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ARTICLE

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Fungal communities are passengers in community development of dune ecosystems, while bacteria are not

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Abstract

An increasing number of studies of above-belowground interactions provide a fundamental basis for our understanding of the coexistence between plant and soil communities. However, we lack empirical evidence to understand the directionality of drivers of plant and soil communities under natural conditions: 'Are soil microorganisms driving plant community functioning or do they adapt to the plant community?' In a field experiment in an early successional dune ecosystem, we manipulated soil communities by adding living (i.e., natural microbial communities) and sterile soil inocula, originating from natural ecosystems, and examined the annual responses of soil and plant communities. The experimental manipulations had a persistent effect on the soil microbial community with divergent impacts for living and sterile soil inocula. The plant community was also affected by soil inoculation, but there was no difference between the impacts of living and sterile inocula. We also observed an increasing convergence of plant and soil microbial composition over time. Our results show that alterations in soil abiotic and biotic conditions have long-term effects on the composition of both plant and soil microbial communities. Importantly, our study provides direct evidence that soil microorganisms are not "drivers" of plant community dynamics. We found that soil fungi and bacteria manifest different community assemblies in response to treatments. Soil fungi act as "passengers," that is, soil microorganisms reflect plant community dynamics but do not alter it, whereas soil bacteria are neither "drivers" nor "passengers" of plant community dynamics in early successional ecosystems. These results are critical for understanding the community assembly of plant and soil microbial communities under natural conditions and are directly relevant for ecosystem management and restoration.

KEYWORDS
bacteria, coexistence, ecosystem dynamics, ecosystem restoration, field experiment, fungi, plant community, soil microbial community, sterilization, whole-soil inoculation

INTRODUCTION

Over the past two decades, a wealth of studies has demonstrated that plants and soil microorganisms are core components of ecosystems (Bever et al., 2015; Castle et al., 2016; Kardol et al., 2006). Soils comprise the most diverse and complex microbial communities on Earth, and one handful of soil may contain more than 5000 species (Anthony et al., 2023). Interactions between these soil communities and the aboveground communities can contribute to the coexistence of species and the maintenance of organism diversity within an ecosystem (Bever et al., 2015; Castle et al., 2016; Kardol et al., 2006). Understanding how the dynamics of soil microbial community composition influence this co-assembly of plants and microbes, provides insights into how plant and soil biodiversity affects ecosystem processes (van der Heijden et al., 2008; Wagg et al., 2019). Although there is accumulating knowledge about how plant and soil microbial assemblages are associated with one another over longer timescales (Fukami & Nakajima, 2013; Lekberg et al., 2018), the exact role of soil microbial communities in the development of plant communities remains unclear.

Interactions with soil pathogens, decomposers, and mutualists affect the diversity and composition of plant communities through the modification of ecological niches and soil legacy effects (Eisenhauer et al., 2012; Heinen et al., 2020; Kardol et al., 2018; Yan et al., 2022). For instance, the presence of soil pathogens can reduce the abundance of fast-growing plants, which are assumed to invest less in defense and, consequently, can lead to a decline in competition among plant species (Kardol et al., 2007; Mordecai, 2011). Associations between slow-growing plants and soil symbionts, like arbuscular mycorrhizal fungi (AMF), can also alter plant–plant competition and influence the strength and direction of vegetation succession (Klironomos et al., 2000; Koziol & Bever, 2017; Wubs et al., 2016). Several studies have shown that AMF diversity has the potential to influence plant composition by improving the resource acquisition of plants and mediating resource partitioning among plants belowground (Van der Heijden et al., 1998). Because plant species vary in the degree to which they benefit from associating with various AMF (Klironomos, 2003), certain AMF may allow particular species greater access to soil resources than others and thus alter competition among plant species (Bauer et al., 2020; Scheublin et al., 2007; Urcelay & Díaz, 2003).

