Plant-soil interactions determine ecosystem aboveground and belowground processes in primary dune ecosystems

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Chapter 2

Shifts in soil community influence the establishment of arbuscular mycorrhizal fungi

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Abstract

Arbuscular mycorrhizal (AM) fungi interact with multiple soil communities under natural conditions. Several studies have demonstrated that specific groups of soil organisms influence the symbiotic interactions between plant roots and AM fungi. However, the influence of entire soil communities on AM fungal communities remains rarely understood. Here, we tested the influence of alternation in entire soil communities on AM fungal composition, root-AM fungal association and growth of phytometer plant *Plantago lanceolata* by using the soil inoculation method in a field experiment. The effects of shifts in soil community on the root AM fungal composition were detectable one year later, whereas the impact on soil AM fungal communities was not apparent anymore. In addition, these treatments had no significant impacts on root AM fungal colonization intensity and performance of the host plant growth, except for an increase in root biomass. We conclude that the legacy effects of changes in soil communities on soil AM fungi fade away gradually but persist in plant roots which might be a less competitive environment than soils in a short term.

Highlights

- Effect of changes in the whole soil community on the establishment of AM fungi was studied in a field experiment.
- The legacy effects of altering soil community on AM fungal composition remained persistent in the roots of *P. lanceolata* for a longer time than in soil during early ontogeny.
- The alteration in the root AM fungal composition did not influence plant performance which relied more on the nutrient availability in the early ecosystem.
- The establishment of AM fungi was associated with the structure of the soil community despite no response in the colonization percentage of the root AM fungi.

2.1 Introduction

AM fungi are a ubiquitous group of soil fungi capable of establishing a symbiotic association with approximately 78% of vascular plant species from terrestrial ecosystems (Tedersoo *et al.* 2020). The mycorrhizal symbiosis has multiple beneficial influences on plant nutrient and water acquisition as well as plant protection against various stresses (Jia et al., 2020; Qiu et al., 2022; van der Heijden et al., 2008). Although the establishment and functioning of the plant-AM fungal association have been studied extensively (Šmilauer et al., 2020; van der Heijden et al., 2015), most studies have focused on understanding the influence of abiotic factors including nutrient availability, climate and environmental conditions on the plant-AM fungal association (Kim *et al.* 2015; Soudzilovskaia *et al.* 2015). How AM fungi and other members of the soil community interact and how these interactions drive ecosystem functioning is less well understood (Bonfante and Anca, 2009; Ferreira et al., 2021; van der Heijden et al., 2015).

In natural ecosystems AM fungi are surrounded by complex soil microbial communities. The interaction between AM fungi and other soil microorganisms can assist the formation of AM fungal symbioses with plants (Kapulnik 2000; Bonfante & Anca 2009; Miransari 2011). For example, the "mycorrhiza helper bacteria" (MHB) can form biofilms on AM fungal spores and mycelia and positively influence the establishment of AM fungi (Garbaye 1994; Frey-Klett *et al.* 2007). These bacteria influence AM fungal activities and functioning through promoting AM fungal spore germination, improving mycelia growth and enhancing host recognition (Bruzos 2017; Bastías *et al.* 2020). The exudates of soil microorganisms can also promote AM fungal spore germination and mycelial growth, thereby stimulating the AM fungal colonization percentage (Scervino *et al.* 2009). In addition, recent research indicates that fungal antagonists, such as Acidobacteria, may compete with AM fungi for resources, resulting in negative impacts on AM fungi (Svenningsen *et al.* 2018).

The importance of the interactions between AM fungi and other soil organisms on plant growth and consequently ecosystem functioning is increasingly recognized (Becklin *et al.* 2012; Ferreira *et al.* 2021). The variable interactions between AM fungi and other soil microbiota suggest that the changes in the structure and diversity of the soil community may either promote or inhibit AM fungal colonization (Duffy *et al.* 2007; Wagg *et al.* 2014). However, it is challenging to decipher the interactions between AM fungi and the whole soil community due to the difficulty in manipulating soil community composition under natural conditions. Here, we addressed this challenge by using a novel method of soil inoculation (Wubs *et al.* 2016, 2018) in the field to experimentally investigate how changes in soil community influence the AM fungal community composition, the intensity of root AM fungal colonization and growth of host plant. Earlier field experiments demonstrated that inoculation with soil communities originated from distinct ecosystems can substantially change the soil microbial structure and composition (Wubs *et al.* 2016, 2019), and this

method is effective for examining the effects of whole soil community composition on plant growth and composition (Middleton & Bever 2012; van de Voorde *et al.* 2012; Wubs *et al.* 2016).

The main aim of our study was to investigate how changes in soil communities influence the AM fungal establishment. We manipulated soil communities of a sandy dune ecosystem by introducing living soil inocula and sterilized soil inocula from different donor dune ecosystems, including primary dunes, dune grasslands and dune forests into a new artificially created dune ecosystem. We used a common dune vascular plant *Plantago lanceolate*, a forb which is highly associated with AM fungi (Ayres *et al.* 2006) as the AM fungal phytometer plant to address whether the shifts in soil communities influence AM fungal establishment after soil inoculation. We assessed the community composition of AM fungi in soil and root of *P. lanceolata*, root colonization percentage by AM fungi, the biomass and functional root traits of *P. lanceolata*. We hypothesized that: (1) Alternation in the soil community via soil inoculation would influence AM fungal composition in soil and plant root. Specifically, the soil inocula originated from later succession (e.g. dune grasslands, dune forests) had stronger effects on the AM fungal composition; (2) The presence of added soil biota (living soil inocula) would result in stronger effects on the AM fungal composition; (3) manipulation of soil communities through soil inoculation would affect the colonization intensity of host roots and plant performance.

