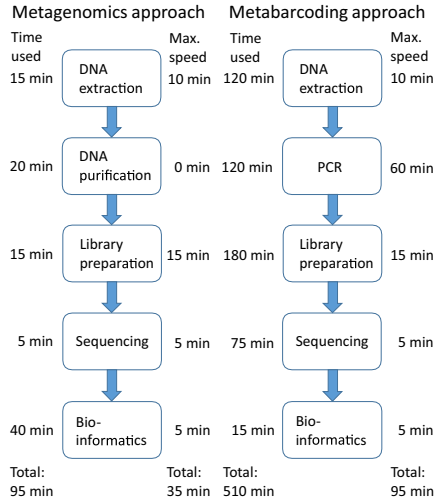

FIG 3 Schematic overview of work flow and speed of pathogen diagnostics in this study (time used) and maximum potential speed under optimal conditions using metagenomics and metabarcoding approaches with the MinION instrument. Note that the time used here was compiled across our successful runs and does not indicate any individual analyses.

sequencing revealed *L. esculentum* (72.2% of sequences), *S. tuberosum* (14.4%), and *T. cucumeris* (4.1%) as the only putative pathogens after 145 min of overall laboratory analyses and 5 min of bioinformatics analyses (Table 5). Notably, the sequencing process was suboptimal because of the small amount of DNA obtained, which resulted in <20% of pores effectively used at termination of this run. Sanger sequencing from four subsamples revealed *T. cucumeris* (all four subsamples) and Pyronemataceae sp. (two subsamples; Ascomycota).

For the ONT2j run, we grew potato plants inoculated with a suspension of *Phytophthora infestans* (Oomycota) and *B. exigua* isolates in growth chamber MLR-351H (Sanyo, Osaka, Japan). Based on visual examination, the plant individuals did not become infected, apart from bearing tiny brown spots on leaves (sample KL038). The leaf samples were disrupted in liquid N_2 instead of bead beating. The analysis revealed *L. esculentum* (76.6%), *Escherichia coli* (5.4%), *Shigella* sp. (1.2%) and *Salmonella enterica* (0.3%) (all *Bacteria*) and *P. infestans* (0.015%) at low relative abundance. However, Sanger sequencing revealed the presence of both *P. infestans* and *B. exigua*. Because of

TABLE 5 Details of the rapid pathogen diagnostics experiments using the MinION instrument[c]

Sample/run (min)	DNA extraction method[a] (min)	DNA purification (min)	No. of raw/passed reads	Bioinformatics: no. of quality-filtered reads (min)	Pathogen identification (% of sequences)	Total analysis time (min)
KL037/ONT2i (50)	BB, Phire lysis (15)	FavorPrep (25)	1,142/436	97 (40)	*Thanatephorus cucumeris* (4.1)	150
KL038/ONT2j (5)	LN, Phire lysis (40)	FavorPrep (60)	20,000/9,974	6,750 (409)	*Phytophthora infestans* (0.015)	434
KL039/ONT2k (30)	LN, Phire lysis (40)	FavorPrep (55)	5,044/4,046	849 (79)	*Fusarium* aff. *fujikuroi* (0.12)	224
KL040/ONT2l (5)	LN, Phire lysis (35)	FavorPrep (55)	2,000/1,420	669 (132)	*Albugo laibachii* (0.2)	247
KL040/ONT2m (60)	LN, Phire lysis (35)	FavorPrep (55), AMPure (30)	1,074/223	104 (10)	None	ND[b]
KL041/ONT2n (4)	LN, $(NH_4)_2SO_4$ lysis (20)	FavorPrep (20), AMPure (30)	4,000/2,457	1,236 (97)	*Rhizoctonia solani* (2.0)	191

[a]Abbreviations: BB, bead beating; LN, liquid N_2.
[b]ND, not determined because AMPure purification was performed on a subsequent day.
[c]The time used for library preparation and data interpretation took 15 min and 5 min, respectively.

contamination and marginal abundance of one of the two inoculated pathogens, we consider this MinION run unsuccessful.

The ONT2k run of wilted cucumber (*Cucumis sativus* L.) roots (sample KL039) was dominated by *L. esculentum*, a contaminant from the previous run (43.9%), followed by *C. sativus* (11.9%) and *C. melo* (11.8%). Apart from the pathogen *Fusarium* aff. *fujikuroi* (0.12%), the microbiome was dominated by bacteria *Rhodanobacter denitrificans* (3.8%) and *Pseudomonas* aff. *umsongensis* (1.6%), all of which were undetected in the previous run. Sanger sequencing failed because of unreadable chromatograms. Given the lethal effect on plants and low proportion of the pathogen but also a high level of contamination from the previous run, it remains unclear if fusariosis caused wilting.

A diseased *Cucumis sativus* leaf with white powdery infection (sample KL040) was used for the ONT2l run. Rapid analysis of the first 2,000 sequences revealed that *C. sativus* (58.7%) and *C. melo* (27.5%) dominate, but only a single putative pathogen, *Albugo laibachii* (Oomycota), is present (0.2%). Analysis of 20,000 subsequent sequences revealed similar results, with *A. laibachii* contributing 0.07% of sequences. However, Sanger sequencing of the same sample revealed *Peronospora violae* (Oomycota) instead, along with an unidentified mite (*Acari*). Because genomes of *Peronospora* species are unavailable, it is likely that the metagenomic matches to *A. laibachii* actually represent *P. violae*.

For the ONT2m run, the DNA of sample KL040 was further purified using AMPure XP beads. In this run, we had an issue with flow cell loading that resulted in very low accumulation of sequences, which may be attributable to the fourth use of the flow cell. Bioinformatics analysis revealed *C. sativus* (40.4%) and *C. melo* (34.6%) but no putative pathogens among the 104 quality-filtered sequences.

For the ONT2n run, a diseased *S. tuberosum* stem sample was powderized in liquid N_2 and DNA was extracted using $(NH_4)_2SO_4$ lysis, followed by double purification with FavorPrep and AMPure XP beads to rule out the possibility that the latter procedure inhibited the last run. On a new flow cell, sequences accumulated rapidly, revealing *S. esculentum* (75.4%), a *Pseudomonas* sp. (16.7%), and the putative pathogen *T. cucumeris* (2.0%), which matched the symptoms.

We also intended to analyze several infected samples of pine needles, but the protocols used here and in other commercial kits recovered too low a DNA concentration (<5 ng μl^{-1}). Therefore, we decided not to sequence these samples.

DISCUSSION

Use of third-generation sequencing instruments for DNA metabarcoding. Using the same amplicon pools and additional morphology-based or molecular diagnosis, we had a unique opportunity to evaluate the relative performance and biases of MinION compared with Sequel for taxonomic identification. These instruments revealed contrasting results in metabarcoding of Pinaceae species and *S. tuberosum* samples. The results of Sequel were generally consistent with morphology-based and species-specific oligonucleotide PCR-based diagnosis (Pinaceae species samples) and Sanger sequencing results (*S. tuberosum* samples) but failed to distinguish the closely related needle pathogens *D. pini* and *D. septosporum*. This was resolved when sequences were reclustered at 99% sequence similarity. Apart from the species of *Coniothyrium* contaminating the MinION data sets, the two platforms revealed different taxa (by names and INSD accession numbers) prevailing in the same samples. Mostly these MOTUs correspond to closely related sister taxa that share the UNITE Species Hypothesis at 2% distance level, as confirmed by manual comparisons of best-matching reads.

Our MinION runs utilizing washed, reused flow cells, and the same indexed primers suffered from carryover of 13 to 44% of reads originating from a previous run. Furthermore, we could recover traces of a positive control used in the next-to-previous run at 0.2% relative abundance. In MinION flow cells, a sequence carryover rate of 6% has been reported (40). Notably, such carryover contamination is not unique to nanopore technology, as it also occurs in reusable chips of the Ion Torrent platform (41).

In our analyses, MinION had an issue of contamination with a fungal MOTU matching a *Coniothyrium* sp. that was not observed in Sequel runs, negative controls, and our previous data sets. This contamination occurred in two R9.4 flow cells (1 and 2) supplied as a single batch with the MinION instrument but not in other R9.4 batches (flow cells 3, 4, and 5) or the R9.5 batch. Flow cells 1 and 2 were used over 6 months, rendering several independent laboratory contamination events unlikely. Therefore, we suspect that this contamination was inadvertently introduced during manufacture.

Chimeric reads were common in both Sequel and MinION data. UCHIME effectively detected chimeric molecules in the Sequel data, but it performed poorly on the MinION data. We speculate that the error-rich MinION reads were too different from each other to be recognized as chimeric using the algorithm parameters. In contrast to Sequel, a large proportion of long MinION reads represented self-chimeras that were not recognized by UCHIME. In addition, the MinION data included a substantial proportion of chimeric molecules with parents from different samples, representing a unique hybrid issue of index switching and chimera formation that could be detected only manually. This may be related to the formation of chimeric molecules during the library preparation or artifacts of sequencing when molecules pass through nanopores. Since MinION reads are typically mapped to the reference, we estimate that the abundant chimeric molecules create virtually no bias, except for those with switched indexes.

Index switches during library preparation or sequencing make a strong and perhaps predominant contribution to sample contamination (42). The observed index switching rate of 3.6 to 3.8% in MinION compares poorly with that of Sequel ($<$0.2% in this study) and various Illumina instruments (0.1 to 10%) (43). Here, double-size indexes performed equally poorly, suggesting that index switches are attributable to processes in library preparation or sequencing artifacts rather than sequencing errors. In particular, the high rate of index switching spilled the dominant MOTUs in the deeply sequenced MinION data sets across nearly all samples. This partly contributed to the 2-fold- to 4-fold-greater richness per sample compared with the Sequel data sets. The high error rate and inaccurate mapping-based method of MOTU construction certainly add to this difference. Conversely, the clustering of Sequel data at 98% sequence similarity may have been too conservative, because many putative plant-pathogenic fungal species differ from each other by only a few bases in the ITS region (e.g., the Pinaceae-specific needle pathogens *D. pini* and *D. septosporum*) (44); therefore, several species with distinct ecology and pathology may be lumped into a single taxon (45). Reanalysis of Sequel sequence data indicated that these species can be successfully separated at 99% sequence similarity (not shown). In spite of substantial disparity in the taxon-rich ITS1catta-ITS4ngsUni data subsets, the two instruments revealed a great overlap in the species-poor ITS1Oo-ITS4ngsUni data subset.

