Boreal soil microbial diversity and seed onion mycorrhizal colonization is unaffected by preceding one season crop cultivation

Aku Pakarinen [a], Hannu Fritze [b], Sari Timonen [a], Pirjo Kivijärvi [c], Sannakajsa Velmala [b,*]

[a] *Department of Microbiology, P.O.Box 56, 00014 University of Helsinki, Finland*
[b] *Natural Resources Institute Finland, Latokartanonkaari 9 00790 Helsinki, Finland*
[c] *Natural Resources Institute Finland, Lönnrotinkatu 7 50100 Mikkeli, Finland*

ARTICLE INFO

ABSTRACT

Arbuscular mycorrhizal fungi (AMF) enhance plant phosphorus uptake, increase soil water holding abilities, reduce soil erosion and can protect their hosts from soil-borne pathogens. Hence, AMF play an important part in improving sustainable agricultural practices, and information about the effects of different preceding crop species on the following crop's AMF well-being is crucial for designing crop rotations.

We studied onion root and soil microbial diversity and onion root AMF colonization rates after being preceded by three AMF hosting and one non-hosting green manure crop species in a boreal climate organic field.

One-season cultivation of different preceding green manure crops did not have a strong effect on AMF colonization or microbial diversity in onion roots nor in the surrounding soil. Onions had high AMF colonization and microbial diversity after all four preceding crops. The overall fungal and bacterial populations of the soil reacted more strongly to seasonal variations than preceding crops.

The study suggests that one season is a too short time to influence the AMF community in boreal climate organic fields with conventional tillage. Thus, non-host preceding crops can also be used in rotations, especially together with AMF host crops.

1. Introduction

Although mycorrhizal fungi generally benefit plant growth and health, they are rarely taken into consideration in conventional, high-input agricultural practices. Plants abandon their mycorrhizal symbionts under heavy mineral fertilization [1]. Furthermore, heavy tillage breaks soil fungal hyphal mycelia [2,3], reduce spore densities, changes the mycorrhizal community composition [4,5] and disturbs the soil bacterial community [3]. In sustainable agriculture the potential of arbuscular mycorrhizal fungi (AMF) is recognized and practices to facilitate mycorrhizal well-being and to benefit soil and rhizosphere bacterial and fungal communities are developed [6].

Crop rotation is an important tool in sustainable agriculture. Different plant species in rotation can be harvested for yield, while green manure plants, when ploughed into soil, improve soil quality, prevent erosion and nutrient leaching, manage weeds, pests and diseases, while also improving soil microbial biodiversity [7]. Long-term green manuring has been shown to increase soil fungal and bacterial biomass and diversity [8]. Many legumes (family Fabaceae) host nitrogen (N)

fixing rhizobia bacteria inside their root nodules and their use as green manure increase soil N content, improve yields [9] and suppress weeds by overgrowth or by allelopathy. Plants from other genera, such as *Brassica* and *Tagetes*, are also used for weed and pest control [10–12] as they secrete weedicides and antimicrobial glucosinolates, thiophenes and other allelochemicals that may suppress soilborne diseases, other plants or fungi [13,14]. However, many excreted compounds are short lived (weeks or months) in non-sterile soils [15].

Mycorrhizal symbiosis is an important factor in sustainable agriculture as most crop plants form symbiosis with AMF [16]. AMF enhance the plant's nutrient uptake [1], and high AMF colonization rates have been shown to correlate with increased phosphorus (P) uptake, plant growth and yield [17,18]. AMF provide many indirect services in agriculture as hyphae increase soil water holding abilities and control erosion [19]. AMF can also protect their hosts against soil-borne pathogens [20]. Efficient nutrient usage and AMF derived erosion control help in creating sustainable, low-input agricultural systems with minimum environmental impact [21].

AMF are not believed to be particularly host-specific but the

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efficiency of the symbiosis in P uptake is fungus and plant-species specific [22]. Plants which are usually colonized simultaneously by multiple AMF species allocate more of their photosynthates to the more efficient symbionts [23]. Thus, a change in plant community can cause significant shifts in soil AMF species composition and affect spore densities [24]. AMF species favored by a certain plant species produce more spores and hyphae and are more capable of colonizing new host plants than AMF species that are not favored by that plant. Some plant genera, e.g. *Brassica, Beta, Fagopyrum, Isatis, Urtica, Camelina* and *Lupinus* include crop species that are known to be insusceptible to AMF colonization [25–27]. Incompatibility between plants and AMF can be caused by the lack of stimulus from plant roots to form symbiosis, or fungitoxic exudates and their breakdown products that prevent hyphal growth [28]. Both have been suggested for example to lie behind the incompatibility of white lupin (*Lupinus alba*) and AMF; the inhibitory effect is proposed to be AMF species and strain specific [29]. Moreover, it has been reported that the use of AMF non-hosts as preceding crops, negatively affect the cash crop root AMF colonization compared to AMF host crops in maize (*Zea mays*) [30].

Although the effect of tillage and fertilization on soil microbes and AMF has been amply studied, only few studies have addressed how crop rotation affect the indigenous soil microbial and especially AMF communities [31], and even fewer studies have been conducted in the boreal climate [27,32]. For developing efficient and sustainable crop rotations, we studied the effect of four different preceding green manure crops on AMF community composition and abundance in onion roots and mycorrhizosphere soil in the following year, as well as the impact on bacterial and fungal microbial communities in the spring bulk and autumn mycorrhizosphere soil. We assessed AMF colonization of onion roots with microscopic methods and studied the AMF community composition from onion roots and soil with high-throughput amplicon sequencing of the small subunit 18S ribosomal RNA gene. AMF spore biomass in soil was estimated by neutral lipid fatty acid analysis. The soil communities of fungi and bacteria were analyzed by amplicon sequencing of ribosomal 16S and ITS2 regions. We hypothesized that: (1) AMF non-host as preceding green manure crop decrease onion root AMF colonization and diversity in roots compared to an AMF host preceding crop; (2) at the end of the growing season AMF communities in onion roots and surrounding soil differ in plots with different green manure crops cultivated in the previous season; (3) the species and the symbiotic associations of preceding crops influence both fungal and bacterial communities in soil.

2. Materials and methods

2.1. Study setup, site and soil characteristic

The study was a randomized block design with four treatments and four replications. The treatments were four different green manure crops cultivated in the first season in each of the plots. There were 4 blocks of 10 × 12 m (10 m from each other) each having 4 treatment plots of 10 × 1.5 m with 0.5 m distance from each other (total of 16 plots). Each block had two side beds on the fringes. The site had been previously fertilized with organic fertilizers for several seasons. Before the experiment, from 2014 to 2015, timothy *(Phleum pratense),* red clover (*Trifolium pratense*) and meadow fescue (*Festuca pratensis*) were cultivated in the site for green manuring. In 2016, the site was cultivated with spring wheat (*Triticum aestivum*) with Italian ryegrass (*Festuca perennis*) as a catch crop.

The study was carried out over two growing seasons in the same site at the Natural Resources Institute Finland's experimental field, located in Mikkeli, eastern Finland (61.677°N 27.219°E), in the boreal climate. The soil type is fine sand till, rich in organic matter (Dystric Cambisol). Soil fertility was assessed in October 2016 by Eurofins Scientific (Finland) (Supplementary Table 1). In May 2018, after the cultivation of the preceding crops, soil C/N ratio, organic matter, microbial activity,

nitrogen supply capacity and total nitrogen were assessed by Eurofins Scientific (Finland) with near-infrared spectroscopy (Supplementary Table 2). Soil pH that was determined in August 2018 for each block from a soil-water (1:2.5 v/v) suspension according to ISO10390 (Soil Quality – Determination of pH) using the pH meter Symphony SB70P (VWR International, Helsinki, Finland) varied between pH 5.9 and 6.0.