2.2 Materials and methods

- *Experimental design*

The experiment was carried out in the TERRA-Dunes experiment in 2019. The outline of the experimental design is presented in general introduction chapter of this thesis (Chapter 1).

- *Collection and measurements of samples*

Plant sampling was conducted in July 2019. First, we counted the leaves of all *P. lanceolata* individuals in each plot and then selected three individuals that had the same number of leaves as the average number of leaves per plant in that plot. The selected plants in each plot were carefully excavated to obtain the whole plant with an intact root system and then put into plastic bags with dry ice. In the lab, the host plants were thoroughly cleaned under tap water. For each sample, the plants were divided into two parts; roots and aboveground shoots. Aboveground shoots were dried at 65°C for 48 h and weighed. All roots in each sample were cut into 1-2 cm sections and mixed for later measurements. 0.1 g sections of fine root were selected from each sample and kept in 50% ethanol to measure the intensity of AM fungal colonization (AMFC). Subsamples of 0.5 g fresh roots were stored at -20 °C for quantification of the AM fungi. The remaining roots were weighed and scanned using a

Cannon LiDE 210 scanner (600 dpi). Thereafter, the root samples were oven-dried at 60°C for 48 hr and weighed. Total root length, volume and average diameter (AD) were determined using the scanned images by the software of WinRhizo (Regent Instruments, Quebec, Canada). Specific root length (SRL) was calculated as root total length divided by its dry mass. Root tissue density (RTD) was calculated as root volume divided by its dry mass. We estimated the AM fungal colonization percentage by quantifying the degree to which plant roots were colonized by AM fungal structures. The AM fungal structures were stained with Trypan blue using a standard protocol (Freschet et al., 2021). Roots were cleared with 5% KOH solution in a 75 °C water bath for 30 minutes. Roots were then acidified in 1% HCl solution for 30 minutes and subsequently stored in 0.01% Trypan blue for 30 minutes in a 75 °C water bath. Roots were stored in 50% glycerol for microscopic investigation. The AM fungal colonization percentage of the roots was estimated according to the gridline intersection method (McGonigle et al., 1990).

Soil samples were collected using a soil corer from 9 locations (two at each side of the plot and one in the center) in each plot, pooled, homogenized and immediately transported to the lab within an ice box. Each soil sample was further divided into two parts. Ten grams of the soil was stored at -20 °C for molecular analysis. The rest was sieved and kept for chemical analysis. Total C and N were analyzed using a Flash EA 1112 elemental analyzer (Thermo Scientific, Rodana, Italy). Mineral N was extracted by shaking 3 g dry soil in 30mL 0.01M $CaCl_2$ solution for two hours at 250 rpm, centrifuged for 10 min at 300 rpm and NO_3^- -N and NH_4^+ -N content were determined in the supernatant using a Skalar Continuous Flow Analyzer. Micronutrients were determined on the ICP-OES with 130µL 69% HNO_3. The complete results of soil chemistry can be found in Table S2-1, indicating an overall higher nutrient availability in plots inoculated with dune grassland soils and dune forest soils, compared to that of primary dunes.

- *AMF community composition in the roots*

Root samples were analyzed with 40 selected plots that enable at least 4 replications under soil inoculation treatments (Table S2-2). Root DNA was extracted from root samples of P. lanceolata using the Power Plant DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and cleaned with OneStep PCR Inhibitor Removal kit (Zymo Research). A nested PCR targeting the 18S rRNA region was conducted to amplify AM fungi using two primer pairs NS31/AML2 (NS31: 5'-TTGGAGGGCAAGTCTGGTGCC-3'; AML2: 5'-GAACCCAAACACTTTGGTTTCC-3') (Morgan and Egerton-Warburton, 2017) and NS31_glo3ngs/AML2 (NS31_Glo3: 5'-TTGYTGCRGTTAAAAAGCTCG-3'; AML2: 5'-GAACCCAAACACTTTGGTTTCC-3') (Kolaříková et al., 2021). The first PCR was performed with primer pair NS31/AML2 within a 25-µl reaction system using the following PCR conditions: initial denaturation at 98 °C for 30 s, followed by 40 cycles of denaturation at 98 °C for 10 s, annealing at 63 °C for 30 s,

extension at 72 °C for 30 s, and a final extension at 72 °C for 10 min. Subsequently, the products from the first PCR (5-50 ng) were used as a template for the second PCR using the second primer pair NS31_glo3ngs/AML2 with the same conditions except for 30 cycles for the second step. Gel electrophoresis was performed on all amplicons to confirm the amplicon size and quality, and the DNA concentration of each sample amplicon library was checked with Qubit 2.0 fluorometer (Life Technologies), followed by pooling and purification of amplicons with MinElute PCR Purification Kit (Qiagen). Finally, the end pool for all amplicons was processed for illumina MiSeq sequencing to generate 2×250 base paired-end reads.