The average error rate of MinION reads was 10 to 15%, depending on the proportion of homopolymers in the marker gene region of particular species. MOTUs possessing homopolymer-rich ITS markers were up to 2 orders of magnitude less common in the MinION (ONT2) than in the Sequel (Sequel2) run. This is supported by the observation that these MOTUs were relatively more common in the failed bin than other MOTUs. In particular, the most homopolymer-rich positive-control sample was 16-fold more common in the failed bin than expected. Possibilities to solve this include lowering the initial quality threshold (Phred score) or including the failed reads in analyses.

We observed discrimination against longer reads when sequencing potato amplicons using the $1D^2$ approach (ONT2f), which confirms a previous report (40). Preferential recovery of shorter reads seems to be inherent to both PCR and all sequencing instruments, including Sequel (6, 7, 12).

Rapid molecular diagnostics. We tested both metagenomics- and amplicon-based approaches of MinION sequencing for rapid identification of pathogens. Most taxa recovered in the metagenome run were rare in the amplicon data set and vice versa. Although we detected severe biases in MinION amplicon sequencing, we believe that amplicon-based analyses are more accurate than metagenomics analyses and that

reference bias accounts for much of the difference; i.e., in metagenomics analyses, species with available reference genomes have a much greater chance of accumulating hits than species with no available genomes. In our analyses, this is illustrated by misidentification of a potato as a tomato. Mapping reads of an ascomycete pathogen to genomes of several others, as in our study, is likely to remain cryptic. A solution may be sufficiently deep metagenomics sequencing to secure coverage of mitochondrial or ITS markers that occur in multiple copies per genome. Because most bacterial and fungal human pathogens have available genome sequences, nanopore metagenomics-based identification may be better suited to medical samples, but this situation is likely to improve very soon in plant pathology.

In spite of multiple quality issues in MinION runs, we demonstrate that accurate molecular identification from sample collection through DNA extraction, concentration, library preparation, sequencing, bioinformatics, and taxonomic interpretation takes as little as 2.5 h using nanopore sequencing in the metagenome mode (Fig. 3). With no specific DNA extraction step, identification of bacterial human pathogens from urine was performed in 4 h (36). Multiple other studies report on running the full analysis work flow in 1 day (22, 37, 46). When accounting for all technological advances in molecular and bioinformatics analyses, the nanopore analysis work flow for plant and animal tissues potentially can be reduced to <2 h for metagenomes and ca. 3 h for amplicons (Fig. 3). However, it requires (i) that the DNA is easily extractable in high quantity and purity (47), (ii) amplification is performed by methods alternative to those of conventional slow polymerases (48), (iii) nanopore library preparation follows methods for rapid library kits, and (iv) the sequencing process is limited to ca. 15 min after obtaining a critical number of reads (37). These express diagnostics rates of MinION cannot be beaten by instruments of other HTS platforms that require several hours for library preparation and at least 1 day for sequencing (49).

However, there is a clear trade-off between overall analysis time and data reliability in MinION sequencing. Shortened DNA extraction protocols may yield lower quality and quantity of DNA, whereas culled incubation and centrifugation steps in library preparation may result in dilute and poorly indexed libraries overrepresented by short fragments. Although we successfully identified microorganisms from *S. tuberosum* using a library 13-fold more dilute than recommended, it may be useful to add a certain amount of so-called carrier DNA to secure preparation of high-quality libraries (50). We anticipate that sample preparation, bioinformatics, and interpretation processes take longer for multiplexed samples, which may be necessary to reduce the overall analytical costs per sample by an order of magnitude when using Oxford Nanopore's commercial multiplexing kits or by 3 orders of magnitude by applying custom multiplexing methods of indexed primers (51). To reduce the chances of carryover of previous molecules, contamination-aware indexes, i.e., different indexes across runs (e.g., runs ONT2 and ONT2a in this study), could be used.

Technical and analytical issues. Although several authors have reported species-level identification of bacterial taxa in complex communities using MinION (35, 52), these interpretations are not convincing, because mapping of sequences with 10 to 25% errors to reference reads of high similarity is inaccurate (described above). We demonstrate that even when using the relatively rapidly evolving fungal ITS region and reference sequences differing from each other by at least 2%, positive-control samples and plant material yield multiple MOTUs, sometimes recovering strongest hits to different species or genera. Conversely, species absent from databases may be mapped to one or more closely related species, which may provide incorrect taxonomic implications. This is of particular importance in molecular diagnostics, necessitating inclusion of marker genes of all potential target species in the reference database to prevent incorrect interpretation. The metagenomics approach requires a comprehensive database of genomes of all potential target organisms, which strongly depends on whole-genome sequencing initiatives. Exome compilations are suboptimal, because much of the eukaryotic DNA is noncoding. Besides target organisms, metagenomics databases

also require inclusion of specific interacting taxa (e.g., *S. tuberosum*), contaminants (*Homo sapiens* and species propagated in laboratories), and various bacteria that contribute much to the metagenomic DNA.

A major concern with novel sequencing techniques is the paucity of reports on analytical shortfalls and the nature of artifacts, which may partly be derived from the lack of controls or inappropriate sampling design (53). The MinION instrument has been used for 5 years, but so far there is limited information about analytical errors and biases, and a few authors mention checking chimeras, index-switching artifacts, or unsuccessful runs (however, see references 40, 54, and 55). It is important to raise the awareness of the scientific community about the limitations and potential issues when choosing among analytical methods and interpreting data.

Perspectives of third-generation sequencing technologies. Both Oxford Nanopore and PacBio sequencing platforms are rapidly evolving in terms of read length and base-calling accuracy. At the moment, Sequel seems to be the best choice for metabarcoding of regular-size (600 to 1,000 bp) and long (up to 4.5 kb) amplicons (6, 7, 12, 56–58) and for barcoding using ultralong markers (up to 7 kb) (59). The recently released M8 chemistry has reduced the per read cost of PacBio sequencing roughly threefold. Declining costs and greater throughput, read length, and quality continue to promote Sequel for metabarcoding, metagenomics, and metatranscriptomics (https://www.pacb.com/videos/webinar-sequence-with-confidence-introducing-the-sequel-ii-system/). Although Sequel enables reliable separation of species at 99% sequence similarity based on the full-length ITS region, it may be hard to convince users of Illumina MiSeq to switch to another platform and adopt alternative bioinformatics work flows.

Use of MinION for metabarcoding offers little promise considering the current state-of-the-art technology, with unacceptably high error rates. The error rates should be reduced to $<$0.1%, i.e., to the level of Illumina instruments and circular consensus of Sequel for use in routine biodiversity assessments. Several methods of tandem repeat (concatemer) sequencing enable the reduction of error rates to 1 to 3% (27–29). Double barcoding of each size-selected RNA or DNA molecule followed by generation of consensus sequences yields quality improvements comparable to tandem repeat sequencing (60), but it would require ultrahigh sequencing depth to reach a 1% error rate and to be able to multiplex over several biological samples. Combining these methods may facilitate reducing error rates toward the critical threshold but also adds to time and cost of analysis.

For regular barcoding, the third-generation sequencing tools offer great promise in situations where their throughput and read length are much superior to double-pass Sanger sequencing, i.e., for barcodes of $>$1,000 bases and multiplexing hundreds of samples to secure cost efficiency (16, 51). Sanger sequencing handles poorly the alleles or copies of markers with read length polymorphism, which is common in noncoding regions of eukaryotes. The third-generation HTS technologies are able to recover various alleles as well as pseudogenes (26) from mixed or contaminated samples by sequencing single DNA molecules. Both Sequel and MinION are capable of handling DNA amplicons of $>$7,000 bases, requiring generation of consensus reads for reliable results (59). Although we could reach up to 99.5% accuracy with $>$100 MinION reads, 100 reads were considered sufficient for generating principally error-free barcodes for animals using the $1D^2$ approach (30). For PacBio instruments, a single read may be enough for reads of around 2,000 bases, but three or more may be needed for longer fragments and to average over PCR errors (58).

Unlike Sequel and other HTS technologies, MinION is well suited to rapid diagnostics of pathogens and invasive species, especially in groups that are well-known and well referenced in public sequence databases. Besides detection of pathogenic species, MinION has the potential to recover antibiotic resistance genes and pathogenesis-related genes as well as single mutations in the metagenomics mode (26, 61). By using multiplex amplicons or a metagenomics/genomics approach, it will be possible to

detect harmful organisms and their specific pathogenicity-related genomic features in less than 1 day (36). Besides enabling users to trace taxon/gene exchange between different habitats (62), this approach has important implications for improving diagnosis and implementing countermeasures, e.g., releasing biocontrol agents, spraying biocides, or arranging quarantine.

Nanopore-based detection methods are flexible for incorporating additional options, such as recording epigenetic modifications (11, 63) and primary structure of RNA (64), proteins (65), and polysaccharides (66). Alternative nanopore-based DNA sequencing methods are also evolving (67). The potential of different nanopores to record various biomolecules indicates great promise of nanopore-based molecular diagnostics in the future.