2.2. Site setup during the experiment: 1st and 2nd season

In 2017, all four different green manure crops were cultivated once in each block: *Lupinus albus* 'Feodora', white lupin which is a non-host of AMF and a nitrogen-fixer. *Tagetes patula,* French marigold which is an AMF host and not a nitrogen-fixer. *Trifolium incarnatum* 'Contea', crimson clover which is an AMF host and a nitrogen-fixer. A mixture of *Vicia sativa* 'Ebena', common vetch and *Vicia villosa* 'Savane', hairy vetch, both of which are AMF hosts and nitrogen-fixers. Sow densities and yields of the preceding green manure crops are presented in Supplementary Table 2. The field was fertilized with the organic fertilizer Arvo 4-1-2 NPK (Novarbo; N 4 wt%, P 1 wt%, K 2 wt%) with a rate of 1000 kg/ha and ploughed on May 24th, 2017. The field was harrowed, and the green manure crops were sowed on June 8th, 2017 and after sowing the field was rolled. The green manure was terminated with a flail mower and ploughed on October 25th, 2017 to a depth of 15 cm. The field was left uncultivated for the winter.

Onions were outplanted on May 22nd, 2018. The onion cultivar was *Allium cepa* 'Hylander', from H.C. Diener Jungpflanzen Germany. The planted seedlings were certified organic press pot (4 × 4 × 5 cm) plants grown in a mix of peat, compost and wood fiber for a month in a glass house and watered with tap water. Planting density was 15 cm in the row and 35 cm between the rows, with three 6-m-long rows in a plot. Weeds, mainly couch grass (*Elymys repens*), were controlled by hand pulling and with mechanical hoeing regularly.

2.3. Sampling and preparation of samples

Soil was sampled twice in 2018 from all 16 plots. First, in May after the field had been harrowed, three soil cores were taken by pressing an open 50 ml Falcon tube to 10 cm depth per plot, resulting in 48 spring bulk soil samples (Table 1). Second, in autumn, onions and root surrounding soil were collected in early August from soil clods surrounding the sampled onion roots (five/plot): each entire onion plant was picked manually by hand from approximately 10 cm depth, taking the whole root ball and attached soil. This resulted in 80 autumn mycorrhizosphere soil samples and 80 onion root samples (Table 1). Mixing of samples was prevented by changing disposable vinyl gloves between each sample.

Onions and soil were transported in coolers from the site in Mikkeli to the laboratory in Helsinki and stored at +4 °C until further processing within the next seven days. A subsample of three replicate onions per plot, altogether 48 onion root samples (Table 1), were randomly selected for microscope slide preparations. One 14 ml Falcon tube was filled with onion roots of each sample. Roots were washed with tap water and stored in 60% ethanol at +4 °C. The remaining roots were cut off from the onions and the roots from the five onions from the same plot were combined and carefully mixed resulting in 16 pooled root samples. A small 100 mg (fresh weight) sub-sample was taken for DNA extraction to a 5 ml Eppendorf-tube from each composite root sample and stored at −20 °C prior to freeze-drying. All mycorrhizosphere soil samples were kept separate and stored at −20 °C prior to DNA extraction or other analysis. Bulbs and leaves were dried in paper bags at +70 °C for two days. The dried bulbs and leaves were left to further dry at room temperature for four months. After air drying, the bulbs and leaves were dried for one day at +50 °C and their dry weight was measured.

Table 1
Soil and root samples from altogether 16 plots including 4 treatments and 4 repetitions processed in the experiment.

Sample timepoint and type	Sample nr	DNA extractions	AMF samples sequenced	Bacterial and fungal samples sequenced	NLFA	Root colonization analyses
Spring, bulk soil 3 replicate samples	48	48	16	16	16	NA
Autumn, mycorrhizosphere soil, 5 replicate samples	80	80*	80	16	16	NA
Autumn, onion roots, 5 replicate samples	80	16	16	NA	NA	48

*Large number of samples to compensate for the sparse abundance of AMF spores and hyphae in small 500 mg soil samples.
NA not assessed.

2.4. Staining of roots, preparation of microscope slides, and counting of colonization

Roots of the subsampled 48 onions were stained with the ink-vinegar method [33] after one-week storage in 60% ethanol. The roots were cleared in 10% KOH at room temperature for 72 h, instead of boiling for a shorter time. Roots were rinsed in double distilled (dd) water from 5 to 7 times until the pH of the washing solution was between 7 and 8. Roots were stained with preheated black ink (Sheaffer Skrip®) and 5% acetic acid for 10 min at +60 °C and mixed twice by inversion. Excess ink was rinsed with rinsing solution (dd water with 0.5% acetic acid) and left in acid water at +4 °C to de-stain for 24 h. Stained roots were stored in acid water up to 30 days and then transferred to lactoglycerol (equal parts 95% glycerol: 90% lactic acid: dd water). Stained roots were cut into small pieces approximately 1 cm in length. 30 young root pieces with small (<1 mm) diameters were randomly selected and placed onto two microscope slides from each sample.

For counting of AMF intraradical hyphae, arbuscules and vesicles were executed with the magnified intersection method [34] using a transillumination microscope with 20x magnification. 100 intersections were counted from each microscope slide evenly distributed between the three columns of root pieces. Slide total counts of intraradical hyphae, arbuscules and vesicles were divided by the amount of reads per slide (100) to get the percentage of colonization.

We also ensured that no nursery originating AMF colonization is present in press pot seedlings in May (H.C. Diener Jungpflanzen Germany) by staining and microscopy of a fresh batch of *Allium cepa* 'Hylander' (data not shown).

2.5. DNA extraction, and amplicon sequencing

2.5.1. DNA extraction

DNA was extracted from soil (spring 48 bulk samples and autumn mycorrhizosphere 80 samples) and root samples (16 composite samples) (Table 1). Soil DNA was extracted with the Nucleospin® Soil kit according to the manufacturer's instructions [35] from 500 mg of fresh soil with two repetitions from each sample: in total 256 soil DNA extractions. Root DNA was extracted with Nucleospin® Plant II kit according to the manufacturer's instructions [36] with two repetitions from 16 freeze-dried composite root samples that had been grinded with Fast-Prep 4 × 30 s 5 m/s (MP biomedicals) into fine powder with the use of ceramic spheres.

2.5.2. Amplicon sequencing of ribosomal 18S, ITS2 and 16S V4 regions

Amplification and amplicon sequencing (Illumina MiSeq V3, paired end 2 × 300 bp, 8bp dual index, MCS 2.5.0.5 and RTA 1.18.54.0) was done at the Institute of Genomics, University of Tartu, Estonia. DNA from technical replicates, and replicates within each plot were pooled prior to sequencing and shipped for sequencing. The only exception was the 80 autumn mycorrhizosphere soil DNA extractions that were not combined within the plot but kept separate for the AMF community analysis. For AMF analysis, altogether 16 combined spring bulk soil samples and 80 autumn mycorrhizosphere soil samples, 16 combined autumn root samples, a soil kit control and a plant kit control were

amplified for 28 PCR cycles (KAPA HiFi HotStart ReadyMix (2X) by Kapa Biosystems, Wilmington, MA. 95 °C 3 min; cycling 95 °C 30 s, 55 °C 30 s, 72 °C 30 s; 72 °C 5 min) with the universal eukaryotic primer WANDA (CAGCCGCGGTAATTCCAGCT [37] and AMF specific AML2 (GAACCCAAACACTTTGGTTTCC [38] to target the 18S rDNA region at the sequencing facility. For other microbial groups, 16 combined spring and 16 combined autumn DNA samples were amplified with primers gITS7 and ITS4 primers [39,40] to target the fungal internal transcribed spacer region ITS2, and with 515 and 806 primers [41] to target the bacterial 16S V4 region, at the sequencing facility with 24 PCR cycles, respectively, and using the above mentioned conditions.

