agronomy

MDPI

Article

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

Received: 28 August 2020; Accepted: 7 October 2020; Published: 9 October 2020

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).

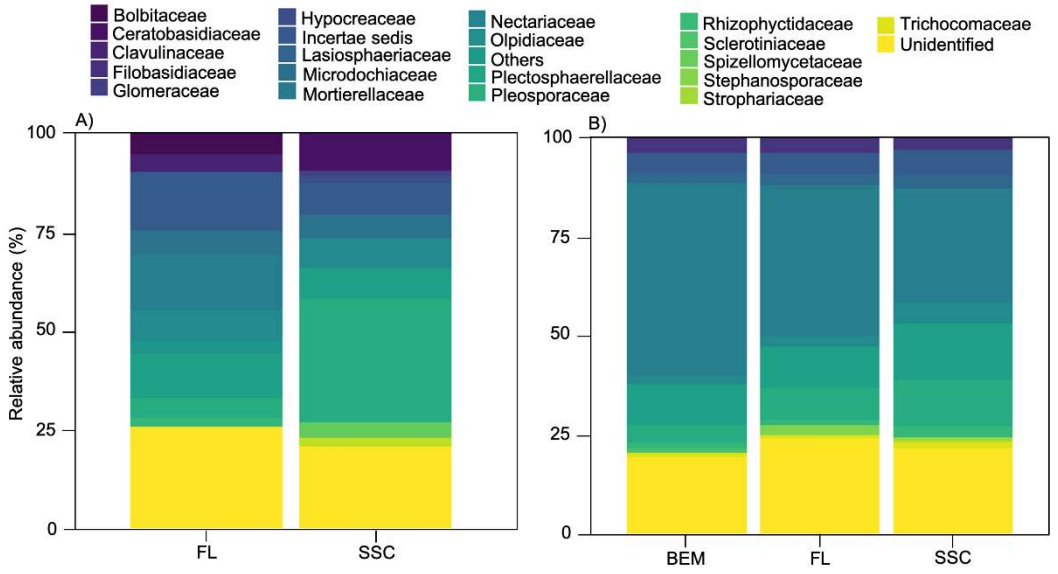

Figure 1. Taxonomic composition of root (**A**) and soil (**B**) fungal communities in different sampling times across all cultivars and replication blocks. BEM, before emergence; FL, flowering stage; SSC, senescence stage.

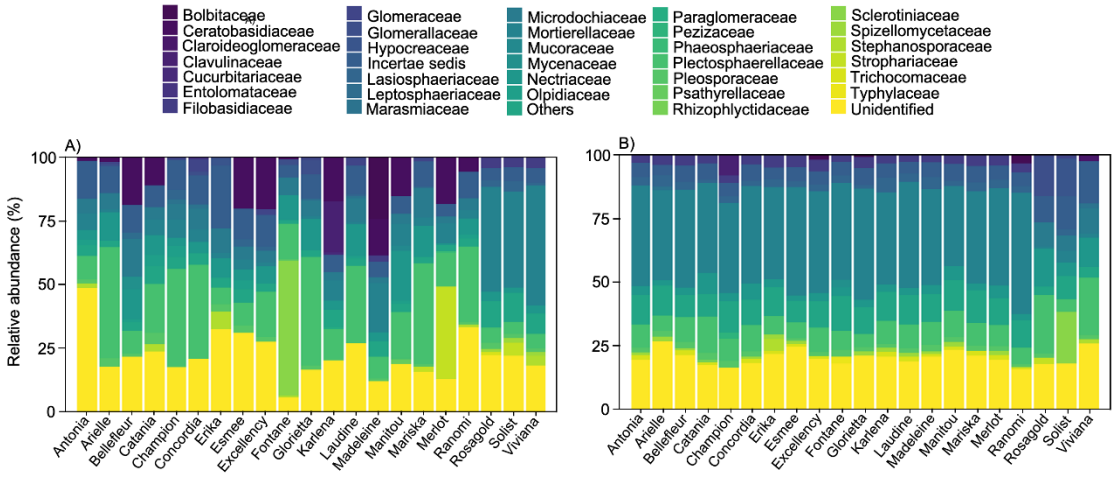

Figure 2. Taxonomic composition of root (**A**) and soil (**B**) fungal communities in different potato cultivars across all sampling times and replication blocks.

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).