Conclusions. We demonstrate that the MinION device is well suited for rapid PCR-free diagnosis of fungal and oomycete pathogens and other eukaryotic organisms, which may take as little as 150 min from sample preparation (including DNA extraction, library preparation, sequencing, and bioinformatics) to data interpretation. However, care should be taken to secure profound reference sequence data to avoid misdiagnosis. Amplicon-based diagnostics take longer but are more accurate if genomes of potential pathogens, hosts, or other associated organisms are unavailable. For whole-community metabarcoding, Sequel performs much better than MinION in terms of data quality and analytical biases. Although tandem repeat sequencing and read consensus sequencing have been developed for MinION, their error rate of 1 to 3% is still insufficient for exploratory metabarcoding analyses of biodiversity, but this may change in the coming years.

MATERIALS AND METHODS

Sample preparation. The *S. tuberosum* subset includes 27 samples of leaves and 10 samples of tubers with symptoms of disease (Table 6). We also included a DNA sample of two Australian Tuberaceae (Ascomycota) species as a positive control. The Pinaceae species subset includes 36 distinct needle samples from eight species of *Pinus* and two species of *Picea* that represent material with symptoms of needle blight or no symptoms. The foliar samples of natural, planted, or recently imported trees were collected in Estonia in 2011 to 2018 (Table 7). We included a cultured isolate of *D. pini* (146889), *D. septosporum* (150931), and the closely related *Lecanosticta acicola* (150943; all Ascomycota) as positive controls. Unequal mixtures of DNA from these cultures served as a simple mock community. In both subsets, we included a negative-control sample.

In Pinaceae species samples, DNA from 0.2 g plant material and fungal cultures was extracted using the GeneJET genomic DNA purification kit (Thermo Fisher Scientific, Waltham, MA, USA). In *S. tuberosum* samples, DNA from 0.1 g diseased fresh leaf tissue was extracted using a lysis buffer [0.8 M Tris-HCl, 0.2 M $(NH_4)_2SO_4$, 0.2% (wt/vol) Tween 20; Solis BioDyne, Tartu, Estonia].

Molecular identification. Pinaceae species samples were screened for specific pathogenic fungi *Dothiostroma pini*, *D. septosporum*, and *Lecanosticta acicola* using species-specific primer pairs by following the developer's protocols (68). Samples from potato and cultured needle fungi were amplified using the ITS1F + ITS4 primer pair for Fungi (69, 70). The potato samples were also amplified for oomycetes using the ITS1Oo + ITS4 primer pair (71, 72). For sequencing of fungi and oomycetes, ITS5 (69) and ITS1Oo primers, respectively, were used. Some amplicons were resequenced using primers ITS2, ITS3, and ITS4 (69).

For the metabarcoding approach, we used forward primer ITS1catta (5′-ACCWGCGGARGGATCATT A-3′) (73) and reverse primer ITS4ngsUni (74) to be able to selectively amplify fungal DNA and simultaneously avoid the 18S rRNA gene intron bias. Located in the terminus of the 18S rRNA gene, the ITS1catta primer covers nearly all Ascomycota and Basidiomycota as well as selected groups of zygomycetes and early diverging lineages but discriminates against plants and most other eukaryote groups (including fungal taxa Mortierellomycota and Tulasnellaceae) in the last 3′ position. For Oomycota, we used the ITS1Oo primer in combination with the ITS4ngsUni primer for the potato data set in parallel. Forward primers were tagged with one of the 12-base Golay indexes with at least four differences from any other index (12). Because of issues in data recovery, we also amplified a subset of eight *S. tuberosum* samples (KL001 to KL008) using ITS1catta and ITS4ngsUni primers in which the forward primer was equipped with a tandem repeat barcode of double length (securing at least an 8-base difference) to increase resolution among samples. Because the $1D^2$ nanopore sequencing method requires DNA fragments of >3 kb, we amplified these *S. tuberosum* samples (roughly 3.2-kb amplicons) using the indexed ITS1catta primer combined with the LR14 primer (75). For comparing the metabarcoding approach to the metagenomics method, we used ITS1catta in combination with the LR11 primer (75), which yielded stronger amplicons than LR14. We used negative and positive controls as described above.

PCR was performed in a 25-μl volume comprising 0.5 μl each of the tagged primer (20 μM), 5 μl HOT FIREPol blend master mix (Solis Biodyne), 1 μl DNA extract, and 18 μl double-distilled H_2O. Thermocycling conditions included an initial 15-min denaturation at 95°C, 30 cycles of 30 s of denaturation, 30 s of annealing

TABLE 6 Details of needle samples collected in Estonia

Sample ID	Collection locality	Collection date (day.mo.yr)	Host	Substrate[b]	Disease symptom
115	Tallinn Botanic Garden	17.11.2011	*Pinus sylvestris*	Needle	*Dothistroma*-like
117	Tallinn Botanic Garden	17.11.2011	*P. sylvestris*	Needle	*Dothistroma*-like
118	Pirita	17.11.2011	*P. mugo*	Needle	*Dothistroma*-like
119	Tallinn Botanic Garden	17.11.2011	*P. sylvestris*	Needle	*Dothistroma*-like
123	Tallinn Botanic Garden	17.11.2011	*P. uncinata*	Needle	*Dothistroma*-like
125	Tallinn Botanic Garden	17.11.2011	*P. rigida*	Needle	*Dothistroma*-like
127	Tallinn Botanic Garden	17.11.2011	*P. contorta*	Needle	*Dothistroma*-like
139	Tallinn Botanic Garden	15.08.2011	*P. x rotundata*	Needle	*Dothistroma*-like
141	Tallinn Botanic Garden	15.08.2011	*P. mugo*	Needle	*Dothistroma*-like
142	Tallinn Botanic Garden	15.08.2011	*P. x rotundata*	Needle	*Dothistroma*-like
148	Tallinn Botanic Garden	15.09.2011	*P. mugo* var. *pumilio*	Needle	*Dothistroma*-like
154	Tallinn Botanic Garden	15.09.2011	*P. rhaetica*	Needle	*Dothistroma*-like
2404	Petrivka, The Ukraine	10.09.2013	*P. nigra* subsp. *pallasiana*	Living culture: DoPi	NA
3904	Kärevere	20.01.2015	*P. mugo*	Living culture: LeAc	NA
3906	Kärevere	20.01.2015	*P. mugo*	Living culture: DoSe	NA
4154	NA[a]	09.10.2014	NA	Mock: DoPi, DoSe, LeAc	NA
4162	Levala	09.10.2014	*P. sylvestris*	Needle	*Dothistroma*-like
4180	Kolli	13.10.2014	*P. sylvestris*	Needle	*Dothistroma*-like
4181	Mustumetsa	13.10.2014	*P. sylvestris*	Needle	*Dothistroma*-like
4192	Soohara	07.10.2014	*P. sylvestris*	Needle	*Dothistroma*-like
4194	Värska	07.10.2014	*P. sylvestris*	Needle	*Dothistroma*-like
4195	Vastse-Kuuste	07.10.2014	*P. mugo*	Needle	*Dothistroma*-like
4197	Partsi	07.10.2014	*P. sylvestris*	Needle	*Dothistroma*-like
4220	Sääre	15.10.2014	*P. sylvestris*	Needle	*Dothistroma*-like
4221	Unimäe	15.10.2014	*P. sylvestris*	Needle	*Dothistroma*-like
4222	Tori	16.10.2014	*P. mugo*	Needle	*Dothistroma*-like
4223	Tori	16.10.2014	*P. mugo*	Needle	*Dothistroma*-like
5136	Imported: Netherlands	03.11.2015	*P. mugo* var. *pumilio*	Needle	Asymptomatic
5137	Imported: Netherlands	17.12.2015	*Picea omorika*	Needle	Asymptomatic
5146	Imported: Netherlands	03.11.2015	*P. mugo*	Needle	Asymptomatic
5148	Imported: Netherlands	03.11.2015	*P. mugo*	Needle	Asymptomatic
5151	Imported: Netherlands	03.11.2015	*P. sylvestris*	Needle	Asymptomatic
5186	Imported: Netherlands	26.10.2015	*P. peuce*	Needle	Asymptomatic
5194	Imported: Netherlands	26.10.2015	*P. koraiensis*	Needle	Asymptomatic
5195	Imported: Netherlands	26.10.2015	*P. mugo*	Needle	Asymptomatic
5297	Imported: Germany	17.12.2015	*Picea pungens*	Needle	Asymptomatic
5307	Imported: Germany	17.12.2015	*Picea omorika*	Needle	Asymptomatic
14374	Agali	16.02.2018	*P. sylvestris*	Needle	*Lecanosticta*-like
14378	Agali	16.02.2018	*P. mugo*	Needle	*Lecanosticta*-like

[a]NA, not applicable.
[b]Abbreviations for species: DoPi, *Dothiostroma pini*; DoSe, *D. septosporum*; LeAc, *Lecanosticta acicola*.

at 55°C, and 60 s of elongation at 72°C, with a final 10-min elongation before hold at 4°C. The number of cycles was increased to 35 or 38 for some samples to yield a visible amplicon on 1% agarose gel. For the ITS1Oo + ITS4ngsUni primer combination, 40 PCR cycles at 50°C annealing were used to secure greater product recovery. The two replicate amplicons were pooled, checked on a gel, and mixed with amplicons of other samples in roughly equal proportions based on visual estimates of band size.

Third-generation sequencing. The mixed amplicons of *S. tuberosum* and those of Pinaceae species needles were separately split into library preparation for Sequel and MinION. The two PacBio libraries were sequenced on a Sequel instrument using the same SMRT cell (SMRT cell 1M, v2, Sequel Polymerase v2.1, sequencing chemistry v2.1, loading by diffusion, movie time of 600 min, preextension time of 30 min). The PacBio CCS reads (minPasses = 3, MinAccuracy = 0.9) were generated using SMRT Link v 6.0.0.47841 (Pacific Biosciences). Subsequent bioinformatics were performed using PipeCraft 1.0 (76), which included steps of demultiplexing (2 mismatches to primer and 1 mismatch to tag), extraction of the ITS region (ITSx: default options) (77), chimera removal (UCHIME: *de novo* and reference-based methods combined) (78), additional filtering option "remove primer artifacts," which removes chimeric sequences where the full-length primer is found somewhere in the middle of the read, clustering (UPARSE: 98% sequence similarity threshold) (79), and taxonomic identification (BLASTn: E value = 0.001, word size = 7, reward = 1, penalty = −1, gap open = 1, gap extend = 2;) (80) against the UNITE 7.2 (45) reference database. We used the criteria of a BLAST E value of $<e^{-40}$ and sequence similarity of >75% for the kingdom-level identification and E value of $<e^{-50}$ for phylum- and class-level identification.