2.6. Bioinformatics

Raw sequences of 128 samples are stored in NCBI GenBank BioProject PRJNA634803 with accessions SAMN15009306–SAMN15009433.

Sequencing reads were assembled, pre-processed, chimera filtered and clustered with PipeCraft 1.0 [42] bioinformatic pipeline implementing mothur v1.36.1 [43], VSEARCH v1.11.1 [44], CD-HIT v4.6 [45], and Swarm v2.1.8 [46]. Forward and reverse reads were combined using a minimum of 15 bp overlap and trunc qual 0 for AMF and 10 for fungi and bacteria. Chimeric sequences (annotation 0.97 and abskew 2) and sequences without a correct primer were filtered out. Primers were cut off from the sequences. Fungal ITS2 reads were filtered against Unite ITS2 ref v7.1 database, and the ITS2 region was extracted from reads with ITSx [47].

Similar sequence reads of AMF were clustered with CD-hit as operational taxonomic units (OTU) with a threshold of 0.95 similarity, minimum 3 sequences per OTU and minimum length of sequences as 300 bp. 95% similarity was used to reduce the number of highly similar OTUs (as opposed to using e.g. 99% similarity). Martínez-García et al. [48] concluded that the 95% threshold is sufficient in determining ecological patterns of AMF. For fungi and bacteria parameters were threshold 0.97 and min size 2.

We made a BLAST comparison of OTUs with the parameters: word size = 7; e = 0.001; reward = 1; penalty = −1; gap open = 1; gap extend = 2 against MaarjAM database [49] (including unpublished sequences from November 2017) of 18S sequences of Glomeromycotina [50] and the SILVA database [51] (SILVA 132 SSU).

Second quality filtering was done based on the results of the sequence alignments against the MaarjAM and Silva-databases. The following 18S based OTUs were excluded: identity less than 80% (and e-value > E−52) based on the MaarjAM database together with the OTUs that got higher BLAST matches from the Silva database or had an affiliation in non-target taxa other than Glomeromycotina. Fungal ITS2 OTUs were annotated against UNITE database [52,53] (sh general release dynamic s 04.02.2020). We further filtered out fungal OTUs that had an identity less than 70% (and > E−20) with the database match, and OTUs that had affiliation other than fungi. Moreover, OTUs referring to the exact same species hypothesis SH [54] were consolidated. Bacterial 16S OTUs were annotated against the SILVA database [51] (SILVA 138 SSU tax silva trunc). We further filtered out bacterial OTUs that had an identity less than 70% (and > E−20) with the database match, and OTUs that had an affiliation other than bacteria. Moreover,

bacterial OTUs with the same GenBank GB accession number were consolidated.

2.7. Neutral lipid fatty acid 16:1ω5 analysis

Neutral lipid fatty acid (NLFA) analysis 16:1ω5 was done to estimate the amount of AMF spores in the soil [55]. NLFA samples were taken from the same samples as for DNA extraction separately from spring bulk soil (16 samples, subsamples within each plot were pooled together, Table 1) and from autumn mycorrhizal soil (16 samples). Prior to the analyses, soil samples were stored frozen (−20 °C). The neutral lipid extraction and analysis of NLFAs were carried out as described for phospholipid fatty acid analyses [56]. Briefly, 2.5 g of fresh soil was extracted with a chloroform:methanol:citrate buffer mixture (1:2:0.8) and thereafter, lipids were separated into neutral lipids, glycolipids and phospholipids on a silicic acid column. The neutral lipids were subjected to a mild alkaline methanolysis and methyl nonadecanoate (19:0) was added as an internal standard for quantification. The fatty acid methyl ester of 16:1ω5 was detected by gas chromatograph (GC) using a flame ionization detector and 50-m HP-5 capillary column (see GC run configuration in Pennanen et al. [57]).

2.8. Statistical analyses

Statistical analyses were performed with R version 3.6.0 [58]. We used a linear mixed-effect model, with the function lme () from the package nlme [59]. The differences in onion dry weights and proportions of the colonization, arbuscules and vesicles in relation to the preceding crop were fitted with preceding crop as the explanatory factor and the block as the random effect (Supplementary Table 3). Differences in the NLFA 16:1ω5 concentrations and OTU numbers were studied for spring bulk soil and autumn mycorrhizosphere samples separately with preceding crop as the explanatory factor and the block as the random effect. Tukey's post hoc test from package multcomp [60] was used to study pairwise comparisons. We used Shapiro-Wilks test and visual observation of R's regression diagnostic plots to assess the normality of the residuals and used logarithmic transformation as the normalization method for onion dry weight, and arcsin square root transformation for proportions of the colonization and arbuscules that did not meet the assumptions.

We normalized the amplicon sequencing based OTU diversity data matrices with geometric mean of pairwise ratios (GMPR [61]) which is robust and deals with zero-inflated community data and preserves differences in relative abundances of taxa. We ran the community analyses also with rarefied data (data not shown), and this did not affect our results or our main conclusions.

We used adonis () function from vegan package [62] to perform a permutational multivariate analysis of variance (PERMANOVA) of microbial communities using a Bray-Curtis distance matrix with block as strata separately for spring, autumn and root samples, and for all sample types together. We visualized the changes in AMF community structures with nonmetric multidimensional scaling (NMDS) with the function metaMDS () from the vegan package. Differential abundances of AMF, bacterial and fungal OTUs were studied with DESeq2 package [63] using GMPR size factors. Both timepoint and preceding crop plants were included in the full model. Contrasts with all combinations of the timepoints and onion roots, and of all preceding crops were studied.

3. Results

3.1. AMF colonization of onion roots

All onion roots, regardless of the preceding crop, displayed high AMF hyphal and Arum-type arbuscular colonization rates in the autumn (mean > 80% and >70%, respectively). Onion root AMF hyphal colonization varied in different preceding crop treatments ($F = 3.49$, $P =$

0.024); it was highest after *L. albus* and *V. sativa/V. villosa* and was significantly lower after *T. incarnatum* when compared to *V. sativa/V. villosa* (Fig. 1a; Supplementary Table 3). Thus, AMF hosts as preceding crops did not increase onion root AMF colonization compared to the *L. albus* AMF non-host. Onion root arbuscule proportion (Fig. 1b), assessed from the same slides followed a similar, but only indicative trend. Moreover, there were only few vesicles visible on the slides (<7% mean) and the proportion was the same regardless of treatment (Fig. 1c).

Dry weight of onion samples (Fig. 1d) as well as onion yield per plot (Supplementary Table 2) was low and did not vary between the preceding crop treatments.

3.2. Neutral lipid fatty acid 16:1ω5 analysis

Soil NLFA 16:1ω5 concentrations, that give an estimate of AMF spore quantity in soil, were on average lower in spring bulk soil (mean and SD; 2.17 ± 0.96, 3.37 ± 0.54, 3.20 ± 1.49, 2.32 ± 0.46) than in autumn mycorrhizal soil (4.26 ± 0.98 SD, 3.76 ± 1.52, 2.66 ± 1.09, 3.10 ± 0.83) but the difference was not statistically significant (Supplementary Table 3).

3.3. AMF species composition

Amplicons of the 18S region clustered with threshold 0.95 into 2781 OTUs and after second quality filtering we accepted 32 OTUs with altogether 196 932 sequences as glomeromycetes (Supplementary Table 4).