At the same time, there is increasing evidence suggesting that soil microbes follow the dynamics of a plant community, as plant species can shape the composition of the soil community via the quantity and quality of rhizodeposits, and litter (Bever et al., 1996; De Deyn et al., 2011; Leff et al., 2018; Zhalnina et al., 2018). For instance, Schmid et al. (2021) reported that the association between plants and soil microbes is related to the plant species composition and its functional groups. In an experiment conducted in a seminatural grassland, both fungi and bacteria richness and evenness changed following the absence and presence of grasses, and only fungal diversity responded to the absence and presence of legumes (Schmid et al., 2021). Moreover, the abundance, activity, and composition of soil decomposer communities have been shown to vary markedly with different plant species because of plant species-specific variation in the quality and quantity of plant materials that enter the soil (De Deyn et al., 2011; Philippot et al., 2013; Urbanová et al., 2015).

While the examples above illustrate that plant and soil communities are interrelated across different scales and circumstances, the question remains open regarding the role of soil microbial community in the development of plant communities: 'Are soil microbes "drivers" in affecting the plant community or "passengers" following plant community development?' Recently, several studies about the relationship between AMF and plant communities have been done in the context of the driver-passenger hypothesis (AMF driving plant communities or AMF following plant community assembly) (Horn et al., 2017; Kokkoris et al., 2020). For example, AMF symbiosis was demonstrated to play a driving role in determining the community assembly of plants (Neuenkamp et al., 2018). However, a study investigating AMF communities in a European seminatural grassland yielded a different result, as it revealed that both plant and AMF communities were shaped by abiotic conditions (Van Geel et al., 2018), supporting the so-called habitat hypothesis, which states that plant and AMF communities co-vary with changes in their habitat (Zobel & Öpik, 2014). Collectively, these studies advance our understanding of the dynamics of covariation between plant and AM fungal communities. However, to our knowledge, there is no empirical evidence of how the composition of soil microbial communities as a whole is associated with the dynamics of plant communities under field conditions.

The reason for a precluding understanding of the driver-or-passenger role of soil microorganisms lies in the methodological challenge to manipulate the structure and composition of the soil community under natural conditions (Klironomos et al., 2011; Zobel & Öpik, 2014). Here, we took the challenge to explore this relationship in an early successional dune ecosystem, by manipulating the soil community using soil inoculation. We added soil inocula originating from different donor dune ecosystems, including primary dunes, dune grasslands, and dune forests, into experimental plots in a new experimentally created

dune ecosystem. From each donor ecosystem, we used two types of soil inocula—living inocula (i.e., bearing propagules of entire soil community typical of the giving donor ecosystems), and sterilized inocula (the inocula that had all abiotic properties of the living one, but no soil community). The sterilization setup allowed us to tease apart the effects of introduced soil biota versus changes in soil abiotic properties on the assembly of the plant and soil community.

We examined four alternative hypotheses: H_1, soil microbes act as "drivers" of plant community dynamics during the early successional stage. H_2, soil microbes act as "passengers" rather than "drivers" of plant community dynamics during the early successional stage, where soil microbial community dynamics mirrored fluctuations in the plant community. H_3, plant and soil microbial communities follow the same direction of development, but there is no clear evidence for "driver" or "passenger" relationships. This hypothesis was expected to be proven if we had observed that the experimental treatments had no impact on either plant communities or soil microbial communities, while a significant relationship was observed between them (due to variation in the abiotic conditions). H_4, there is no relationship between the development of plant and soil microbial communities. In this case, we expected that the sterilization of soil inocula only influences the soil microbial community but not the plant community. During the 3 years following the experimental additions of soil inocula, we annually measured plant, fungal, and bacterial communities to assess the dynamics of their responses to the soil inoculation treatments.