For the MinION instrument, amplicon library preparation was performed using the 1D amplicon/cDNA by a ligation (SQK-LSK109) kit (ONT) using R9.4 flow cells by following the manufacturer's instructions. For long fragments, we also used the $1D^2$ sequencing of genomic DNA (SQK-LSK308) kit on an R9.5 flow cell by following the producer's protocols (run ONT2f). Flow cells were used 1 to 3 times,

TABLE 7 Details of *S. tuberosum* and *C. sativa* samples collected in Estonia

Sample ID	Collection locality	Collection date (day.mo.yr)	Cultivar[a]	Substrate	Disease symptom[b]
KL001	Õssu	02.08.2017	St Ants	Leaf	DCL
KL002	Õssu	02.08.2017	St Ants	Leaf	DCL
KL003	Õssu	02.08.2017	St Ants	Leaf	DCL
KL004	Õssu	02.08.2017	St Ants	Leaf	DCL
KL005	Õssu	02.08.2017	St Sarpo Mira	Leaf	DCL
KL006	Õssu	02.08.2017	St Sarpo Mira	Leaf	DCL
KL007	Õssu	02.08.2017	St Sarpo Mira	Leaf	DCL
KL008	Õssu	02.08.2017	St Sarpo Mira	Leaf	DCL
KL009	Õssu	02.08.2017	St Toluca	Leaf	DCL
KL010	Õssu	02.08.2017	St Toluca	Leaf	DCL
KL011	Õssu	02.08.2017	St Toluca	Leaf	DCL
KL012	Õssu	02.08.2017	St Toluca	Leaf	DCL
KL013	Õssu	02.08.2017	St Toluca	Leaf	DCL
KL014	Õssu	02.08.2017	St Toluca	Leaf	DCL
KL015	Õssu	02.08.2017	St Toluca	Leaf	DCL
KL016	Õssu	02.08.2017	St Toluca	Leaf	DCL
KL017	Õssu	02.08.2017	St Kelly	Leaf	DCL
KL018	Õssu	02.08.2017	St Kelly	Leaf	DCL
KL019	Õssu	02.08.2017	St Kelly	Leaf	DCL
KL020	Õssu	02.08.2017	St Kelly	Leaf	DCL
KL021	Karala	02.08.2017	St unknown	Leaf	DCL
KL022	Karala	02.08.2017	St unknown	Leaf	DCL
KL023	Karala	02.08.2017	St unknown	Leaf	DCL
KL024	Karala	02.08.2017	St unknown	Leaf	DCL
KL025	Metsaküla	12.08.2017	St unknown	Leaf	DCL
KL026	Metsaküla	12.08.2017	St unknown	Leaf	DCL
KL027	Metsaküla	12.08.2017	St unknown	Leaf	DCL
KL028	Väljataguse	11.04.2018	St Elfe	Tuber	Potato gangrene
KL029	Väljataguse	11.04.2018	St Elfe	Tuber	Potato gangrene
KL030	Väljataguse	11.04.2018	St Elfe	Tuber	Potato gangrene
KL031	Õssu	11.04.2018	St Laura	Tuber	Potato gangrene
KL032	Suur-Rahula	11.04.2018	St Gala	Tuber	Potato gangrene
KL033	Tagaküla	11.04.2018	St Laura	Tuber	Potato gangrene
KL034	Tagaküla	11.04.2018	St Laura	Tuber	Potato gangrene
KL035	Padise	11.04.2018	St Marabel	Tuber	Potato gangrene
KL036[c]	Õssu	30.08.2018	St Carolus	Tuber	*Rhizoctonia*-like
KL037[d]	Õssu	30.08.2018	St Carolus	Tuber	*Rhizoctonia*-like
KL038[d]	Tartu	16.05.2019	St Carolus	Leaf	DCL
KL039[d]	Eistvere	20.05.2019	Cs Carambole	Roots	Wilted
KL040[d]	Luunja	22.05.2019	Cs Petrifin	Leaf	White powdery spots
KL041[d]	Roiu	30.05.2019	St Flavia	Stem	*Rhizoctonia*-like

[a]St, *Solanum tuberosum*; Cs, *Cucumis sativa*.
[b]DCL, dark circular lesions.
[c]Used only for the ONT2g and ONT2h runs.
[d]Used only for the rapid identification run.

being cleaned once or twice using the wash kit (EXP-WSH002; ONT). Sequencing was performed in the laboratory at room temperature, connecting the MinION device to a plugged-in, Internet-connected laptop computer with four processors and 32 GB RAM. For base calling in MinKnow 3.1.19 software (ONT), we used the default Phred score of 11, which placed the reads into passed and failed bins. The passed FASTA-formatted reads (additionally failed reads in runs ONT2g and ONT2h) were further subjected to bioinformatics analysis using PipeCraft. The options in PipeCraft included demultiplexing of metabarcoding reads allowing no mismatches to the barcode, followed by BLASTn search using default settings. The sequencing adaptors were removed by a custom script.

Given the poor overall sequence quality, traditional MOTU-based approaches are not applicable to the MinION data; therefore, we mapped reads based on their best matches to database sequences in the UNITE reference database by following the principles of previous nanopore sequencing studies (33, 35). Limitations of this approach are outlined in Discussion.

The rate of index switching was calculated based on the average of the distribution of MOTUs corresponding to the positive-control sample in experimental samples and the distribution of other MOTUs in the positive-control sample relative to the overall amount of sequences in positive-control samples (12). It assumes that reads belonging to different MOTUs and samples exhibit equal probability of switching indexes. We also assessed potential index switching in the putatively chimeric sequences by manual BLAST searches of these sequences against the INSD by following Hyde et al. (4). Chimeric reads that had one part belonging to a parent not found in the particular sample were considered cross-sample chimeras.

To evaluate the relative efficiency of metagenomics approaches on pathogen recovery, we used DNA from the *S. tuberosum* tuber sample KL036 (run ONT2g compared to the amplicon run ONT2h from the same sample) (Table 1). For library preparation, the rapid sequencing kit (SQK-RAD004; ONT) was used by following the manufacturer's instructions. Base calling was performed as described above. Both passed and failed sequences were used in further bioinformatics analyses as implemented in PipeCraft. We relied on the entire INSD as a metagenomics reference database.

Rapid pathogen diagnostics. We further elaborated on the metagenomics approach to maximize the speed of pathogen diagnosis using the MinION instrument. For ONT2i, ONT2j, ONT2k, ONT2l, ONT2m, and ONT2n runs (Table 1), we used ca. 20 mg of infected tissue of *S. tuberosum* or *Cucumis sativa* samples and optimized rapid extraction protocols (Table 5). We used ca. 20 mg fresh material for extraction in two technical replicates in 2-ml Eppendorf tubes that contained either 100 μl Phire plant direct PCR kit lysis buffer (Thermo Fisher Scentific) or 500 μl $(NH_4)_2SO_4$-based lysis buffer [0.8 M Tris-HCl, 0.2 M $(NH_4)_2SO_4$, 0.2% (wt/vol) Tween 20; Solis BioDyne]. The Phire lysis protocol was shortened by reducing the step of lysis to 2 to 5 min, preceded by tissue disruption using bead beating (5 min at 30 Hz) or a mortar and pestle in liquid N_2, followed by brief centrifugation at 5,000 $\times$ *g*, incubation at 30°C for 5 min, and a final centrifugation at 11,000 $\times$ *g* for 1 min. The $(NH_4)_2SO_4$ lysis included incubation time reduced to 5 min, followed by incubation at 98°C for 2 min. The DNA was concentrated from lysate using the FavorPrep gel/PCR purification kit (Favorgen Biotech Corp., Vienna, Austria) by following the manufacturer's instructions, except including centrifugation steps for 1 min and final elution using 50 μl water. Some samples were further subjected to Agencourt AMPure XP bead purification (Beckman Coulter, East Windsor, NJ, USA) to remove DNA fragments of <500 bases. DNA concentration was measured using QUBIT. For library preparation, we used the SQK-RAD004 kit and followed the manufacturer's protocols, except with shortened fragmentation time at 30°C of 30 s. MinION runs were performed on R9.4 flow cells, and the data were downloaded after obtaining sufficient amounts of sequences (Table 5). The runs were interrupted in 1 to 2 h (Table 1). Only the passed sequences were used in further bioinformatics analyses using PipeCraft by following the options for the ONT2g metagenomic run.

Data availability. Sanger sequences of potato samples have been deposited in the UNITE database (https://unite.ut.ee/) and INSD databases (GenBank accession numbers MN065746 to MN065768). Representative Sequel sequences of 99% similarity consensus were generated using PipeCraft and uploaded to the UNITE database, which is the only repository that accepts quality-filtered HTS reads. Raw sequence data of MinION and Sequel are available from the PlutoF digital repository and the Sequence Read Archive (PRJNA545967), respectively. Sample-by-MOTU tables used in these analyses can be accessed from the PlutoF digital repository (https://plutof.ut.ee/#/filerepository/view/3431905).