In spring the bulk soil samples, having 28 AMF OTUs (Fig. 2; GMPR normalized data in Supplementary Table 5), the most common genera were *Paraglomus, Glomus, Scutellospora* and *Diversispora*. In autumn the mycorrhizosphere soil samples had 31 OTUs of which the four most common genera were *Paraglomus*, *Diversispora*, *Claroideoglomus* and *Glomus*. Onion root samples had 23 AMF OTUs, with most of the sequences from the genera *Glomus* and *Clareideoglonus* (Table 2). Although, the primer pair WANDA-AML2 is referred to as AMF specific in literature, in soil samples many OTUs matched to Mortierellomycotina, Ascomycotina, and Chytridiomycota (Supplementary Table 4) and in root samples most sequences were identified as onion.

The AMF taxa and their relative abundances were similar after different preceding crops (Fig. 2). Most sequences in root samples were from the genus *Glomus* and the relative abundances of AMF genera in root samples were clearly different from soil samples (Fig. 2, Supplementary Table 5). This is also visible in the 3D NMDS where AMF communities cluster together according to the sample type rather than the preceding crop (Fig. 3).

Both sample type and preceding crop had some explanatory effect on the AMF community (PERMANOVA, sample type $R^2 = 0.28$, $P = 0.001$; preceding crop $R^2 = 0.052$, $P = 0.001$). However, the preceding crop did not have a statistically significant effect on the AMF community structures in the spring bulk soil samples. Nevertheless, of the autumn mycorrhizosphere soil samples, the preceding crop explained 10% of the AMF taxa variation (PERMANOVA, $R^2 = 0.102$, $P = 0.001$). Furthermore, the preceding crop explained 28% of the variation of the AMF community in roots (PERMANOVA, $R^2 = 0.276$, $P = 0.011$).

We used differential abundance testing to find indicative OTUs for specific conditions. In the normalized AMF data 25 OTUs were differentially abundant. *Ambispora* and *Archaespora* were much more abundant in soil samples than in onion roots, particularly in spring (Table 2). *Glomus* species were indicative for roots as they were much more abundant in roots than in soil (Table 2). Most *Clareideoglomus* species along with *Glomus mosseae* were abundant in roots and autumn rhizosphere samples when compared to spring soil samples (Table 2). When differential abundances were studied for the different preceding crops, only three OTUs appear to be significantly indicative. *Ambispora fennica* was four times as abundant in *T.patula* than in *V.sativa/V.villosa*. Ambispora sp. was on the contrary four times as abundant in
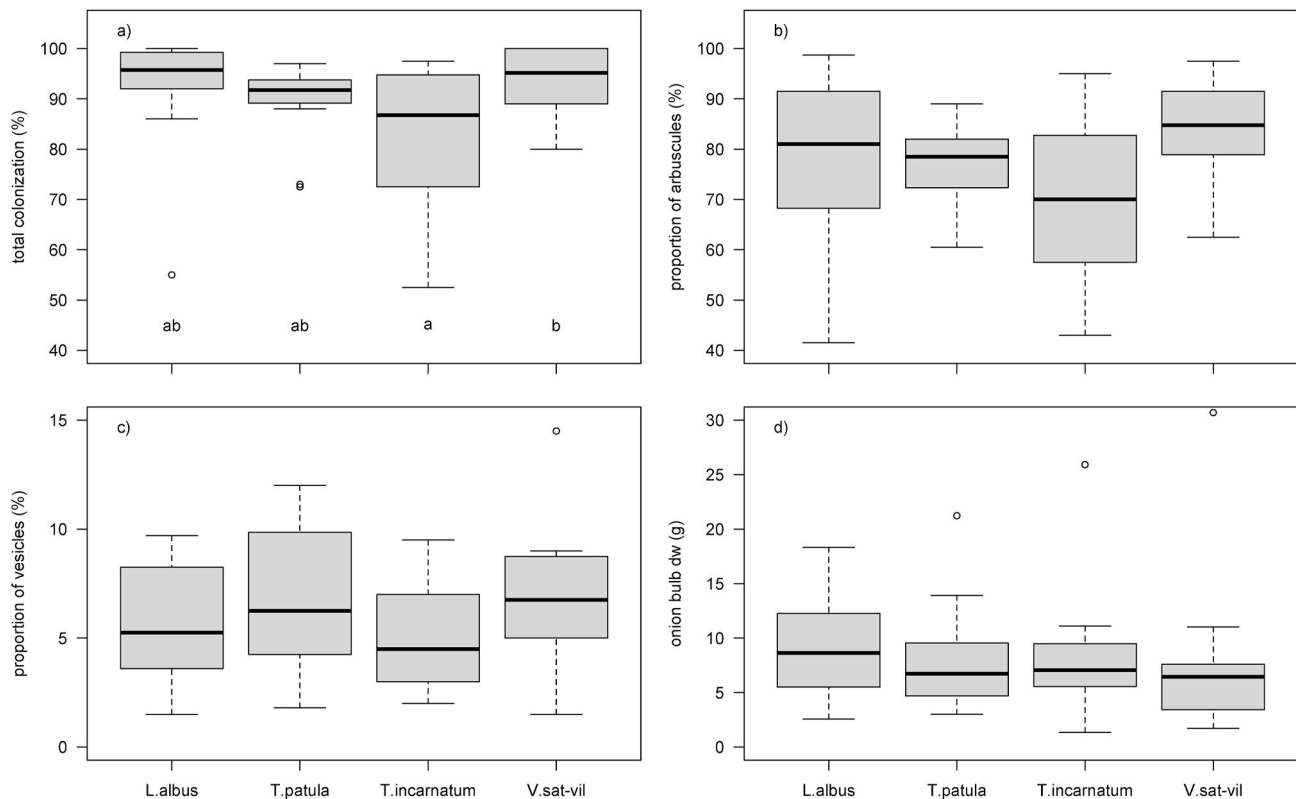
Fig. 1. Boxplots of a) proportion of total AMF colonization (hyphal colonization) of onion root, b) proportion of arbuscules in onion roots, c) proportion of vesicles in onion roots, d) average dry weight of an onion bulb and top in the sample, all on the y-axis. Preceding crop (*L. albus, T. patula, T. incarnatum, V. sativa/V. villosa*) is labeled on the x-axis. Different letters below boxplots indicate significant difference (P < 0.05) compared with the other treatments. The boxes represent the middle 50% of the data with thick lines showing the medians. Whiskers represent values outside middle 50% that are inside 1.5x interquartile range (height of the box) and open circles are values that do not fit inside the whiskers.

T. incarnatum than in *T.patula*. Scutellospora was twice as abundant after *L. albus* than after *T. incarnatum*.

3.4. Fungal and bacterial communities

3.4.1. Fungal community

ITS2 amplicons clustered with a threshold value of 0.97 initially into 3678 fungal OTUs (Supplementary Table 4). The second quality filtering resulted 1951 OTUs/SHs. The library sizes varied from 12 645 to 54 412 reads with a median of 29 232. Of fungal sequences 13.5% belonged to Glomeromycetes.

The mean fungal OTU numbers were slightly lower in the spring bulk soil than in the autumn mycorrhizosphere soil. No differences between fungal OTU numbers could be detected between preceding crop treatments (Table 3). More than half of fungal OTUs were shared between spring bulk soil and autumn mycorrhizosphere soil (Supplementary Fig. 1a). A fifth of the OTUs were unique for the two samplings, respectively (OTUs present at least once). In the spring bulk soil, there were ca. 100 unique fungal OTUs for each preceding crop treatment, and 689 OTUs were shared between all treatments. *T. patula* and *V. sativa/V. villosa* shared the highest number of unique OTUs (Supplementary Fig. 1b). The highest mean fungal OTU count was found in the autumn mycorrhizosphere soil after *T. patula* (Table 3). Autumn mycorrhizosphere soil had the same number of unique OTUs as the spring bulk soil (Supplementary Fig. 1c). Again, *T. patula* and *V. sativa/V. villosa* shared the highest number of unique OTUs. Moreover, 650 OTUs were shared between all treatments in autumn mycorrhizosphere soil.