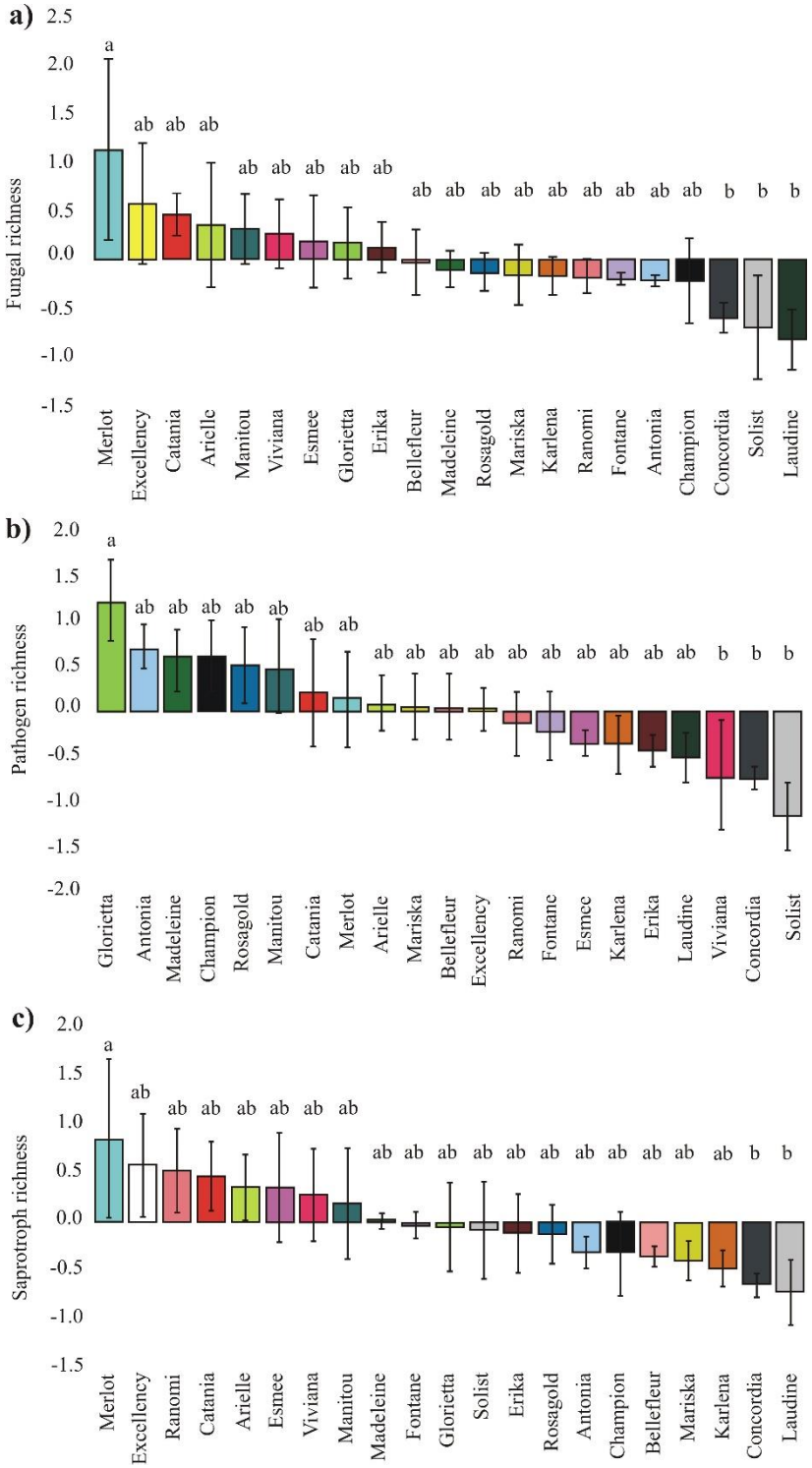

Figure 3. Results of a general linear model estimating the effect of cultivar for root (**a**) overall fungal, (**b**) pathogen, (**c**) saprotroph richness collected across all sampling times and replication blocks. Colours represent the cultivars. Different letters indicate statistically significant 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. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

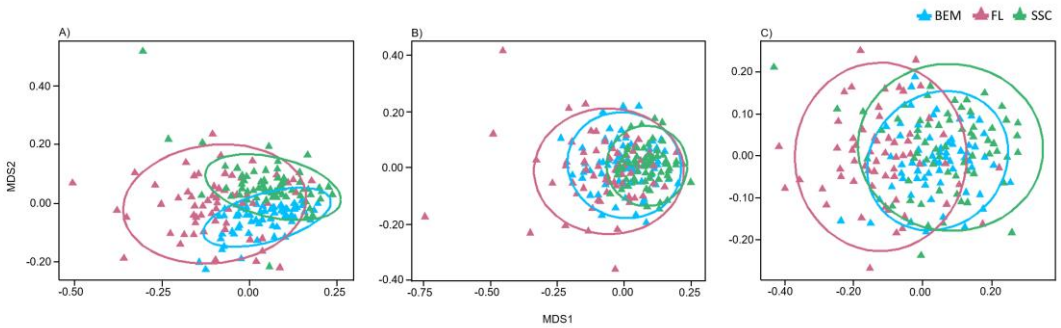

Figure 4. Non-metric multi-dimensional scaling, to model the effect of time on soil (**A**) overall fungal, (**B**) pathogen and (**C**) saprotroph community composition collected at different sampling points across all cultivars and replication blocks. BEM, before emergence; FL, flowering stage; SSC,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

Funding: This research was funded by Estonian Science Foundation grant no 9432, the European Regional Development Fund (project RESIST 3.2.0701.11-0003) and Center of Excellence EcolChange: Ecology of global change: natural and managed ecosystems), the Estonian University of Life Sciences project (base funding P190259PKTT), the Institutional Research Funding [project no. IUT36-2 of Estonian Research Council], European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No 817819 and European Union's European Regional Development Fund (Estonian University of Life Sciences ASTRA project "Value-chain based bio-economy").

Acknowledgments: We thank R. Kiiker, K. Leitaru, S. Anslan, R. Puusepp and Einola Farm for technical support, and Jonathan Willow for language editing.

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

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]