- *The biomass and composition of soil AM fungal community*

Biomass of soil AM fungal community was estimated with the samples collected in September 2018 and 2019, using neutral lipid fatty acid analysis (NLFA). Extracted phospholipids were separated using solid-phase extraction cartridges (LiChrolut Si 60, Merck) and samples were subjected to mild alkaline methanolysis (Šnajdr *et al.* 2008). The resulting phospholipid fatty acid and neutral lipid fatty acid methyl ethers were measured by gas chromatography-mass spectrometry (450-GC,240-MS ion trap detector, Varian, Walnut Creek, CA, USA). The content of AM fungal biomass was estimated using the 16:1 ω 5 concentration in the NLFA fraction (Bååth 2003).

Genomic DNA was extracted from soil samples collected in all plots in 2019 using the PowerSoil Plant DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The nuclear internal transcribed region (ITS2) was targeted for PCR reaction. A fungal universal primer pair gITS7/ ITS4 (gITS7: 5'-GTGARTCATCGARTCTTTG -3' (Ihrmark *et al.* 2012); ITS4: 5'-TCCTCCGCTTATTGATATGC -3') (White *et al.* 1990) was used to amplify the ITS2 region using the following PCR conditions: initial denaturation at 94 °C for 30 s, followed by 35 cycles of denaturation at 94 °C for 10 s, annealing at 49 °C for 30 s, extension at 72 °C for 40 s, and a final extension at 72 °C for 10 min. Gel electrophoresis was performed on all amplicons to confirm the amplicon size and quality, and the DNA concentration of each sample amplicon library was checked with Qubit 2.0 fluorometer (Life Technologies), followed by pooling and purification of amplicons with MinElute PCR Purification Kit (Qiagen). Finally, the pool of amplicons was used for sequencing library preparation with the TruSeq PCR-free kit (Illumina) and sequenced on illumine MiSeq to generate 2×250 base paired-end reads.

After demultiplexing based on Nextera indexes, paired reads of each sample were merged and poor quality sequences (error rate > 0.5) were filtered by Vsearch. Merged sequences were separated based on primer sequences and subsequently trimmed from primers using CUTADAPT 1.0 (Saeidipour & Bakhshi 2013). Chimeric sequences were trimmed by the

UCHIME chimera detection program (de novo algorithm) (Edgar *et al.* 2011). After quality filtering and chimera removal, fungal OTUs were clustered based on a 97% similarity threshold using Vsearch. Global singletons (i.e., OTUs representing only one sequence in the whole dataset) were removed because they may reduce the accuracy of diversity estimates (Ihrmark *et al.* 2012; Waud *et al.* 2014). The remaining OTUs were assigned with taxonomic identities to the highest taxonomic rank possible by Usearch using the latest released Unite reference dataset (utax_reference_dataset_10.05.2021.fasta) as annotation resources. A total of 487,104 high-quality-filtered sequences were obtained from all soil samples, of which 3,173 sequences were identified as AM fungal taxa of Glomeromycota (Van Der Heijden *et al.* 2015).

- *Data analysis*

To allow for a full factorial analysis, all 22 control plots were a-*priori* randomly assigned as controls associated with living or sterile soil inocula. We examined the composition of AM fungi in plant roots and soils separately at the family level (estimated with the number of reads of each AM fungal family). Before this analysis, samples that had no OTUs were removed (for root, 2 samples were removed; for soil, 10 samples were removed). To test the effect of the soil inoculation treatments on the AM fungal community composition, we applied permutational analysis of variance (PERMANOVA) based on a Bray-Curtis dissimilarity matrix in R package "vegan" (Oksanen *et al.* 2013). A pair-wise PERMANOVA analysis was applied to examine significant differences within in a factor (Martinez Arbizu 2017). The AM fungal community structure was visualized using non-metric multidimensional scaling (NMDS) through the "metaMDS" command in R package "vegan". The impacts of the imposed treatments on the species-level AM fungal composition were also analyzed with the same method (Table S2, Table S3). We used generalized linear mixed model with the data on individual AMF families to further explore which AM fungal families in plant roots were affected by soil treatments. A generalized linear mixed model was used because the data were not normally distributed. Since the data had a form of counts and the data was overdispersed, we specified the family as "quasi-poisson" in the Generalized-linear mixed model (Pineda *et al.* 2020). We then used the "anova()" function with "F test" for the analysis of deviance for the model fits.

Effects of soil inoculation treatments on root AM fungal colonization percentage, biomass and root traits of the host plant, and soil AM fungal biomass determined via NLFA were tested using a two-way ANOVA. Model assumptions of normality and homoscedasticity were checked on the model residuals (Kozak & Piepho 2018) and variables were transformed when necessary to meet the assumptions of residuals homogeneity. In the case where the effect of model parameter(s) was significant in an ANOVA, a post-hoc test was performed using the lsmeans package, with the Turkey method for p-value adjustment (Lenth 2016). All analyses were performed in R version 4.0.2 (R Core Team 2020).

2.3 Results

- *Root-AM fungal community*

PERMANOVA analysis revealed that the root-associated AM fungal composition was significantly influenced by soil inocula origin (pseudo-F = 2.42, p = 0.04, 17% explained variation, Table 2.1; pseudo-F = 1.51, p = 0.04, 12% explained variation, Table S2-4) (Figure 2.1a). There were significant differences (Pairwise PERMANOVA, p=0.04) between the pair of control and grassland soil inocula (Table S2-4). There was no significant difference in the AMF composition subjected to sterilization treatment (pseudo-F = 1.92, p = 0.15, Table 2.1; pseudo-F = 0.80, p = 0.64, Table S2-3). Soil inocula origin significantly influenced the abundance of Claroideoglomeraceae, Diversisporaceae, Gigasporaceae, Paraglomeraceae, Archaeosporaceae and unclassified families in the root of phytometer plant (Table 2.2). There was no overall effect of the added soil biota (i.e. inoculation in absence of the sterilization treatment) on community composition, while the abundance of Diversisporaceae, Gigasporaceae, Paraglomeraceae were significantly influenced by soil sterilization (Table 2.2). Among the AM fungi, Glomeraceae and Claroideoglomeraceae were the dominant families (Figure 2.2; Figure S2-1). The Glomeraceae had the highest relative abundance in control plots without soil inocula, while the second most abundant family, Claroideoglomeraceae was abundant in plots with soil inocula originated from dune grasslands (Figure 2.2). The abundance of neither of these dominant families was influenced by soil sterilization treatment.