Almost no fungal OTUs were found to be indicative to a certain preceding crop treatment; one OTU representing the genus *Ambispora* appeared to be more abundant in *L. albus* plots in autumn rhizosphere

soil. *Microbotryomycetes* sp., *Phenoliferia psychrophila*, *Chalara longipes*, *Glarea lozoyensis*, and *Chytridiomycota* sp. were more abundant in spring bulk soil (Table 4). *Alternaria alternata*, *Arthrobotrys amerospora*, *Aureobasidium pullulans*, *Candida* sp., *Cyphellophora reptans*, *Penicillium amphipolaria*, *Phialemonium atrogriseum*, *P. inflatum*, *Sarocladium strictum*, *Wickerhamomyces onychis*, *Cutaneotrichosporon guehoae*, *C. moniliiforme*, *Filobasidium magnum*, *Leucosporidium yakuticum*, *Sebacinaceae* sp., *Serendipita* sp., *Sporidiobolales* sp., *Sporobolomyces ruberrimus*, *Dissophora ornata*, *Mortierella gemmifera*, *M. hyalina*, and *M. parvispora* were indicative for autumn mycorrhizosphere soil as they were much more abundant in autumn compared to spring (Table 4).

Moreover, the sampling timepoint and not the preceding crop explained the separation of the fungal community (PERMANOVA, $R^2 =$ 0.44, P = 0.001). Similarly, in separate analysis for the two timepoints the preceding crop treatment only indicatively explained a quarter of the variation in the autumn fungal community structure (PERMANOVA, $R^2 =$ 0.23, P = 0.088).

3.4.2. Bacterial community

Bacterial 16S amplicons clustered with a threshold of 0.97 into 13 444 OTUs, and the second quality filtering resulted in 8219 bacterial OTUs/GB (Supplementary Table 4). The library sizes varied from 18 814 to 31 620 reads with a median of 26 115.

Bacterial OTU numbers were lower in the spring bulk soil than in autumn mycorrhizosphere soil in all treatments. Altogether, 80% bacterial OTUs were shared between spring bulk soil and autumn mycorrhizosphere soil, and 7% OTUs were unique for the spring bulk soil and 13% for the autumn mycorrhizosphere soil (Supplementary Fig. 2a). This difference was particularly striking after *L. albus* (Table 3). *L. albus* spring bulk soil samples differed in pairwise comparisons from *T. patula*
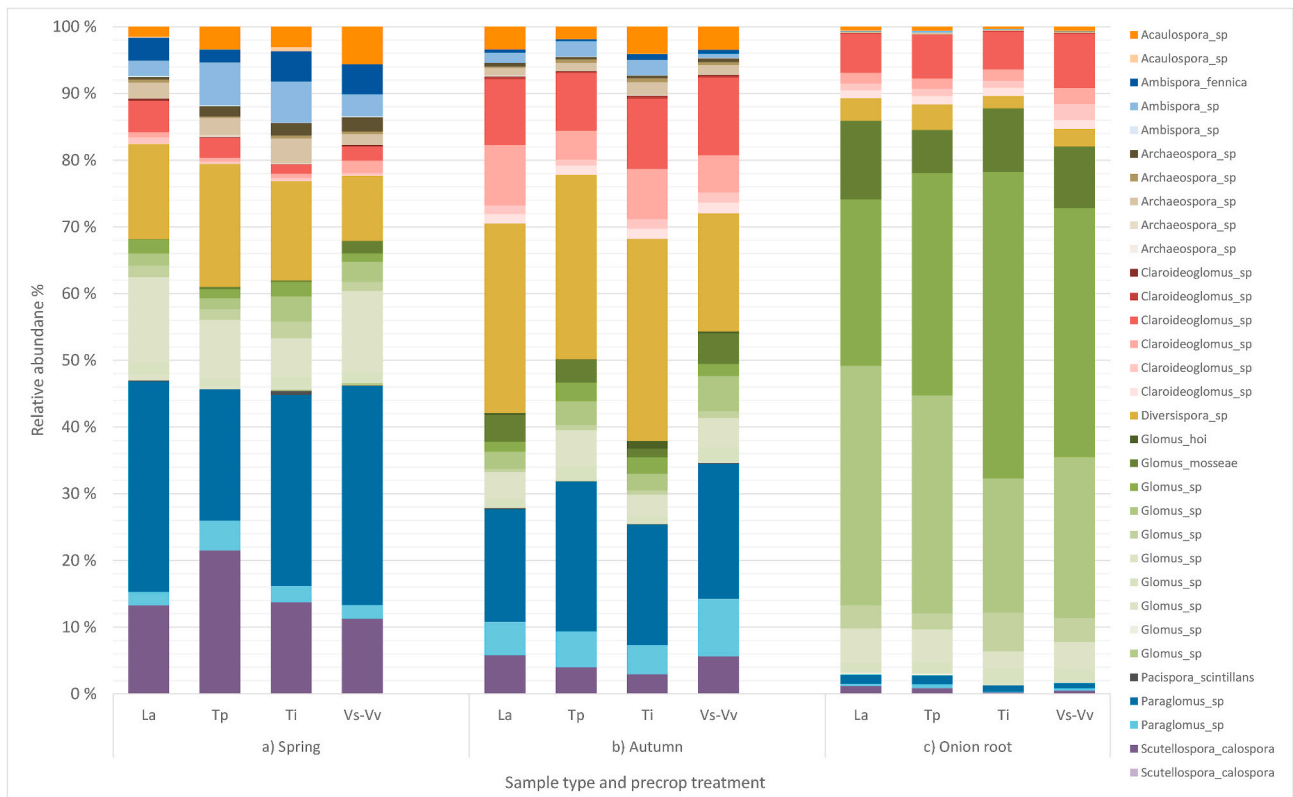
Fig. 2. Relative abundances of AMF virtual taxa sorted according to the preceding crop treatment (La = *L. albus*, Ta = *T. patula*, Ti = *T. incarnatum*, Vs-Vv = *V. sativa/ V. villosa*) in a) spring bulk soil samples collected on May 9th, 2018; b) autumn mycorrhizosphere soil samples and c) root samples collected on August 2nd, 2018.

Table 2
Differential Abundance Analysis (DAA) using DESeq2. Values presented are Log2FoldChange comparison of treatments, and only statistically significant values are shown, Padj ≤0.05 for all comparisons. Positive Log2FoldChange tells that the abundance is higher in the main group than in the group it is compared to and vice versa.