MATERIALS AND METHODS

The experiment was carried out in the TERRA-Dunes experiment (Meijendel Nature Reserve, Wassenaar, The Netherlands, 52°07′50.4″ N; 4°20′27.6″ E). A detailed outline of the experiment is provided elsewhere (Gao et al., 2022) and a scheme of the experimental design is presented in Appendix S1: Figure S1. Briefly, the TERRA-Dunes experiment is a long-term field experiment that was established in 2018 with a factorial soil inoculation design. Soil inocula were collected from different donor dune ecosystems, including primary dunes, dune grasslands and dune forests, and each donor type was collected from four independent sites (Appendix S1: Figure S2). Half of the collected soil for inoculation was left intact and half was sterilized. Soil sterilization was performed with gamma radiation (>25 kGy gamma radiation, Isotron, Ede, The Netherlands). Hereafter we use the term "soil inocula" for addition of sterilized or nonsterilized soil, specifically describing the inocula type every time where it is necessary. These soil inocula were added to plots of 2 × 2 m into a previously bare sand dune area. Before adding each 0.5 cm layer of living soil inoculum, a 1.5 cm layer of sterilized soil from a respective donor ecosystem was added to the plots to ensure the presence of developed soil material on the bare sand, enabling a quicker start-up of plant community development (Appendix S1: Figure S1). The inoculation treatments with living soil inocula enabled alterations of soil abiotic and biotic conditions (Appendix S1: Table S1 and Figures S3–S5), with the former one being altered because donor ecosystem soil is unavoidably added together with living inocula. The inoculation treatment with sterile soil inocula allowed us to tease apart the effects of introduced soil biota versus changes in soil abiotic properties on the plant and soil community assembly. Seeds of 30 plant species (Appendix S1: Table S2) typical for European coastal dune ecosystems were sown into the experimental plots simultaneously with soil inocula additions (Appendix S1: Figure S1). This was done to introduce a diverse and homogeneous species pool of plants in all plots. One control treatment (control 1, 10 plots) entailed no seed additions and no inocula. A second control treatment (control 2, 22 plots) entailed seed additions and no inoculum; this control treatment was included in the current analysis. The addition of ectomycorrhizal fungi (EMF) failed (Appendix S1: Section S1) and in the current work we opted to ignore this treatment, and used the EMF-treated plots as additional replicates of other treatments. The ultimate replication of our experiment was 12 plots for each combination of soil inoculum origin and sterilization treatment (Appendix S1: Figure S1). We expected that the soil treatments will influence the establishment, performance, and temporal changes of the plant community in the different plots, as shown in another soil inoculation field experiment (Heinen et al., 2020).

Vegetation assessment and soil sampling

The taxonomic composition of the plant community was recorded annually in the first week of September from 2018 to 2021. The percentage of vegetation cover was estimated visually in all plots for each plant species. Soil samples were also collected annually, immediately after the vegetation survey. Nine soil cores (0–10 cm depth, diameter 18 mm) were collected randomly in each plot to characterize abiotic parameters and for molecular analysis of the soil microbial composition. These nine soil cores were pooled per plot and homogenized. A subsample of soil from each plot was transferred to a 2 mL tube

on the day of sampling and stored at −20°C. The remaining soil was sieved (2 mm mesh size) for analysis of soil chemical parameters. Soil samples from each plot were weighed to measure abiotic parameters following protocols in Appendix S1: Section S1. The complete set of results for the soil chemical analysis is presented in Appendix S1: Table S1 and Figure S3.

Soil microbial analysis

Genomic DNA was extracted from soil samples collected in all plots in 2018, 2019, and 2020 using the PowerSoil Plant DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The nuclear internal transcribed region (ITS2) of fungi and part of 16S of bacteria were targeted for PCR reaction. A fungal universal primer pair gITS7/ITS4 (gITS7: 5′-GTGART CATCGARTCTTTG-3′ (Ihrmark et al., 2012); ITS4: 5′-TCCTCCGCTTATTGATATGC-3′ (White et al., 1990)) and a bacterial primer pair 515F/806R (515F: 5′-GTG CCAGCMGCCGCGGTAA-3′; 806R: 5′-GGACTACHVH HHTWTCTAAT-3′) was used to amplify the ITS2/16S 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 using the 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 an Illumina MiSeq system to generate 2 × 250 base paired-end reads.