Table 2.1 Summary statistics of a Permanova, testing the effects of soil inocula orogin (Inoculum, I), soil sterilization (Sterilization, S) and their interactions on the AMF community family composition in the root of *P. lanceolata* and soil (Inoculum, I. Sterilization, S). Presented are degrees of freedom, variance explained (R^2), F-values and *p*-values. Significant effects ($p < 0.05$) are presented in bold.

Taxonomy	Treatments	df1,df2	F-value	R^2	*p*-value
Root AM fungi	**Inoculum**	**3, 37**	**2.42**	**0.17**	**0.04**
	Sterilization	1, 37	1.92	0.05	0.15
	I x S	3, 37	0.93	0.07	0.49
Soil AM fungi	Inoculum	3, 83	0.78	0.03	0.67
	Sterilization	1, 83	1.18	0.01	0.33
	I x S	3, 83	0.49	0.02	0.93

Table 2.2 Effects of soil inocula origin (Inoculum, I), soil sterilization (Sterilization, S) and their interaction on the abundance of root-associated AM fungal families measured as read number. Significant effects ($p < 0.05$) are presented in bold.

	Variance					
	Inoculum		Sterilization		I × S	
	F	*p*	*F*	*p*	*F*	*p*
Claroideoglomeraceae	**8.52**	**<0.01**	0.01	0.99	0.74	0.54
Diversisporaceae	**19.95**	**<0.01**	**38.79**	**<0.01**	0.03	0.99
Gigasporaceae	**7.67**	**<0.01**	**15.58**	**<0.01**	0.68	0.57
Glomeraceae	1.25	0.31	0.34	0.56	0.68	0.60
Paraglomeraceae	**9.53**	**<0.01**	**14.28**	**<0.01**	0.00	1.00
Archaeosporaceae	**5.85**	**<0.01**	**6.25**	**0.02**	0.03	0.99
Unclassified	**6.35**	**<0.01**	0.01	0.94	0.03	0.99

When a generalized mixed linear model was used, no residual df is given because of computational issues.

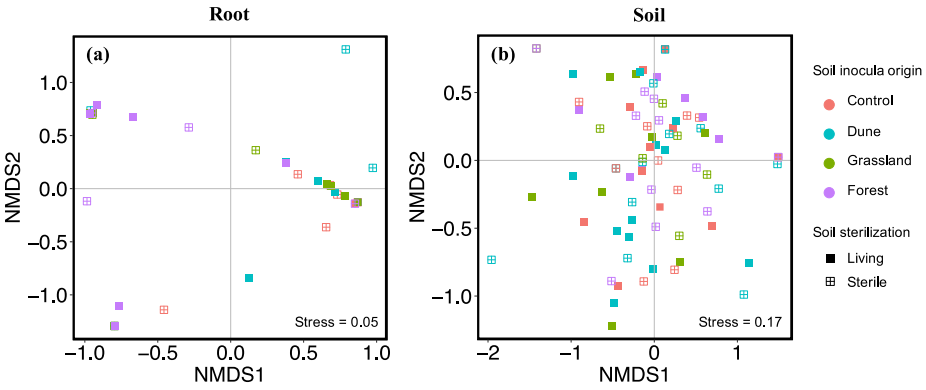

Figure 2.1 Non-metric multidimensional scaling (NMDS) ordination plots showing arbuscular mycorrhizal fungal community composition in response to soil inocula origin and soil sterilization in root (a) and soil (b) based on relative proportions of AM fungal families.