| Genus	Species	Virtual taxa	OTU	Compared to Onion root		Compared to spring bulk soil
				Spring bulk soil	Autumn rhizosphere	Autumn rhizosphere
Acaulospora	sp.	VTX00030	Acau_Otu0885	1.73	1.85	
Ambispora	sp.	VTX00242	Ambi_Otu0937	7.96	5.50	-2.46
Ambispora	fennica	VTX00283	Ambi_Otu0979	4.12	2.74	-1.39
Ambispora	sp.	VTX00242	Ambi_Otu1336	3.34		
Archaeospora	sp.	VTX00009	Arch_Otu0281	6.64	5.46	
Archaeospora	sp.	VTX00245	Arch_Otu1059		1.97	
Archaeospora	sp.	VTX00005	Arch_Otu1107	2.65	2.04	
Claroideoglomus	sp.	VTX00193	Clar_Otu0106	3.59	3.66	
Claroideoglomus	sp.	VTX00056	Clar_Otu0238	-2.65		3.27
Claroideoglomus	sp.	VTX00057	Clar_Otu0474	-2.42		2.03
Claroideoglomus	sp.	VTX00056	Clar_Otu0479	-1.91		2.87
Claroideoglomus	sp.	VTX00340	Clar_Otu0645	-2.67	-1.03	1.65
Claroideoglomus	sp.	VTX00402	Clar_Otu0857	-5.29		4.52
Diversispora	sp.	VTX00306	Dive_Otu0882	1.15	2.19	1.04
Glomus	mosseae	VTX00067	Glom_Otu0931	-5.82	-2.89	2.93
Glomus	sp.	VTX00114	Glom_Otu0954	-5.74	-5.35	
Glomus	sp.	VTX00108	Glom_Otu0986	-4.83	-4.29	
Glomus	hoi	VTX00199	Glom_Otu0996	-2.71	-3.79	
Glomus	sp.	VTX00125	Glom_Otu1002		-1.18	-1.08
Glomus	sp.	VTX00143	Glom_Otu1029	-1.94	-1.63	
Glomus	sp.	VTX00125	Glom_Otu1425		-4.73	
Paraglomus	sp.	VTX00281	Para_Otu1088	3.44	3.11	
Paraglomus	sp.	10000107	Para_Otu1253	1.73	3.20	1.47
Scutellospora	calospora	VTX00052	Scut_Otu1041	3.62	1.97	-1.66
Scutellospora	calospora	VTX00052	Scut_Otu2040		3.01	
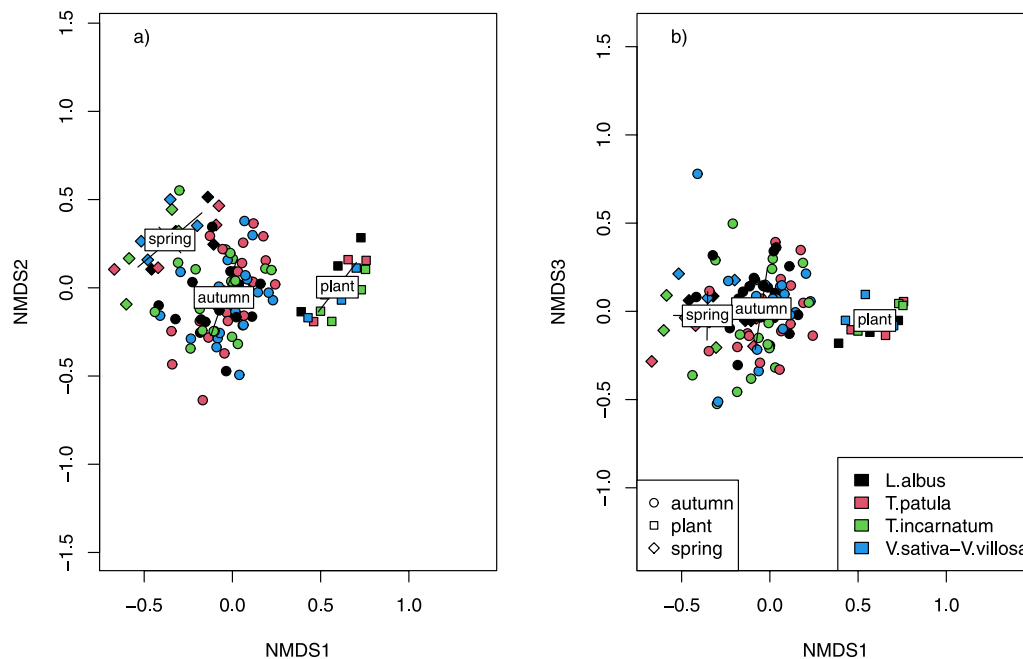
Fig. 3. Three-dimensional NMDS ordination plot of the AMF OTU community in spring bulk soil samples (diamond), autumn mycorrhizosphere soil samples (circle) and *Allium cepa* root samples (square) in autumn for a) axes 1 and 2, and b) axes 1 and 3. Crossed error bars show the standard deviation of point scores. GMPR normalized data is presented in Supplementary Table 5.

Table 3
Mean OTU/species number (SD) of bacteria and fungi in soil after different preceding green manure crops. Statistically significant differences (Turkey's test) between preceding crop treatments separately for spring and autumn are indicated with letters (P < 0.05).

Microbe	Soil	*Lupinus albus*	*Tagetes patula*	*Trifolium incarnatum*	*Vicia sativa/V. villosa*	F (DF 3, 9)	*P*-value
Fungi	Spring, bulk	607 (23)	655 (87)	582 (38)	653 (77)	1.9	0.2
Fungi	Autumn, mycorrhizosphere	650 (20)	672 (43)	619 (75)	661 (7)	1.1	0.4
Bacteria	Spring, bulk	2905 (202)a	3259 (94)b	3148 (94)b	3208 (208)ab	3.9	0.05
Bacteria	Autumn, mycorrhizosphere	3502 (245)	3455 (256)	3515 (108)	3378 (80)	0.52	0.68

spring bulk soil samples. In the spring bulk soil, 47% OTUs were shared between all preceding crop treatments (Supplementary Fig. 2b). *T. patula*, *T. incarnatum*, and *V. sativa/V. villosa* had around 5% of unique OTUs and *L. albus* less than 4%. In autumn mycorrhizosphere soil, more than half of bacterial OTUs were shared between treatments (Supplementary Fig. 1c). *T. patula T. incarnatum V. sativa/V. villosa* and *L. albus* all had around 6% of unique OTUs each.

Preceding crop did not explain differences in bacterial community structure in the spring soil nor in the autumn mycorrhizosphere soil. Bacteria from orders Rhizobiales and Burkholderiales, that include species forming symbiotic relationships with legumes, were not more abundant in the spring after leguminous preceding host compared to *T. patula*. Over fifty bacterial OTUs were found to be statistically more abundant in the autumn mycorrhizosphere soil than in spring bulk soil, but no bacterial OTUs were more abundant in spring bulk soil (Table 5): OTUs referring to *Rhodanobacter*, *Pantoea* and unknown bacteria from Chitinophagales, Flavobacteriales, and Rhizobiales genera were indicative for autumn mycorrhizosphere soil.

4. Discussion

4.1. AMF colonization of onion roots after different preceding crop species

Onion is particularly dependent on its mycorrhizal symbionts and has high overall AMF colonization rates due to its poorly branched root system that lacks root hairs [64,65]. When P availability is low, the plant's need for fungal symbionts increases [1]. In this experiment, the need for fungal symbionts was supposedly high as the available P concentration in the soil was low (6.5 mg/l in 2016), and the high overall colonization in onion roots was evident in our results.

The preceding green manure crop had little effect on AMF colonization. This is in contradiction to our hypothesis, which predicts that onion root colonization rate is lower compared to the three AMF hosts when the non-host *L. albus* is used as a preceding crop. Surprisingly, onion root AMF colonization rate was lowest after *T. incarnatum* at the end of the growing season. Our results did not agree with previous studies made with other crop plants. A lower AMF colonization rate after non-mycorrhizal preceding crops has been observed in maize [66] and barley roots [67] in temperate climate. However, a slight tendency toward increased AMF colonization after non-host preceding crops has also been reported [68].

The biological significance of differences in hyphal colonization in our study seems marginal as a colonization rate of over 80% signals an intense symbiosis [69]. This was also reflected in our results, as the onion yields were similar after all preceding crops. Furthermore, there was no difference in the relative abundance of arbuscules, the main sites of nutrient exchange between symbionts, after different preceding crops.

We used foreign seed onions watered with tap water as the cash crop, and it cannot be excluded that the substrate contained some carry over microbes. We could not use onion sets (i.e. small bulbs) because of the fungal disease load we had experienced with them in the previous season. Several onion pathogens transmit through infected seeds, seedlings, sets or bulbs [70], and are likely to be introduced with the main crop. However, the major growth substrate of onion seedlings used in the

Table 4

Differential Abundance Analysis (DAA) of fungi in spring bulk soil vs. autumn mycorrhizosphere soil samples (Log-2-fold change>|2|; P < 1E-10). All species with positive log-2-fold change are more abundant in autumn and species with negative log-2-fold change more abundant in spring. Log-2 fold change indicate the magnitude (log-ratio) of difference in abundance.