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REFERENCES

1. Comtet T, Sandionigi A, Viard F, Casiraghi M. 2015. DNA (meta)barcoding of biological invasions: a powerful tool to elucidate invasion processes and help managing aliens. Biol Invasions 17:905–922. https://doi.org/10.1007/s10530-015-0854-y.
2. Kashyap PL, Rai P, Kumar S, Chakdar H, Srivastava AK. 2017. DNA barcoding for diagnosis and monitoring of fungal plant pathogens, p 87–122. *In* Singh BP, Gupta VK (ed), Molecular markers in mycology. Springer-Verlag, New York, NY.
3. Grosdidier M, Aguayo J, Marçais B, Ioos R. 2017. Detection of plant pathogens using real-time PCR: how reliable are late Ct values? Plant Pathol 66:359–367. https://doi.org/10.1111/ppa.12591.
4. Hyde KD, Udayanga D, Manamgoda DS, Tedersoo L, Larsson E, Abarenkov K, Bertrand Y, Oxelman B, Hartmann M, Kauserud H, Ryberg M, Kristiansson E, Nilsson RH. 2013. Incorporating molecular data in fungal systematics: a guide for aspiring researchers. Curr Res Environ Appl Microbiol 3:1–32. https://doi.org/10.5943/cream/3/1/1.
5. Bik HM, Porazinska D, Creer S, Caporaso JG, Knight R, Thomas WK. 2012. Sequencing our way towards understanding global eukaryotic biodiversity. Trends Ecol Evol 27:233–243. https://doi.org/10.1016/j.tree.2011.11.010.
6. Nilsson RH, Anslan S, Bahram M, Wurzbacher C, Baldrian P, Tedersoo L. 2019. Mycobiome diversity: high-throughput sequencing and identification of fungi. Nat Rev Microbiol 17:95–109. https://doi.org/10.1038/s41579-018-0116-y.
7. Tedersoo L, Drenkhan R, Anslan S, Morales-Rodriguez C, Cleary M. 2019. High-throughput identification and diagnostics of pathogens and pests: overview and practical recommendations. Mol Ecol Resour 19:47–76. https://doi.org/10.1111/1755-0998.12959.
8. Mosher JJ, Bowman B, Bernberg EL, Shevchenko O, Kan J, Korlach J, Kaplan LA. 2014. Improved performance of the PacBio SMRT technology for 16S rDNA sequencing. J Microbiol Methods 104:59–60. https://doi.org/10.1016/j.mimet.2014.06.012.
9. Schloss PD, Jenior ML, Koumpouras CC, Westcott SL, Highlander SK. 2016. Sequencing 16S rRNA gene fragments using the PacBio SMRT DNA sequencing system. Peer J 4:e1869. https://doi.org/10.7717/peerj.1869.
10. Weirather JL, de Cesare M, Wang Y, Piazza P, Sebastiano V, Wang X-J, Buck D, Au KF. 2017. Comprehensive comparison of Pacific Biosciences and Oxford Nanopore Technologies and their applications to transcriptome analysis. F1000Res 6:100. https://doi.org/10.12688/f1000research.10571.2.
11. Jain M, Koren S, Miga KH, Quick J, Rand AC, Sasani TA, Tyson JR, Beggs AD, Dilthey AT, Fiddes IT, Malla S, Marriott H, Nieto T, O'Grady J, Olsen HE, Pedersen BS, Rhie A, Richardson H, Quinlan AR, Snutch TP, Tee L, Paten B, Phillippy AM, Simpson JT, Loman NJ, Loose M. 2018. Nanopore sequencing and assembly of a human genome with ultra-long reads. Nat Biotechnol 36:338. https://doi.org/10.1038/nbt.4060.
12. Tedersoo L, Tooming-Klunderud A, Anslan S. 2018. PacBio metabarcoding of fungi and other eukaryotes: biases and perspectives. New Phytol 217:1370–1385. https://doi.org/10.1111/nph.14776.
13. Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, Peluso P, Rank D, Baybayan P, Bettman B, Bibillo A, Bjornson K, Chaudhuri B, Christians F, Cicero R, Clark S, Dalal R, Dewinter A, Dixon J, Foquet M, Gaertner A, Hardenbol P,

Heiner C, Hester K, Holden D, Kearns G, Kong X, Kuse R, Lacroix Y, Lin S, Lundquist P, Ma C, Marks P, Maxham M, Murphy D, Park I, Pham T, Phillips M, Roy J, Sebra R, Shen G, Sorenson J, Tomaney A, Travers K, Trulson M, Vieceli J, Wegener J, Wu D, Yang A, Zaccarin D, Zhao P, Zhong F, Korlach J, Turner S. 2009. Real-time DNA sequencing from single polymerase molecules. Science 323:133–138. https://doi.org/10.1126/science.1162986.