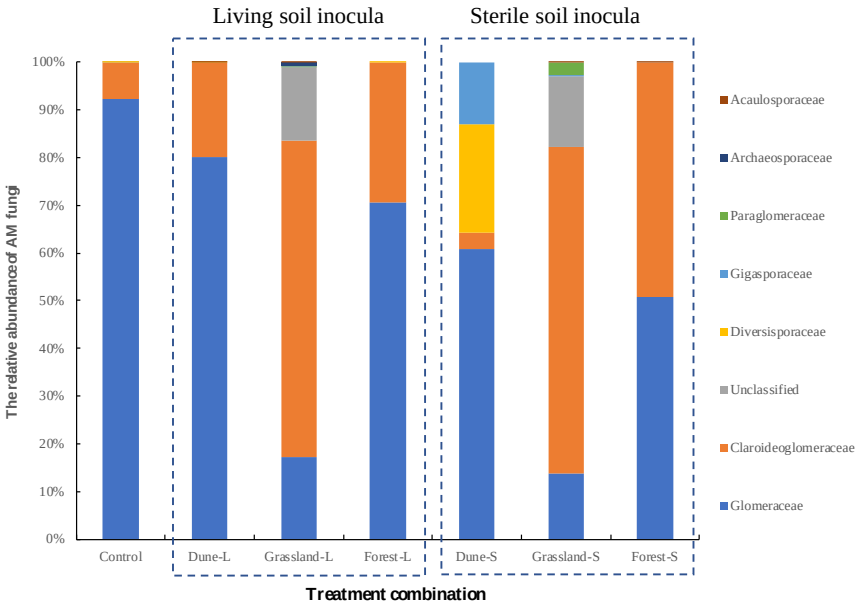

Figure 2.2 The abundance of root-associated AM fungal families (measured as read number) in each treatment combination. *Control* no addition of soil inocula; *Dune* addition of soil inocula originated from primary dune ecosystems; *Grassland* addition of soil inocula originated from dune grasslands; *Forest* addition of soil inocula originated from dune forests; *L* addition of living soil inocula; *S* addition of sterile soil inocula.

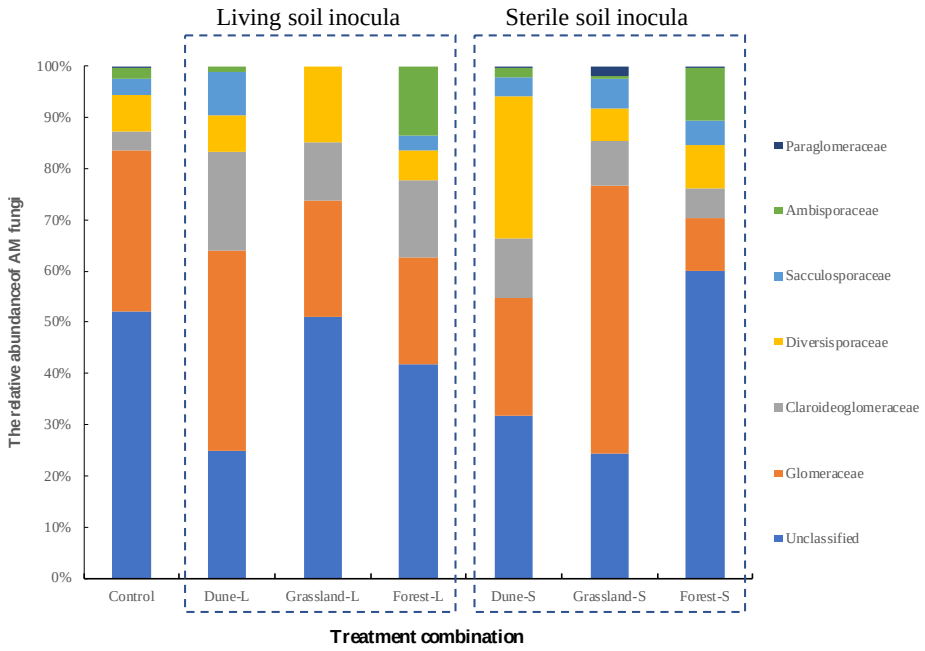

Figure 2.3 The abundance of soil AM fungal families (measured as read number) in each treatment combination. *Control*: no addition of soil inocula; *Dune*: addition of soil inocula originated from primary dune ecosystems; *Grassland*: addition of soil inocula originated from dune grasslands; *Forest*: addition of soil inocula originated from dune forests; *L*: addition of living soil inocula; *S*: addition of sterile soil inocula.

- *Soil AM fungal community*

Based on PERMANOVA, we found that soil inocula origin (pseudo-F = 0.78, p = 0.67, Table 2.1; pseudo-F = 0.93, p = 0.63, Table S2-3) and soil sterilization (pseudo-F = 1.18, p = 0.33, Table 2.1; pseudo-F = 0.81, p = 0.71, Table S2-3) had no influences on the soil AM fungal composition. While, consistent with the root AM fungal composition, the Glomeraceae and Claroideoglomeraceae were the most abundant families in soil among the classified OTUs (Figure 2.3). The soil inocula origin significantly influenced soil AMF abundance in 2018 ($F_{3,86} = 4.35$, $p < 0.01$, Table 2.3). The highest AM fungal abundance occurred in plots with living soil inocula from dune grasslands (Figure 2.4a). Yet, soil inocula origin did not influence soil AM fungal biomass in 2019 ($F_{3,86} = 0.17$, $p = 0.92$, Table 2.3). Similarly, PERMANOVA results indicated that soil inocula origin had no significant impact on the soil AM fungal composition (Table 2.1 and Table S2-3).

Table 2.3 Effects of soil inocula origin (Inoculum, I), soil sterilization (Sterilization, S) and their interaction (I × S) on the biomass of AM fungal communities in soil, as revealed by 2018 and 2019 NLFA analysis. Outcomes of a two-way ANOVA (F, F-value; *p*, P-value; η^2, eta squared). Significant effects ($p < 0.05$) are presented in bold. Data were transformed for normality using squared root.