Phyla	Genus	Species	log2FC	Soil
Ascomycota	Chalara	longipes	−4.24	Spring, bulk
	Glarea	lozoyensis	−3.57	
Basidiomycota	*Microbotryomycetes*	sp.	−7.58	
	Phenoliferia	*psychrophila*	−6.69	
Chytridiomycota	Chytridiomycota	sp.	−3.03	
	Chytridiomycota	sp.	−3.44	
unidentified	Fungi	sp.	−2.02	
Ascomycota	*Alternaria*	*alternata*	8.14	Autumn, mycorrhizosphere
	Arthrobotrys	*amerospora*	8.37	
	Arthrographis	kalrae	5.5	
	Aureobasidium	pullulans	8.15	
	Botrytis	californica	5.75	
	Cadophora	luteo-olivacea	4.51	
	Candida	saitoana	5.91	
	Candida	sp.	8.31	
	Candida	subhashii	6.18	
	Cladosporium	*flabelliforme*	5	
	Cyphellophora	*reptans*	8.41	
	Dothioraceae	sp	4.82	
	Epicoccum	*proteae*	2.15	
	Fusarium	*fujikuroi*	6.49	
	Gibberella	*baccata*	4.64	
	Nectriaceae	sp.	2.9	
	Penicillium	*amphipolaria*	11.03	
	Penicillium	subrubescens	6.52	
	Phialemonium	*atrogriseum*	13.49	
	Phialemonium	*inflatum*	12.94	
	Phoma	herbarum	7.25	
	Plectosphaerella	*niemeijerarum*	2.54	
	Sarocladium	*strictum*	8.16	
	Stemphylium	*vesicarium*	7.57	
	Wickerhamomyces	*onychis*	9.81	
Basidiomycota	*Apiotrichum*	*porosum*	7.06	
	Bulleromyces	*albus*	4.94	
	Cutaneotrichosporon	*guehoae*	11.65	
	Cutaneotrichosporon	*moniliiforme*	9.65	
	Cystobasidium	*pinicola*	6.78	
	Cystofilobasidium	*macerans*	6.17	
	Filobasidium	*magnum*	8.15	
	Hymenochaetales	*sp.*	7.94	
	Leucosporidium	*yakuticum*	9.34	
	Rhodotorula	*glutinis*	5.26	
	Rhodotorula	*sp*	5.12	
	Sebacinaceae	sp.	9.72	
	Serendipita	sp.	10.36	
	Serendipita	sp.	8.24	
	Serendipita	sp.	7.89	
	Serendipita	sp	7.29	
	Serendipita	sp	6.21	
	Serendipita	sp	8.24	
	Sporidiobolaceae	sp.	6.55	
	Sporidiobolales	sp.	12.25	
	Sporobolomyces	*ruberrimus*	8.42	
	Tremellomycetes	sp.	2.33	
	Vishniacozyma	carnescens	3.47	
Chytridiomycota	*Kochiomyces*	sp	5.38	
	Sonoraphlyctis	sp	7.03	
	Spizellomyces	pseudodichotomus	2.88	
	Spizellomycetales	sp	6.12	
Mortierellomycota	*Dissophora*	*ornata*	12.36	
	Mortierella	*gemmifera*	10.25	
	Mortierella	*hyalina*	11.86	
	Mortierella	*parvispora*	8.11	
	Mortierella	*pulchella*	6.02	
Mucoromycota	*Mucor*	*circinelloides*	7.29	
Rozellomycota	*Rozellomycota*	sp.	7.01	
	Rozellomycota	sp.	7.01	
unidentified	Fungi	sp.	4.55	
	Fungi	sp.	9.97	
	Fungi	sp.	7.16	
	Fungi	sp.	4.74	
	Fungi	sp.	6.59	

Table 4 (*continued*)

Phyla	Genus	Species	log2FC	Soil
	Fungi	sp.	8.11	
	Fungi	sp.	5.79	
	Fungi	sp.	2.99	

Table 5

Differential Abundance Analysis (DAA) of bacteria in spring bulk soil vs. autumn mycorrhizosphere soil samples (Log-2-fold change>|2|; P < 1E-10). All bacterial species presented in the table were more abundant in autumn mycorrhizosphere. Log-2 fold change indicate the magnitude (log-ratio) of difference in abundance.

Phyla	Order	Genus	log2FoldChange
Acidobacteriota	Acidobacteriales	Granulicella	4.22
	Corynebacteriales	Rhodococcus	4.19
	Frankiales	unknown	2.19
	Unknown	unknown	2.13
	Unknown	unknown	3.67
Bacteroidota	Chitinophagales	Flavisolibacter	2.32
	Chitinophagales	Flavisolibacter	4.05
	Chitinophagales	Segetibacter	2.07
	Chitinophagales	unknown	7.03
	Flavobacteriales	unknown	6.18
	Sphingobacteriales	Mucilaginibacter	3.75
	Sphingobacteriales	Pedobacter	2.25
	Sphingobacteriales	Pedobacter	4.36
	Sphingobacteriales	unknown	4.61
Chloroflexi	Anaerolineales	unknown	4.84
	unknown	unknown	4.71
	unknown	unknown	4.62
Cyanobacteria	Obscuribacterales	unknown	3.53
Deinococcota	Deinococcales	Deinococcus	5.02
Gemmatimonadota	Gemmatimonadales	Roseisolibacter	2.92
	Gemmatimonadales	Roseisolibacter	3.69
	Gemmatimonadales	unknown	5.76
Proteobacteria	Burkholderiales	Achromobacter	4.77
	Burkholderiales	Comamonas	3.53
	Burkholderiales	Massilia	2.27
	Burkholderiales	Methylotenera	2.72
	Burkholderiales	Paralcaligenes	4.85
	Burkholderiales	unknown	4.04
	Caulobacterales	unknown	4.46
	Enterobacterales	Pantoea	7.39
	Micropepsales	Micropepsis	4.13
	Pseudomonadales	Acinetobacter	4.59
	Pseudomonadales	Pseudomonas	2.38
	Rhizobiales	Corticibacterium	4.34
	Rhizobiales	Methylobacterium-Methylorubrum	4.79
	Rhizobiales	Ochrobactrum	5.22
	Rhizobiales	Rhodopseudomonas	5.75
	Rhizobiales	unknown	6.22
	Rickettsiales	Silene	2.23
	Salinisphaerales	Alkanibacter	5.24
	Salinisphaerales	Polycyclovorans	3.12
	Sphingomonadales	Altererythrobacter	4.42
	Sphingomonadales	Novosphingobium	3.12
	Sphingomonadales	Sphingobium	5.19
	Sphingomonadales	Sphingobium	6.86
	unknown	unknown	4.77
	Xanthomonadales	Dyella	4.77
	Xanthomonadales	Luteimonas	4.46
	Xanthomonadales	Luteimonas	2.18
	Xanthomonadales	Lysobacter	4.49
	Xanthomonadales	Rhodanobacter	7.90
	Xanthomonadales	Rhodanobacter	4.68
	Xanthomonadales	Stenotrophomonas	5.97
Verrucomicrobiota	Opitutales	Lacunisphaera	2.15
	Pedosphaerales	unknown	4.23
	Pedosphaerales	unknown	3.05

study was peat with additional compost and wood fiber, and negative effects of peat on AMF have been reported in the literature [71,72]. Furthermore, based on an analysis of a separate onion batch, seedling roots were not mycorrhizal during outplanting. This leads us to presume that only a restricted amount of AMF symbionts accustomed to nursery conditions may have been introduced by the onion itself. It could however partly explain the similarities in AMF communities.