14. Rhoads A, Au KF. 2015. PacBio sequencing and its applications. Genomics Proteomics Bioinformatics 13:278–289. https://doi.org/10.1016/j.gpb.2015.08.002.
15. Giordano F, Aigrain L, Quail MA, Coupland P, Bonfield JK, Davies RM, Tischler G, Jackson DK, Keane TM, Li J, Yue J-X, Liti G, Durbin R, Ning Z. 2017. *De novo* yeast genome assemblies from MinION, PacBio and MiSeq platforms. Sci Rep 7:3935. https://doi.org/10.1038/s41598-017-03996-z.
16. Hebert PDN, Braukmann TWA, Prosser SWJ, Ratnasingham S, deWaard JR, Ivanova NV, Janzen DH, Hallwachs W, Naik S, Sones JE, Zakharov EV. 2018. A Sequel to Sanger: amplicon sequencing that scales. BMC Genomics 19:219. https://doi.org/10.1186/s12864-018-4611-3.
17. Singer E, Bushnell B, Coleman-Derr D, Bowman B, Bowers RM, Levy A, Gies EA, Cheng J-F, Copeland A, Klenk H-P, Hallam SJ, Hugenholtz P, Tringe SG, Woyke T. 2016. High-resolution phylogenetic microbial community profiling. ISME J 10:2020–2032. https://doi.org/10.1038/ismej.2015.249.
18. Wagner J, Coupland P, Browne HP, Lawley TD, Francis SC, Parkhill J. 2016. Evaluation of PacBio sequencing for full-length bacterial 16S rRNA gene classification. BMC Microbiol 16:274. https://doi.org/10.1186/s12866-016-0891-4.
19. Walder F, Schlaeppi K, Wittwer R, Held AY, Vogelgsang S, van der Heijden MG. 2017. Community profiling of Fusarium in combination with other plant-associated fungi in different crop species using SMRT sequencing. Front Plant Sci 8:2019. https://doi.org/10.3389/fpls.2017.02019.
20. Mikheyev AS, Tin M. 2014. A first look at the Oxford Nanopore MinION sequencer. Mol Ecol Resour 14:1097–1102. https://doi.org/10.1111/1755-0998.12324.
21. Lu H, Giordano F, Ning Z. 2016. Oxford Nanopore MinION sequencing and genome assembly. Genomics Proteomics Bioinformatics 14:265–279. https://doi.org/10.1016/j.gpb.2016.05.004.
22. Quick J, Loman NJ, Duraffour S, Simpson JT, Severi E, Cowley L, Bore JA, Koundouno R, Dudas G, Mikhail A, Ouédraogo N, Afrough B, Bah A, Baum JHJ, Becker-Ziaja B, Boettcher JP, Cabeza-Cabrerizo M, Camino-Sánchez Á, Carter LL, Doerrbecker J, Enkirch T, Dorival IG, Hetzelt N, Hinzmann J, Holm T, Kafetzopoulou LE, Koropogui M, Kosgey A, Kuisma E, Logue CH, Mazzarelli A, Meisel S, Mertens M, Michel J, Ngabo D, Nitzsche K, Pallasch E, Patrono LV, Portmann J, Repits JG, Rickett NY, Sachse A, Singethan K, Vitoriano I, Yemanaberhan RL, Zekeng EG, Racine T, Bello A, Sall AA, Faye O, Faye O, Magassouba N, Williams CV, Amburgey V, Winona L, Davis E, Gerlach J, Washington F, Monteil V, Jourdain M, Bererd M, Camara A, Somlare H, Camara A, Gerard M, Bado G, Baillet B, Delaune D, Nebie KY, Diarra A, Savane Y, Pallawo RB, Gutierrez GJ, Milhano N, Roger I, Williams CJ, Yattara F, Lewandowski K, Taylor J, Rachwal P, J Turner D, Pollakis G, Hiscox JA, Matthews DA, Shea MKO, Johnston AM, Wilson D, Hutley E, Smit E, Di Caro A, Wölfel R, Stoecker K, Fleischmann E, Gabriel M, Weller SA, Koivogui L, Diallo B, Keïta S, Rambaut A, Formenty P, Günther S, Carroll MW. 2016. Real-time, portable genome sequencing for Ebola surveillance. Nature 530:228–232. https://doi.org/10.1038/nature16996.
23. Johnson SS, Zaikova E, Goerlitz DS, Bai Y, Tighe SW. 2017. Real-time DNA sequencing in the Antarctic dry valleys using the Oxford Nanopore sequencer. J Biomol Tech 28:2. https://doi.org/10.7171/jbt.17-2801-009.
24. Castro-Wallace SL, Chiu CY, John KK, Stahl SE, Rubins KH, McIntyre ABR, Dworkin JP, Lupisella ML, Smith DJ, Botkin DJ, Stephenson TA, Juul S, Turner DJ, Izquierdo F, Federman S, Stryke D, Somasekar S, Alexander N, Yu G, Mason CE, Burton AS. 2017. Nanopore DNA sequencing and genome assembly on the International Space Station. Sci Rep 7:18022. https://doi.org/10.1038/s41598-017-18364-0.
25. Quick J, Quinlan AR, Loman NJ. 2014. A reference bacterial genome dataset generated on the MinION portable single-molecule nanopore sequencer. GigaSci 3:22. https://doi.org/10.1186/2047-217X-3-22.
26. Cornelis S, Gansemans Y, Vander Plaetsen AS, Weymaere J, Willems S, Deforce D, Van Nieuwerburgh F. 2019. Forensic tri-allelic SNP genotyping using nanopore sequencing. Forensic Sci Int Genet 38:204–210. https://doi.org/10.1016/j.fsigen.2018.11.012.
27. Li C, Chng KR, Boey EJ, Ng AH, Wilm A, Nagarajan N. 2016. INC-Seq: accurate single molecule reads using nanopore sequencing. Gigascience 5:34. https://doi.org/10.1186/s13742-016-0140-7.
28. Calus ST, Ijaz UZ, Pinto AJ. 2018. NanoAmpli-Seq: a workflow for amplicon sequencing for mixed microbial communities on the nanopore sequencing platform. Gigascience 7:140. https://doi.org/10.1093/gigascience/giy140.
29. Volden R, Palmer T, Byrne A, Cole C, Schmitz RJ, Green RE, Vollmers C. 2018. Improving nanopore read accuracy with the R2C2 method enables the sequencing of highly multiplexed full-length single-cell cDNA. Proc Natl Acad Sci U S A 115:9726–9731. https://doi.org/10.1073/pnas.1806447115.
30. Pomerantz A, Peñafiel N, Arteaga A, Bustamante L, Pichardo F, Coloma LA, Barrio-Amorós CL, Salazar-Valenzuela D, Prost S. 2018. Real-time DNA barcoding in a rainforest using nanopore sequencing: opportunities for rapid biodiversity assessments and local capacity building. Gigascience 7:giy033. https://doi.org/10.1093/gigascience/giy033.
31. Kilianski A, Haas JL, Corriveau EJ, Liem AT, Willis KL, Kadavy DR, Rosenzweig CN, Minot SS. 2015. Bacterial and viral identification and differentiation by amplicon sequencing on the MinION nanopore sequencer. Gigascience 4:12. https://doi.org/10.1186/s13742-015-0051-z.
32. Ashikawa S, Tarumoto N, Imai K, Sakai J, Kodana M, Kawamura T, Ikebuchi K, Murakami T, Mitsutake K, Maesaki S, Maeda T. 2018. Rapid identification of pathogens from positive blood culture bottles with the MinION nanopore sequencer. J Med Microbiol 67:1589–1595. https://doi.org/10.1099/jmm.0.000855.
33. Benitez-Paez A, Portune KJ, Sanz Y. 2016. Species-level resolution of 16S rRNA gene amplicons sequenced through the MinION portable nanopore sequencer. Gigascience 5:4. https://doi.org/10.1186/s13742-016-0111-z.
34. Benítez-Páez A, Sanz Y. 2017. Multi-locus and long amplicon sequencing approach to study microbial diversity at species level using the MinION portable nanopore sequencer. Gigascience 6:1–12. https://doi.org/10.1093/gigascience/gix043.
35. Kerkhof LJ, Dillon KP, Häggblom MM, McGuinness LR. 2017. Profiling bacterial communities by MinION sequencing of ribosomal operons. Microbiome 5:116. https://doi.org/10.1186/s40168-017-0336-9.
36. Schmidt K, Mwaigwisya S, Crossman LC, Doumith M, Munroe D, Pires C, Khan AM, Woodford N, Saunders NJ, Wain J, O'Grady J, Livermore DM. 2017. Identification of bacterial pathogens and antimicrobial resistance directly from clinical urines by nanopore-based metagenomic sequencing. J Antimicrob Chemother 72:104–114. https://doi.org/10.1093/jac/dkw397.
37. Votintseva AA, Bradley P, Pankhurst L, del Ojo Elias C, Loose M, Nilgiriwala K, Chatterjee A, Smith EG, Sanderson N, Walker TM, Morgan MR. 2017. Same-day diagnostic and surveillance data for tuberculosis via whole genome sequencing of direct respiratory samples. J Clin Microbiol 55:1285–1298. https://doi.org/10.1128/JCM.02483-16.
38. Bronzato Badial A, Sherman D, Stone A, Gopakumar A, Wilson V, Schneider W, King J. 2018. Nanopore sequencing as a surveillance tool for plant pathogens in plant and insect tissues. Plant Dis 102:1648–1652. https://doi.org/10.1094/PDIS-04-17-0488-RE.
39. Hu Y, Green G, Milgate A, Stone E, Rathjen J, Schwessinger B. 2019. Pathogen detection and microbiome analysis of infected wheat using a portable DNA sequencer. Phytobiomes J 3:92–101. https://doi.org/10.1094/PBIOMES-01-19-0004-R.
40. Cusco A, Catozzi C, Vines J, Sanchez A, Francino O. 2018. Microbiota profiling with long amplicons using nanopore sequencing: full-length 16S rRNA gene and whole rrn operon. F1000Res 7:1755. https://doi.org/10.12688/f1000research.16817.1.
41. Deagle BE, Thomas AC, Shaffer AK, Trites AW, Jarman SN. 2013. Quantifying sequence proportions in a DNA-based diet study using Ion Torrent amplicon sequencing: which counts count? Mol Ecol Resour 13:620–633. https://doi.org/10.1111/1755-0998.12103.
42. Schnell IB, Bohmann K, Gilbert M. 2015. Tag jumps illuminated–reducing sequence-to-sample misidentifications in metabarcoding studies. Mol Ecol Resour 15:1289–1303. https://doi.org/10.1111/1755-0998.12402.
43. Costello M, Fleharty M, Abreu J, Farjoun Y, Ferriera S, Holmes L, Granger B, Green L, Howd T, Mason T, Vicente G, Dasilva M, Brodeur W, DeSmet T, Dodge S, Lennon NJ, Gabriel S. 2018. Characterization and remediation of sample index swaps by non-redundant dual indexing on massively parallel sequencing platforms. BMC Genomics 19:332. https://doi.org/10.1186/s12864-018-4703-0.
44. Barnes I, Nest A, Mullett MS, Crous PW, Drenkhan R, Musolin DL, Wingfield MJ. 2016. Neotypification of *Dothistroma septosporum* and epitypi-