Year		AMF biomass in soil		
		F	P	η^2
2018	**Inoculum**	**4.35**	**<0.01**	**0.006**
	Sterilization	0.19	0.66	<0.001
	I × S	2.58	0.06	0.028
2019	Inoculum	0.17	0.92	0.006
	Sterilization	0.05	0.82	<0.001
	I × S	0.83	0.48	0.028

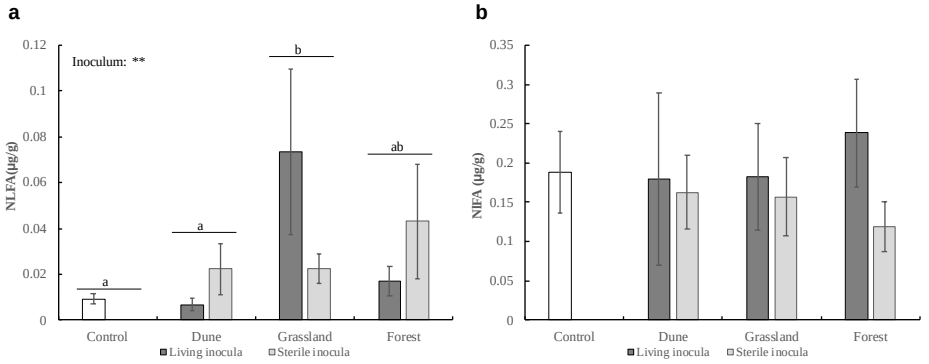

Figure 2.4 Effects of different soil inoculation treatments on (a) AM fungal abundance in 2018, (b) AM fungal abundance in 2019. ***p*<0.01, Data are means ± SE. The white bar indicates the control plots with no soil inoucla, the black bar indicates plots with living soil inocula, and the grey bar indicates plots with sterile soil inocula.

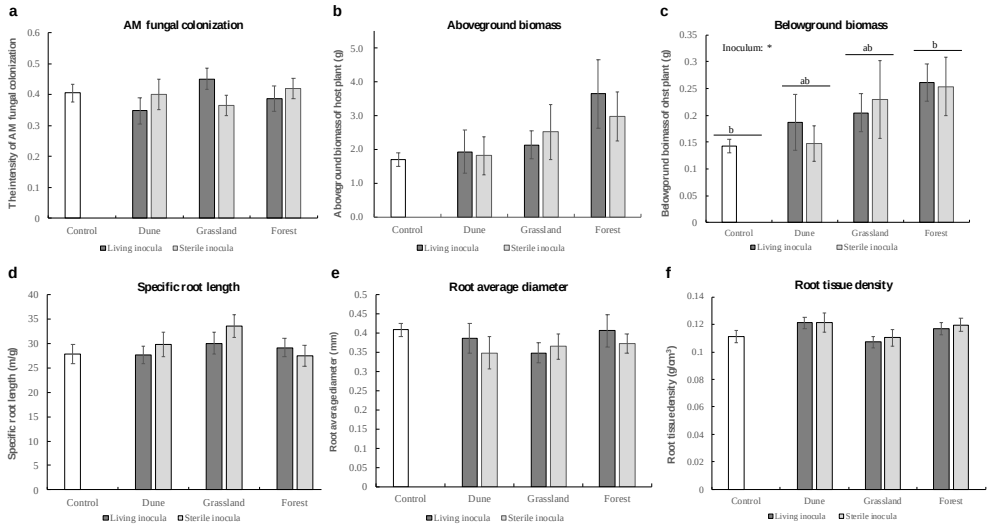

Figure 2.5 Effects of different soil inoculation treatments on (a) AM fungal colonization, (b) aboveground biomass, (c) belowground biomass, (d) specific root length, (e) root average diameter, (f) root tissue density of *P. lanceolata* growing in field plots. **p*<0.05, Data are means ± SE. The white bar indicates the control plots with no soil inoucla, the black bar indicates plots with living soil inocula, and the grey bar indicates plots with sterile soil inocula

- *Root-AM fungal colonization and plant growth*

Contrary to our expectations, soil inocula origin did not influence root-associated AM fungal colonization percentage ($F_{3,86}$ = 0.32, p = 0.81, Table 2.4). Soil inocula origin significantly influenced belowground biomass ($F_{3,86}$ =3.32, p = 0.02, Table 2.4) and marginally affected aboveground biomass ($F_{3,86}$ =2.48, p = 0.07, Table 2.4) of the phytometer plants, with soil sterilization treatment having no effect. Belowground biomass of the *P. lanceolata* was highest in plots with soil inocula that originated from dune forests (Figure 2.5c) which contain more nutrient-rich soil (Table S2-1). There were no significant differences in root traits under different soil inoculation treatments (Table 2.4), besides root tissue density (RTD) being marginally affected ($F_{3,86}$ =2.40, p = 0.07) by soil inocula origin. RTD was highest in plots with soil inocula from early dune ecosystems (Figure 2.5f).

2.4 Discussion

In this study, we explored the effects of changes in whole soil communities on the establishment of AM fungal communities. Our findings show that the legacy effects of changes in soil communities on AM fungi fade away in soil but can be detected in the roots of *P. lanceolate* one year after soil inoculation treatments. Specifically, such changes modified the composition of root-associated AM fungi. This is important as it indicates that

the activity of AM fungi is associated with the structure of the soil community, in particular with those components related to the rhizosphere. At the same time, we did not detect changes in root AM fungal colonization percentage in response to changes in soil communities, suggesting that there might be functional redundancy in the root-associated AM fungal community (Hart & Reader 2002; Maherali & Klironomos 2007; Gosling *et al.* 2016).