AMF can colonize plants from preexisting extraradical hyphae, root intraradical hyphae and spores. AMF hyphae can survive cold winters [73] but the absence of a host plant can kill the hyphae in five months [74]. In this experiment, preceding green manure crops were ploughed in October 2017 and onion was planted in May 2018. Thus, the AMF hyphae in the soil were six months without a host. In *L. albus* plots, the hostless period was even longer. Also, autumn ploughing is known to be detrimental to AMF hyphae [75]. Because of autumn ploughing, the long winter period without a host, spores, either indigenous or originating from the seedling growth substrate, were the most likely source of onion AMF colonization.

Mycorrhizal preceding crops have been observed to increase the amount of AMF spores in the soil [27,76]. AMF colonization was measured only at the end of the growing season, and thus it does not reflect initial effects of preceding crops in early mycorrhizal association of onions. The amount of soil NLFA 16:1ω5 has been shown to correlate with the amount of AMF spores [77], although some correlation between soil AMF hyphae and NLFA 16:1ω5 has also been reported [55]. In our study, there were no statistically significant differences between the NLFA 16:1ω5 content in soil samples in different seasons nor preceding crop treatments. This was somewhat surprising as we expected the soil spore concentration to be reduced during the cultivation of *L. albus* [24, 27,76]. However, some couch grass (*Elymus repens*) grew in the experimental plots that may have acted as hosts for AMF in the *L. albus* plots. Additionally, AMF spores can remain dormant and viable in the soil for several years [78] and buffer the changes in soil AMF inoculum. It is likely that in spring 2018, most spores in the *L. albus* plots were probably derived from earlier seasons, prior to 2017. One season of cultivating a non-host plant did not radically affect the AMF spore bank. Future studies should investigate the roles of spores as a source of inoculum, the stabilizing role of the spore bank against year-to-year fluctuations and the effect of different host species in longer crop rotations. Further, also the role of carry-over AMF from bulbs, seedling roots and even seeds [79] should be addressed carefully to evaluate their inoculum potential in boreal climate.

4.2. Differences in AMF species composition

Plants cause changes in the diversity and community composition of AMF by responding differently to individual fungal species [80]. However, this effect was not visible in our spring soil samples. We hypothesized that AMF communities in onion roots and soil would differ after different preceding crops. The preceding crop affected AMF species composition of autumn mycorrhizosphere soil samples and onion root samples, but the effect of season and sample type was even stronger, and the realized AMF communities were alike after different preceding crops.

AMF communities in roots were distinctive: The genera *Glomus* and *Claroideoglomus* were abundant in root samples compared to soil samples. Our results agree with observations of roots associating with members of Glomerales [81,82], and that onion favors *Glomus*-species [64]. In the study, 80% of the AMF taxa found in onion roots belonged to the genus *Glomus*, whereas only 10–22% of the taxa found in soil samples belonged to *Glomus*. Likely AMF established in roots then spread also to the mycorrhizosphere (e.g. *Clareideoglomus* species along with *Glomus mosseae*) as they were abundant in both roots and autumn soil

samples. The soil AMF communities resemble each other, and we also found the *Paraglomus* to be abundant in soil compared to the roots [82].

4.3. Differences in the broader fungal and bacterial communities

Utilization of green manure molds the fungal and bacterial communities by adding organic matter and nutrients to soil [8]. Long-term green manuring can accumulate several beneficial bacterial genera such as *Acinetobacter* and *Pseudomonas* [83]. Moreover, each plant produces specific chemical compounds that may include promotes of specific microbial groups or even inhibitory antimicrobial exudates. Of the four preceding crops used in our study, *T. patula* secretes antimicrobial and parasitic nematode inhibitory thiophene root exudates to the rhizosphere [10,13]. It is also the only plant that does not fix N. The amounts of Acidobacteria, Gemmatimonadetes and Proteobacteria increase in association with *Vicia villosa* and *Lupinus albus* [84,85]. As members of Fabaceae, *Lupin, Vicia* and *Trifolium* roots secrete promoters of rhizobia nodulation and various secondary compounds for nutrient uptake [9,85]: The gram negative proteobacteria from orders Rhizobiales and Burkholderiales fix N inside the legume root nodules. Especially *L. albus*, the AMF non-host, release huge amounts of organic acids and flavonoids to secure its nutrient uptake. This may have inhibitory effects on some microbes [29].

Thus, we found it surprising that no effect of preceding crop treatment on fungal OTU richness nor community composition was found. Previously, soil fungal communities have been found to be affected by changes in tillage intensity [3], which in our study was the same for all plots and could partly explain the lack of significant differences in the fungal community. *L. albus* plots had lower bacterial richness in spring. Nevertheless, no lasting preceding crop effects on bacterial community composition could be noted. The most striking observation was that we could not observe any significant difference in abundances of rhizobium bacteria after preceding crops *L. albus, T. incarnatum, V. sativa/V. villosa* compared to *T. patula*. In agreement with Cipollini et al. [15], our results suggest that the allelopathic compounds do not persist in soil over the winter. This led to the rejection of our third hypothesis that the preceding crop species and their symbiotic associations influence both fungal and bacterial communities in soil.

We observed seasonal shifts in the microbial communities. *Phenoliferia psychrophila* that is known to thrive in cold environments and is capable of degrading recalcitrant phenolic compounds [86,87], was one of the few bacteria abundantly present in soil in spring. Also, other features typical for spring were saprotrophic plant detritus decomposing soil fungi and soil-dwelling yeasts. In contrast, in the autumn mycorrhizosphere soil there were numerous signature species of which many were common soil-inhabiting filamentous and yeast fungi, including plant endophytes, pathogens and saprotrophs. Several known onion pathogens were abundant in autumn mycorrhizosphere soil, such as the leaf blight causing *Alternaria alternata* [88] and *Stemphylium vesicarium* [89], the bulb rot causing fungi from the *Gibberella fujikuroi* (*Fusarium proliferatum/fujikuroi* [90]) species complex and the blue mold causing *Penicillium* species.

Many bacterial OTUs benefited during the growth season from increased food supply provided by plants, fungi and soil fauna. This was seen in the increase of bacteria belonging to the orders Chitinophagales, Flavobacteriales, Enterobacterales, Pantoea, Rhizobiales, Sphingomonadales, Sphingobium, Xanthomonadales and Rhodanobacter over the season. These taxa include species commonly isolated from soil and root nodules.

5. Conclusions

One season of cultivation of non-host preceding green manure crops did not have a strong effect on AMF colonization and microbial diversity of seed onion roots or in the surrounding soil. Onions had high AMF colonization and diversity irrespective of the four different preceding crops. Based on the results it seems that one season is a too short time to influence the AMF community of organic fields under conventional tillage under boreal climate conditions. The soil AMF community appears to have tolerance for temporal disturbances. AMF might also find a refuge in weed hosts or can be transferred to the cultivation system along with the crop plant bulbs, seedlings, or seeds. Therefore, non-host preceding crops can be used especially in rotations together with mycorrhizal plants. The plant allelopathic compounds were not influencing the spring soil microbial communities, but legacy effects trough microbes cannot be totally excluded as preceding crops seemed to affect the autumn AMF community composition and need to be studied in more detail in a controlled environment. Fungal communities were rather stable throughout the experiment and seasonal fluctuations override the possible effects of preceding crop plants even in the spring soil samples. Also, the bacterial communities showed very little effect, with only the spring bulk soil OTU richness being affected by the preceding crop treatment. This result is probably due to the boreal climate where the long winter diminish the possible differences that appear during the growth season.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejsobi.2021.103335.

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