fication of *D. pini*, causal agents of *Dothistroma* needle blight of pine. Forest Pathol 46:388–407. https://doi.org/10.1111/efp.12304.
45. Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, Douglas B, Drenkhan T, Eberhardt U, Dueñas M, Grebenc T, Griffith GW, Hartmann M, Kirk PM, Kohout P, Larsson E, Lindahl BD, Lücking R, Martín MP, Matheny PB, Nguyen NH, Niskanen T, Oja J, Peay KG, Peintner U, Peterson M, Põldmaa K, Saag L, Saar I, Schüßler A, Scott JA, Senés C, Smith ME, Suija A, Taylor DL, Telleria MT, Weiss M, Larsson K-H. 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol Ecol 22:5271–5277. https://doi.org/10.1111/mec.12481.
46. Charalampous T, Richardson H, Kay GL, Baldan R, Jeanes C, Rae D, Grundy S, Turner DJ, Wain J, Leggett RM, Livermore DM. 2018. Rapid diagnosis of lower respiratory infection using nanopore-based clinical metagenomics. BioRxiv https://doi.org/10.1101/387548.
47. Tomlinson JA, Dickinson MJ, Boonham N. 2010. Rapid detection of Phytophthora ramorum and P. kernoviae by two-minute DNA extraction followed by isothermal amplification and amplicon detection by generic lateral flow device. Phytopathology 100:143–149. https://doi.org/10.1094/PHYTO-100-2-0143.
48. Notomi T, Mori Y, Tomita N, Kanda H. 2015. Loop-mediated isothermal amplification (LAMP): principle, features, and future prospects. J Microbiol 53:1–5. https://doi.org/10.1007/s12275-015-4656-9.
49. Reuter JA, Spacek DV, Snyder MP. 2015. High-throughput sequencing technologies. Mol Cell 58:586–597. https://doi.org/10.1016/j.molcel.2015.05.004.
50. Mojarro A, Hachey J, Ruvkun G, Zuber MT, Carr CE. 2018. CarrierSeq: a sequence analysis workflow for low-input nanopore sequencing. BMC Bioinform 19:108. https://doi.org/10.1186/s12859-018-2124-3.
51. Srivathsan A, Hartop E, Puniamoorthy J, Lee WT, Kutty SN, Kurina O, Meier R. 2019. 1D MinION sequencing for large-scale species discovery: 7000 scuttle flies (Diptera: Phoridae) from one site in Kibale National Park (Uganda) revealed to belong to >650 species. bioRxiv https://doi.org/10.1101/622365.
52. Shin J, Lee S, Go MJ, Lee SY, Kim SC, Lee CH, Cho BK. 2016. Analysis of the mouse gut microbiome using full-length 16S rRNA amplicon sequencing. Sci Rep 6:29681. https://doi.org/10.1038/srep29681.
53. Pontefract A, Hachey J, Zuber MT, Ruvkun G, Carr CE. 2018. Sequencing nothing: exploring failure modes of nanopore sensing and implications for life detection. Life Sci Space Res 18:80–86. https://doi.org/10.1016/j.lssr.2018.05.004.
54. White R, Pellefigues C, Ronchese F, Lamiable O, Eccles D. 2017. Investigation of chimeric reads using the MinION. F1000Res 6:631. https://doi.org/10.12688/f1000research.11547.2.
55. Xu X, Stoyanova EI, Lemiesz AE, Xing J, Mash DC, Heintz N. 2018. Species and cell-type properties of classically defined human and rodent neurons and glia. Elife 7:e37551. https://doi.org/10.7554/eLife.37551.
56. Cline LC, Zak DR. 2015. Initial colonization, community assembly and ecosystem function: fungal colonist traits and litter biochemistry mediate decay rate. Mol Ecol 24:5045–5058. https://doi.org/10.1111/mec.13361.
57. Kyaschenko Y, Clemmensen K, Hagenbo A, Karltun E, Lindahl B. 2017. Shift in fungal communities and associated enzyme activities along an age gradient of managed *Pinus sylvestris* stands. ISME J 11:863–874. https://doi.org/10.1038/ismej.2016.184.
58. Jamy M, Foster R, Barbera P, Czech L, Kozlov AM, Stamatakis AM, Bass D, Burki F. 2019. Long metabarcoding of the eukaryotic rDNA operon to phylogenetically and taxonomically resolve environmental diversity. bioRxiv https://doi.org/10.1101/627828.
59. Wurzbacher C, Larsson E, Bengtsson-Palme J, Van den Wyngaert S, Svantesson S, Kristiansson E, Kagami M, Nilsson RH. 2019. Introducing ribosomal tandem repeat barcoding for fungi. Mol Ecol Resour 19:118–127. https://doi.org/10.1111/1755-0998.12944.
60. Karst SM, Dueholm MS, McIlroy SJ, Kirkegaard RH, Nielsen PH, Albertsen M. 2018. Retrieval of a million high-quality, full-length microbial 16S and 18S rRNA gene sequences without primer bias. Nat Biotechnol 36:190–195. https://doi.org/10.1038/nbt.4045.
61. Bradley P, Gordon NC, Walker TM, Dunn L, Heys S, Huang B, Earle S, Pankhurst LJ, Anson L, De Cesare M, Piazza P. 2015. Rapid antibiotic-resistance predictions from genome sequence data for *Staphylococcus aureus* and *Mycobacterium tuberculosis*. Nat Commun 6:10063. https://doi.org/10.1038/ncomms10063.
62. Bahram M, Hildebrand F, Forslund SK, Anderson JL, Soudzilovskaia NA, Bodegom PM, Bengtsson-Palme J, Anslan S, Coelho LP, Harend H, Huerta-Cepas J, Medema MH, Maltz MR, Mundra S, Olsson PA, Pent M, Põlme S, Sunagawa S, Ryberg M, Tedersoo L, Bork P. 2018. Structure and function of the global topsoil microbiome. Nature 560:233–237. https://doi.org/10.1038/s41586-018-0386-6.
63. Simpson JT, Workman RE, Zuzarte PC, David M, Dursi LJ, Timp W. 2017. Detecting DNA cytosine methylation using nanopore sequencing. Nat Methods 14:407. https://doi.org/10.1038/nmeth.4184.
64. Garalde DR, Snell EA, Jachimowicz D, Sipos B, Lloyd JH, Bruce M, Pantic N, Admassu T, James P, Warland A, Jordan M, Ciccone J, Serra S, Keenan J, Martin S, McNeill L, Wallace EJ, Jayasinghe L, Wright C, Blasco J, Young S, Brocklebank D, Juul S, Clarke J, Heron AJ, Turner DJ. 2018. Highly parallel direct RNA sequencing on an array of nanopores. Nat Methods 15:201–208. https://doi.org/10.1038/nmeth.4577.
65. Restrepo-Pérez L, Joo C, Dekker C. 2018. Paving the way to single-molecule protein sequencing. Nat Nanotechnol 13:786. https://doi.org/10.1038/s41565-018-0236-6.
66. Karawdeniya BI, Bandara YN, Nichols JW, Chevalier RB, Dwyer JR. 2018. Surveying silicon nitride nanopores for glycomics and heparin quality assurance. Nat Commun 9:3278. https://doi.org/10.1038/s41467-018-05751-y.
67. Goto Y, Yanagi I, Matsui K, Yokoi T, Takeda KI. 2018. Identification of four single-stranded DNA homopolymers with a solid-state nanopore in alkaline CsCl solution. Nanoscale 10:20844–20850. https://doi.org/10.1039/c8nr04238a.
68. Ioos R, Fabre B, Saurat C, Fourrier C, Frey P, Marçais B. 2010. Development, comparison, and validation of real-time and conventional PCR tools for the detection of the fungal pathogens causing brown spot and red band needle blights of pine. Phytopathology 100:105–114. https://doi.org/10.1094/PHYTO-100-1-0105.
69. White TJ, Bruns TD, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, p 315–322. *In* Innis MA, Gelfand DH (ed), PCR protocols: a guide to methods and applications. Academic Press, London, United Kingdom.
70. Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes–application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x.
71. Riit T, Tedersoo L, Drenkhan R, Runno-Paurson E, Kokko H, Anslan S. 2016. Oomycete-specific ITS primers for identification and metabarcoding. MycoKeys 14:17–30. https://doi.org/10.3897/mycokeys.14.9244.
72. Riit T, Tedersoo L, Drenkhan R, Runno-Paurson E, Kokko H, Anslan S. 2018. Corrigendum for: "Oomycete-specific ITS primers for identification and metabarcoding" published in MycoKeys. MycoKeys 41:119. https://doi.org/10.3897/mycokeys.41.30558.
73. Tedersoo L, Anslan S. 20 June 2019. Towards PacBio-based pan-eukaryote metabarcoding using full-length ITS sequences. Environ Microbiol Rep https://doi.org/10.1111/1758-2229.12776.
74. Tedersoo L, Lindahl B. 2016. Fungal identification biases in microbiome projects. Environ Microbiol Rep 8:774–779. https://doi.org/10.1111/1758-2229.12438.
75. Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. J Bacteriol 172:4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990.
76. Anslan S, Bahram M, Hiiesalu I, Tedersoo L. 2017. PipeCraft: flexible open-source toolkit for bioinformatics analysis of custom high-throughput amplicon sequencing data. Mol Ecol Resour 17:e234–e240. https://doi.org/10.1111/1755-0998.12692.
77. Bengtsson-Palme J, Ryberg M, Hartmann M. 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods Ecol Evol 4:914–919. https://doi.org/10.1111/2041-210X.12073.
78. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200. https://doi.org/10.1093/bioinformatics/btr381.
79. Edgar RC. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10:996–998. https://doi.org/10.1038/nmeth.2604.
80. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST+: architecture and applications. BMC Bioinformatics 10:421. https://doi.org/10.1186/1471-2105-10-421.

CURRICULUM VITAE

First name: Kaire
Surname: Loit
Citizenship: Estonian
Date of Birth: 07.10.1982
Address: Institute of Agricultural and Environmental Sciences
Estonian University of Life Sciences
Kreutzwaldi 5, 51006 Tartu, Estonia
Phone: +372 5664 2423
E-mail: kaire.loit@emu.ee

Education:

2013-2021 Doctoral studies in Plant Pathology, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences

2006-2009 Master's studies in Horticulture, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences

2002-2006 Bachelor's studies in Horticulture, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences

1999-2002 Tartu Art Gymnasium (Secondary Education)

Professional employment:

2019- Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Chair of Plant Health, Senior Specialist

2016-2019 Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Chair of Plant Health, Junior Researcher

Academic degree:

MSc degree in Horticulture for the thesis "Effect of cultivar, foliar fertilization and glycine betaine on the growth of grapevine" (Estonian University of Life Sciences)

Research interests:

Fungal communities inhabiting crop roots and rhizosphere. A broad interest in the discovery of innovations in agriculture, such as biological

control of soil-borne pathogens and the alert system for rapid detection of airborne diseases.

Administrative work:

2020- Estonian Plant Protection Society, secretary

Foreign languages:

English

Training and special courses:

2019 PERMANOVA+ workshop (California State University, Long Beach, California, USA)

2019 Training School "Linking Soil Biodiversity with Soil Organic Matter Dynamics" (COST Action KEYSOM)

2017 NOVA PhD course "Biological control: Microbial Interactions for Improved Plant Health" (Swedish University of Agricultural Sciences)

2016 NOVA PhD course "Chemical and Genomic Insight to Host-Microbe Symbiotic Interactions" (University of Copenhagen)

Projects:

2020-2023 L200018VLST (616219790074) "Effects of Foliar Fungicide Application on forage grasses"

2020-2022 V190186PKAN (862563) "SMART agriculture for innovative vegetable crop PROTECTion: harnessing advanced methodologies and technologies"

2019-2024 V190002PKMD (817819) "Soil biodiversity enhancement in European agroecosystems to promote their stability and resilience by external inputs reduction and crop performance increase"

2019-2023 T190105PKTE "Innovation Cluster Support Measure 2014-2020" (European Agricultural Fund for Rural Development (EAFRD) and the Estonian Rural Development Plan (ERDP))

2016-2019 8T150054PKTK "IPM2.0 for sustainable control of potato late blight – exploiting pathogen population data for optimised Decisions Support Systems"

2016-2017 8P160194PKML "Interaction of arbuscular mycorrhiza and biochar on crop P uptake"

2015-2020	IUT36-2 “Sustainable crop protection: harnessing ecosystem services for plant production”
2012-2015	ETF9432 “Phenotypic and genotypic characterisation of Baltic and Russian Pskov region populations of Phytophthora infestans; the role of oospores as a source of primary inoculum to late blight pathogen epidemiology”

ELULOOKIRJELDUS

Eesnimi: Kaire
Perekonnanimi: Loit
Kodakondsus: Eestlane
Sünniaeg: 07.10.1982
Aadress: Põllumajandus- ja keskkonnainstituut
Eesti Maaülikool
Kreutzwaldi 5, 51006 Tartu, Eesti
Telefon: +372 5664 2423
E-mail: kaire.loit@emu.ee

Hariduskäik

2013-2021 Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, põllumajanduse eriala, doktoriõpe
2006-2009 Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, aianduse eriala, magistriõpe
2002-2006 Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, aianduse eriala, magistriõpe
1999-2002 Tartu Kunstigümnaasium

Teenistuskäik:

2019- Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Taimetervise õppetool, Peaspetsialist
2016-2019 Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Taimetervise õppetool, Nooremteadur

Teaduskraad: Magistrikraad aianduse erialal. Magistritöö: "Viinapuu kasv sõltuvalt sordist, juurevälisest väetamisest ning glütsiin-betaiini kasutamisest"

Teadustöö põhisuund:

Põllukultuuride juuri ja risosfääri asustavate seenekoosluste uurimine, fütopatoloogia

Teadusorganisatsiooniline ja -administratiivne tegevus:

2020- Eesti Taimekaitse Selts, sekretär

Võõrkeelte oskus: Inglise

Täiendkoolitused:

2019	PERMANOVA+ workshop (California State University, Long Beach, California, USA)
2019	Training School "Linking Soil Biodiversity with Soil Organic Matter Dynamics" (COST Action KEYSOM)
2017	NOVA PhD course "Biological control: Microbial Interactions for Improved Plant Health" (Swedish University of Agricultural Sciences)
2016	NOVA PhD course "Chemical and Genomic Insight to Host-Microbe Symbiotic Interactions" (University of Copenhagen)

Projektid:

2020-2023	L200018VLST (616219790074) "Heintaimiku fungitsiididega töötlemise mõju silo mükotoksiinide sisaldusele, fermentatsioonile ning lüpsilehmade jõudlusele, piima kvaliteedile ja ohutusele"
2020-2022	V190186PKAN (862563) "SMART agriculture for innovative vegetable crop PROTECTion: harnessing advanced methodologies and technologies"
2019-2024	V190002PKMD (817819) "Soil biodiversity enhancement in European agroecosystems to promote their stability and resilience by external inputs reduction and crop performance increase"
2019-2023	T190105PKTE (616118790017) "Leping innovatsiooniklastri tegevuste elluviimiseks - PKTE"
2016-2019	8T150054PKTK "Jätkusuutlik kartuli-lehemädaniku tõrje – patogeeni populatsiooni uuringute andmete alusel nõuandesüsteemi optimeerimine"
2016-2017	8P160194PKML "Arbuskulaarse mükoriisa ja biosöe koosmõju põllukultuuride P omastamisele"
2015-2020	IUT36-2 "Jätkusuutlik taimekaitse: ökosüsteemi teenuste rakendamine taimekasvatuses"
2012-2015	ETF9432 "*Phytophthora infestans* Baltikumi ja Venemaa Pihkva regiooni populatsioonide fenotüübiline ja genotüübiline iseloomustamine; oospooride kui esmase nakkusallika roll lehemädanikutekitaja epidemioloogias"

LIST OF PUBLICATIONS

Publications indexed by ISI Web of Science database (1.1.)

1. **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, 10 (10), ARTN 1535. DOI: 10.3390/agronomy10101535.
2. Tedersoo, L., Anslan, S., Bahram, M., Drenkhan, R., Pritsch, K., Buegger, F., Padari, A., Hagh-Doust, N., Mikryukov, V., Gohar, D., Amiri, R., Hiiesalu, I., Lutter, R., Rosenvald, R., Rähn, E., Adamson, K., Drenkhan, T., Tullus, H., Jürimaa, K., Sibul, I. ... Abarenkov, K. (2020). Regional-scale in-depth analysis of soil fungal diversity reveals strong pH and plant species effects in northern europe. Frontiers in Microbiology, 11, ARTN 1953. DOI: 10.3389/fmicb.2020.01953.
3. Bahram, M., Netherway, Tarquin, H., F., Pritsch, K, Drenkhan, R., **Loit, K.**, Anslan, S., Bork, P., Tedersoo, L. (2020). Plant nutrient-acquisition strategies drive topsoil microbiome structure and function. New Phytologist, 227 (4), 1189–1199. DOI: 10.1111/nph.16598.
4. 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 (1). DOI: 10.1038/s41598-020-74952-7.
5. **Loit, K.**, Kiiker, R., Puidet, B., Soonvald, L., Põldmets, M., Mänd, M. (2020). Assessing bactomix 5 efficacy for clubroot control in naturally infested soil. Plant Protection Science, 57 (1), 14–20. DOI: 10.17221/1/2020-PPS.
6. 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. DOI: 10.1016/j.apsoil.2019.05.003.
7. **Loit, K.**, Adamson, K., Bahram, M., Puusepp, R., Anslan, S., Kiiker, R., Drenkhan, R., Tedersoo, L. (2019). Relative performance of MinION (Oxford Nanopore Technologies) versus sequel (Pacific Biosciences) third-generation sequencing instruments in identifica-

tion of agricultural and forest fungal pathogens. Applied and Environmental Microbiology, 85 (21). DOI: 10.1128/AEM.01368-19.

8. Heinmaa, L., Põldma, P., **Loit, K.**, Kiiker, R., Moor, U. (2019). Physiological disorders affect apple susceptibility to *Penicillium expansum* infection and increase probability for mycotoxin patulin occurrence in apple juice. Agronomy Research, 17 (6), 2269–2276. DOI: 10.15159/AR.19.222.
9. **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). DOI: 10.15159/ar.18.063.
10. Runno-Paurson, E., **Loit, K.**, Hansen, M., Tein, B., Williams, I.H., Mänd, M. (2015). Early blight destroys potato foliage in the northern Baltic region. Acta Agriculturae Scandinavica Section B - Soil Plant Science, 65 (5), 422–432. DOI: 10.1080/09064710.2015.1017003.
11. Runno-Paurson, E., Hansen, M., Tein, B., **Loit, K.**, Jõgi, K., Luik, A., Metspalu, L., Eremeev, V., Williams, I.H., Mänd, M. (2014). Cultivation technology influences the occurrence of potato early blight (*Alternaria solani*) in an organic farming system. Zemdirbyste-Agriculture, 101 (2), 199–204. DOI: 10.13080/z-a.2014.101.026.

Articles published in local conference proceedings (3.5)

1. Ivask, M., Kiiker, R., **Loit, K.**, Põldmets, M., Raamets, J., Shanskiy, M. (2020). Vihmaussikoosluste struktuur ja mulla mikroobikoosluse aktiivsus suvinisu mahe- ja tavatootmispõldudel. Agronoomia (18–24). Eesti Maaülikool.
2. Soonvald, L., **Loit, K.** (2017). Arbuskulaar-mükoriissete seente inokuleerimise tähtsus agroökoloogilise tehnoloogia meetodina. Luule Metspalu, Anne Luik, Elen Peetsman (Toim.). Teaduselt mahepõllumajandusele (139–145).
3. Puidet, B., Kiiker, R., **Loit, K.**, Soonvald, L., Najdabbasi, N. (2017). Kartuli-lehemädaniku ja kuivlaiksuse areng maheviljeluseks soovitatud kartulisortidel. Teaduselt mahepõllumajandusele. Toim. Metspalu, Luule, Luik, Anne, Peetsmann, Elen. Tartu, Eesti: SA Eesti Maaülikooli Mahekeskus, 123–129.

Published conference abstracts (5.1 and 5.2)

1. **Loit, K.**, Kukk, M., Öpik, M., Runno-Paurson, E. (2014). Comparison of the extent of colonization of arbuscular mycorrhiza in plant roots in different cultivation practices. Proceeding of International Congress on Mycorrhizae: Mycorrhizal Symbiosis a key factor for improving plant productivity and ecosystems restoration: International Congress on Mycorrhizae: Mycorrhizal Symbiosis a key factor for improving plant productivity and ecosystems restoration, Marrakesh, Morocco, 15-17 October 2014, 178.
2. **Loit, K.**, Soonvald, L., Runno-Paurson, E., Tedersoo, L. (2016). Arbuscular mycorrhizal community composition across 21 potato cultivars from conventionally managed field. 38th New Phytologist Symposium "Colonization of the terrestrial environment 2016". New Phytologist Trust, 39.
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.
4. **Loit, K.**, Soonvald, L., Astover, A., Tedersoo, L. (2018). Molecular characterization of the pathogen and symbiotic fungal community composition in the rhizosphere of common European potato varieties. Phytopathology, 108 (10), 80-80.
5. Soonvald, L., **Loit, K.**, Astover, A., Tedersoo, L. (2018). Assessment of plant pathogenic fungal and oomycete communities in the soil of a long-term fertilization experiment. Phytopathology, 108 (10), 90–90.

VIIS VIIMAST KAITSMIST

TARMO PILVING

COLLABORATION IN ESTONIAN RURAL TOURISM

KOOSTÖÖ EESTI MAATURISMIS

Vanemteadur **Ants-Hannes Viira**, professor **Tiiu Kull**, nooremprofessor **Monika Suškevičs**
11. juuni 2021

MARIKA KOSE

COASTAL MEADOWS: MAINTENANCE, RESTORATION AND RECOVERY

RANNANIIDUD: SÄILIMINE, TAASTAMINE JA TAASTUMINE

Vanemteadur **Karin Kauer**, vanemteadur **Kadri Tali**
11. juuni 2021

ELINA KARRON

INFLUENCE OF CULTIVATION TECHNOLOGIES ON PATHOGENIC *FUSARIUM* SPP. OCCURRENCE AND PRODUCTION OF MYCOTOXINS IN CEREALS

ERINEVATE KASVATUSTEHNOLOOGIATE MÕJU *FUSARIUM* SPP. ESINEMISELE JA MÜKOTOKSIINIDE TEKKIMISELE TERAVILJADEL

Dotsent **Eve Runno-Paurson**, professor **Ülo Niinemets**, emeriitdotsent **Enn Lauringson**
15. juuni 2021

IMBI NURMOJA

EPIDEMIOLOGY OF AFRICAN SWINE FEVER IN ESTONIA AND CHARACTERIZATION OF ONE VIRUS STRAIN

SIGADE AAFRIKA KATKU EPIDEMIOLOOGIA EESTIS JA ÜHE VIIRUSTÜVE ISELOOMUSTUS

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MERILI TOOM

BIOMASS AND NITROGEN ACCUMULATION BY COVER CROPS DEPENDING ON SPECIES AND SOWING DATE AND THE EFFECT OF COVER CROPS ON SPRING BARLEY YIELD

ERINEVATE VAHEKULTUURIDE BIOMASSI MOODUSTAMISE JA LÄMMASTIKU SIDUMISE VÕIME SÕLTUVALT KÜLVIAJAST NING VAHEKULTUURIDE MÕJU SUVIODRA SAAGILE

Dotsent **Enn Lauringson**, dotsent **Liina Talgre**,
doktor **Andres Mäe** (Eesti Taimekasvatuse Instituut)
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