Although we found that soil inoculation treatments affected the soil AM fungal abundance after the inoculation in 2018 (Table 2.3, Figure 2.4a), the legacy effect of soil inocula on soil AM fungi disappeared in 2019 (Table 2.3, Figure 2.4b). Yet, shifts in soil community composition significantly influenced the root AM fungal composition in 2019. This suggests that the soil legacy effects derived from soil inoculation on AM fungal communities may be transferred during the early stages of establishment of the plants and that they persist longer in host plants than in the associated soil. Compared to the soil, plant roots might constitute AM fungi a less competitive environment, and therefore root community composition is much easier to manipulate. Similar patterns were also found in a recent study in which soil bacterial legacies faded away but were still conserved inside the roots six months after the establishment of the plants (Hannula *et al.* 2021). The difference in the activity of AM fungi in soil and host plant root may contribute to such pattern. In addition, it needs to be noticed that roots were likely to have more active AM fungi, whereas active, inactive, or even dead AM fungi were hard to distinguish by DNA-based sequencing method in soil (Hempel *et al.* 2007; Bainard *et al.* 2014; Babalola *et al.* 2022). Therefore, AM fungal communities in soil showed less responsive to the shifts in the soil community through soil inoculation treatments (Babalola *et al.* 2022). Collectively, our study suggests that the differences in the available pool of microbes (in our case obtained via soil inoculation) can result in different communities inside the roots. Further studies should examine these driver/passenger roles of roots and soil microbes in more detail.

In contrast to our expectations, we did not detect an effect of soil inoculation treatments on the intensity of root AM fungal colonization. This may result from functional redundancy among AM fungal species (Hart & Reader 2002; Maherali & Klironomos 2007). Previous studies have suggested that the intensity of root AM fungal colonization depends on the taxonomic identity of the AM fungal species and the functional traits that characterize them (Hart & Reader 2002; Chagnon *et al.* 2013). Adding other AM fungal species with the same functional attributes may have minor impacts on plants. Second, the intensity of AM fungal colonization featured by the phytometer plant depends on multiple AM fungal species and some of them only provide benefits under stress (Lekberg & Koide 2014). For instance, Gosling *et al*. (2016) found that the beneficial effects of some AM fungal species were apparent only when the host plant was faced with stress. Furthermore, some AM fungal species might not benefit plants despite being present within an AM fungal community (Hart *et al.* 2013), without providing benefits to the host (Yang *et al.* 2017). Finally, we observed

that soil inoculation treatments did not affect the abundance of dominant AM fungal species of the Glomeraceae family in root or soil (Table 2.2)(Figure S2-1), while species of this family typically have high growth rates (Hart & Reader 2002; Chagnon *et al.* 2013).

Although soil inocula did not affect the intensity of AM fungal colonization, the phytometer plants had higher biomass in plots with added soil inocula, especially in plots with the most nutrient-rich soil inocula (originated from dune grassland and forest), even though we added a minimal amount of soil inoculum to each plot only. This suggests that the establishment of *P. lanceolata* may rely more on the soil nutrients than on the root AM fungal community. Our experiment was conducted in an early-successional bare sandy site with limited soil nutrients (Table S2-1). Under such harsh circumstances, nutrients strongly limit plant growth. Although the association with AM fungi can improve plant nutrient acquisition (Smith & Read 2008), investment in forming such relationship may overwhelm plant nutrient strategy in the early successional stage. Plants are likely to invest less in a mutualism that has short-term costs but long-term benefits (Allen & Allen 1984, 1988; Hoeksema *et al.* 2010).

2.5 Conclusions

This empirical study under natural conditions provides evidence that the legacy effects of changes in soil communities on AM fungal composition remain persistent in plant roots but faded away in soil one year later. This finding indicates that the establishment of AM fungi is associated with the structure of the soil community despite no responses in the colonization percentage of the root AM fungi. Furthermore, in contrast to prior studies that demonstrate the beneficial role of AM fungi on plant performance, our study indicated the performance of *P. lanceolata* in an early ecosystem is more dependent on small-scale differences in nutrient availability than on AM fungi.

2.6 Acknowledgements

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2.7 Author contributions

CG, NAS and PMB conceived the idea. NAS, TMB, RM and HH established the experiment of TERRA-Dunes. CG, RM and PK collected and processed the plant and soil samples. CG analyzed the data. CG wrote the first draft, and all authors contributed to editing the manuscript.

2.8 Supporting information

Table S2-1 Mean (± *SE*) for soil abiotic conditions for plots exposed to different soil inocula origins (primary dunes, dune grasslands, and dune forests) and soil sterilization treatments.

	Dune		Grassland		Forest		Control
	Living	Sterile	Living	Sterile	Living	Sterile	No inocula
Fe (mg/kg)	0.14 ± 0.039	0.10 ±0.029	0.12 ± 0.018	0.13 ± 0.013	0.19 ± 0.017	0.16 ± 0.015	0.07 ± 0.006
P (mg/kg)	0.66 ± 0.047	0.64 ± 0.053	0.90 ± 0.048	0.82 ± 0.059	1.09 ± 0.053	1.01 ± 0.073	0.58 ± 0.036
Zn (mg/kg)	0.04 ± 0.003	0.04 ±0.003	0.08 ±0.011	0.07 ±0.009	0.09 ±0.012	0.07 ±0.011	0.03 ±0.003
S (mg/kg)	1.80 ± 0.102	1.66 ± 0.077	2.27 ± 0.177	2.04 ± 0.143	2.73 ± 0.151	2.40 ± 0.144	1.86 ± 0.394
K (mg/kg)	11.20 ± 0.937	10.60 ± 0.555	12.00 ± 0.527	10.40 ± 0.512	14.80 ± 1.280	14.30 ± 1.270	9.85 ± 0.418
Mg (mg/kg)	7.73 ± 0.334	7.94 ± 0.463	15.00 ± 1.13	13.80 ± 1.28	19.80 ± 1.450	19.60 ± 1.940	6.04 ± 0.150
Nitrogen (NO_2^-+NO_3^-) (mg/kg)	3.97 ± 0.245	3.08 ± 0.187	3.73 ± 0.109	3.18 ± 0.109	3.56 ± 0.265	2.97 ± 0.163	3.40 ± 0.152
Nitrogen (NH_4^+) (mg/kg)	6.19 ± 0.589	5.53 ± 0.523	7.24 ± 0.954	5.64 ± 0.656	8.94 ± 1.080	8.03 ± 0.787	4.35 ± 0.302
%N	0.03 ± 0.005	0.05 ± 0.026	0.05 ± 0.010	0.05 ± 0.010	0.05 ± 0.026	0.06 ± 0.008	0.03 ± 0.003
%C	0.59 ± 0.054	0.820 ± 0.284	0.80 ± 0.148	0.91 ± 0.151	1.02 ± 0.153	0.88 ± 0.117	0.56 ± 0.050