After demultiplexing based on Nextera indexes, paired reads of each sample were merged and low-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.fa) for soil fungi and RDP database (rdp_16s_v16_sp.fa) for bacteria as annotation resources (https://drive5.com/usearch/manual/sintax_downloads.html). In total, 13,846 high-quality-filtered sequences were obtained, of which 2692 sequences were identified as fungal taxa, and 11,154 sequences were identified as bacterial taxa.

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. An analysis of plant community dynamics was conducted on the data of abundances of each species. The abundance data were Hellinger pretransformed as the data included many zeros to avoid overemphasizing the impacts of rare species (Legendre & Gallagher, 2001). To test the effect of soil inoculation and sterilization on plant composition, we applied a permutational analysis of variance (PERMANOVA) based on a Bray–Curtis dissimilarity matrix in the R package *vegan* (Oksanen et al., 2013). Sequences of soil fungi and bacteria were analyzed separately. OTU abundances from sequence counts were also standardized prior to the multivariate analysis using Hellinger transformation (Legendre & Gallagher, 2001). The effects of soil inoculation and sterilization on soil microbial composition at the OTU level were estimated using PERMANOVA based on a Bray–Curtis dissimilarity matrix in the R package *vegan*. The plant, soil fungal, and bacterial community structures as affected by the soil inoculation treatments were visualized using nonmetric multidimensional scaling (NMDS) with Bray–Curtis distance measures through the "metaMDS" command in the R package *vegan*.

To visualize the temporal effects of the experimental treatments we constructed a principal response curve (PRC) using the "prc" function of the *vegan* 2.5-6 package (Oksanen et al., 2013) for the plant and soil microbial communities. PRC is based on redundancy analysis (RDA), adjusted for overall changes in community response over time (Moser et al., 2007; Van den Brink & Ter Braak, 1999). The principal components of the treatment effects on individual species are plotted against time (Van den Brink & Ter Braak, 1998). The control plots, which had no soil inoculum, were treated as a reference treatment. We tested for the significance of the experimental treatments, time, and their interactions on plant community composition using multivariate permutation tests. A Mantel test was performed to explore the correlations between plant community and soil microbial

community based on the Bray–Curtis dissimilarity matrices. We used Pearson's correlation coefficients with 999 permutations. All analyses were performed in R version 4.0.2 (R Core Team, 2020).

RESULTS

Effects of soil inoculation on the development of the plant and soil community

During the study, the composition of the plant community changed considerably, and this depended on the origin of the soil inoculum (Table 1, Figure 1). In the first year of the experiment, plant communities that developed in plots with soil inocula originating from dune grassland and dune forest diverged strongly in plant composition from the control (Figures 1 and 2). However, the effects of soil inoculum origin on plant communities were similar for sterile and living inocula, indicating that the presence or absence of living soil biota within added soil inocula had no influence on plant composition. It also suggested that the divergence of plant communities from the control treatment was caused by other factors (likely nutrients present in the inocula). The highest divergence was observed in the second and third year of the experiment (Figure 1). As time passed, the plant communities under different soil inoculation treatments started to converge (Figure 1).

In contrast with the plant community, the soil microbial community was significantly influenced by the sterilization treatment (Table 2, Figure 3). The addition of living soil biota (i.e., inoculation in the absence of the sterilization treatment) significantly influenced the soil fungal composition in plots with dune forest soil inoculum (Figures 3 and 4). In particular fungal communities from the plots with dune forest inoculum showed a major initial divergence from control plots (Figure 3). The added living soil biota had little impact on the fungal community in plots with soil inocula originating from primary dunes and dune grasslands. In contrast with the fungal community, the sterilization treatment had weaker effects on the composition of the soil bacterial community, and this trend persisted over time (Table 2, Figure 3). In addition, compared with the control, both soil fungal and bacterial communities showed larger divergence in plots with forest and grassland soil inocula than in plots with dune inocula. Similar to the plant community, the divergence of soil fungi and bacteria decreased over time (Figures 3 and 4).