Table S2-2 Selected 40 plots for examining the root AMF composition.

	Soil sterilization	
Soil inocula origin	**Living**	**Sterile**
Control (no soil inocula)	10,23,29,45,50,54,58,78,80,86	
Dune	12,15,33,77	19,49,56,68
Grassland	3,24,44,47,67,97	31,53,88,93
Forest	18,21,22,59,89,92	41,48,63,71

At the beginning, the EMF treatment was also considered in the selection of sampling plots. Hence the current selected samples were not balanced under soil inocula origin and sterilization treatments. But this did not influence the statistic power because there were at least four replicates for each treatment.

Table S2-3 Summary statistics of a Permanova, testing the effects of soil inocula origin (Inoculum, I), soil sterilization (Sterilization, S) and their interactions on the AMF community composition in the root of *P. lanceolata* and soil. Presented are degrees of freedom, variance explained (R^2), F-values and p-values. Significant effects (p<0.05) are presented in bold.

Taxonomy	Treatments	df1,df2	F-value	R^2	p-value
Root AM fungi	**Inoculum**	**3, 37**	**1.51**	**0.12**	**0.04**
	Sterilization	1, 37	0.80	0.02	0.64
	I x S	3, 37	0.77	0.06	0.84
Soil AM fungi	Inoculum	3, 83	0.93	0.03	0.63
	Sterilization	1, 83	0.81	0.01	0.71
	I x S	3, 83	0.87	0.03	0.77

Table S2-4 Results of the pairwise PERMANOVA analysis of AM fungal composition (Bray- Curtis dissimilarity indices) between different types of soil inoculum. Significant effects (p<0.05) are presented in bold.

Pairs	df	F	R^2	p-adj
Control -Dune	1	0.50	0.0.3	1.00
Control-Grassland	**1**	**3.53**	**0.17**	**0.04**
Control-Forest	1	1.57	0.08	0.53
Dune-Grassland	1	2.60	0.13	0.07
Dune-Forest	1	0.88	0.05	1.00
Grassland-Forest	1	1.75	0.09	0.53

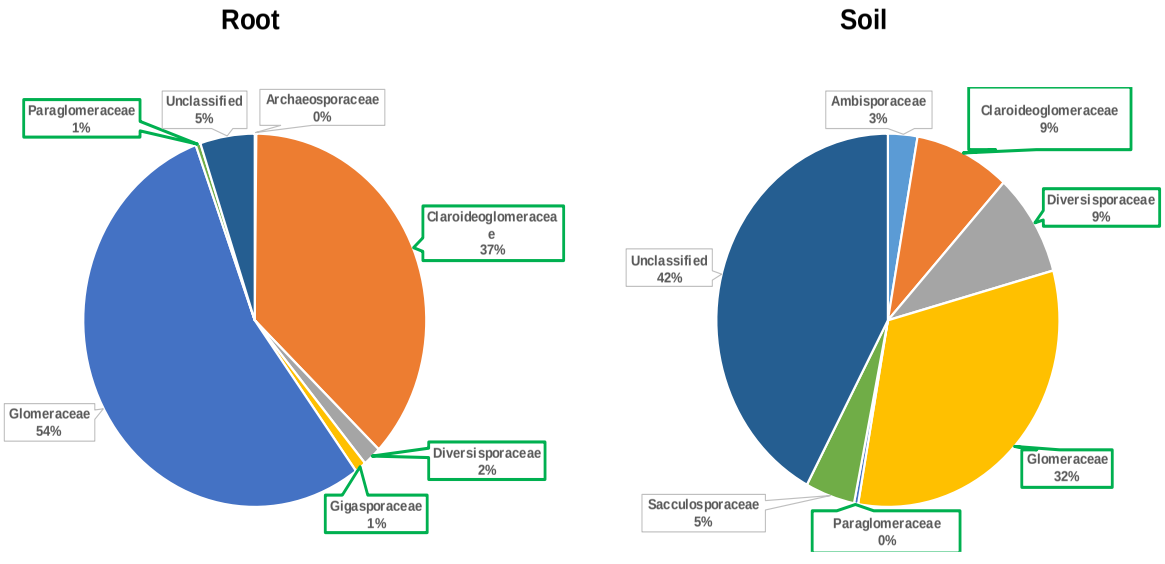

Figure S2-1 Relative abundance of arbuscular mycorrhizal fungal families in root (left) and soil (right). The boxes with green lines indicate the shared AM fungal families in both root and soil.