Correlation between plant and soil microbial community composition

Significant correlations were observed between plant and soil fungal and bacterial communities and these associations were dependent on soil sterilization treatment, especially so for soil bacterial communities (Table 3). In plots with both living and sterile soil inocula, increasing association between soil fungal and plant communities was observed, with the exception of plots with living soil in 2019, where a marginally significant correlation was observed (Table 3). Additionally, an increasing association between soil AM fungal and plant communities was

TABLE 1 Summary statistics of a PERMANOVA testing the effects of different types of soil inoculation, soil sterilization and their interactions on the plant community composition (Inoculum origin, *I*. Sterilization, *S*).

Year	Treatment	df1, df2	*F*-value	R^2	*p*-value
2018	Inoculum origin	**3, 86**	**1.84**	**0.06**	**0.02**
	Sterilization	1, 86	0.58	0.01	0.81
	$I \times S$	3, 86	0.94	0.03	0.52
2019	Inoculum origin	**3, 86**	**3.73**	**0.11**	**<0.01**
	Sterilization	1, 86	0.97	0.01	0.48
	$I \times S$	3, 86	1.04	0.03	0.39
2020	Inoculum origin	**3, 86**	**3.02**	**0.09**	**<0.01**
	Sterilization	1, 86	1.63	0.02	0.07
	$I \times S$	3, 86	1.40	0.04	0.08
2021	Inoculum origin	**3, 86**	**2.66**	**0.08**	**<0.01**
	Sterilization	1, 86	1.27	0.01	0.25
	$I \times S$	3, 86	0.95	0.03	0.54

Note: Presented are degrees of freedom, variance explained (R^2), *F*-values and *p*-values. Significant effects ($p < 0.05$) are presented in bold.

FIGURE 1 First component of the principal response curves (PRC) showing the dynamics of plant community composition over 4 years in response to soil inoculation treatments. The colored lines connect different sample points in the figure. The PRC analysis showed that 6.04% of the total variation in plant composition was explained by the soil treatments, whereas the year effects accounted for 51.23%. The first canonical axis of the PRC captured a significant part (27.21%) of the variance induced by inoculation and year (Monte Carlo permutation test, 999 permutations, $p = 0.001$; Appendix S1: Table S3). The control treatment (no soil inocula) was used as an internal reference. Taxon weights in the ordinations are shown in Appendix S1: Figure S6 on the same axis. Control: Plots with no soil inocula; Primary dunes + Living soil inocula: Plots with living soil inocula originating from primary dunes; Primary dunes + Sterile soil inocula: Plots with sterile soil inocula originating from primary dunes; Dune grasslands + Living soil inocula: Plots with living soil inocula originating from dune grasslands; Dune grasslands + Sterile soil inocula: Plots with sterile soil inocula originating from dune grasslands; Dune forests + Living soil inocula: Plots with living soil inocula originating from dune forests; Dune forests + Sterile soil inocula: Plots with sterile soil inocula originating from dune forests.

observed in plots with both living and sterile soil inocula over time (Appendix S1: Table S4). In contrast to soil fungi, the associations between plant and soil bacterial communities were detected only for plots treated with sterilized inocula, while no significant relationship was found between plant and soil bacteria in plots treated with living soil inocula.

DISCUSSION

We show in a field experiment that manipulation in soil conditions through soil inoculation affects the composition of plant and soil microbial communities. The composition of the plant community was mainly driven by soil abiotic conditions, while the soil fungi and bacteria were influenced by both soil abiotic and biotic conditions. Collectively our results suggest that soil microbes do not act as "drivers" (i.e., soil microbial community patterns drive host plant communities). Increased associations between plant and soil fungal communities suggest that soil fungi acted more as "passengers" (i.e., that soil microbial community dynamics mirrored fluctuations in the plant community), whereas the dynamics of soil bacteria was primarily driven by variation in soil conditions within our experiment. Importantly, the effects of the soil inoculation treatments remained persistent over time for soil fungal communities but not for soil bacterial communities, providing evidence that these communities develop and act at different time scales.

Soil biota are not the driver of plant community dynamics

In this study, we found a significant association between plant and soil microbial communities, which led to the rejection of Hypothesis H_4. However, in contrast with

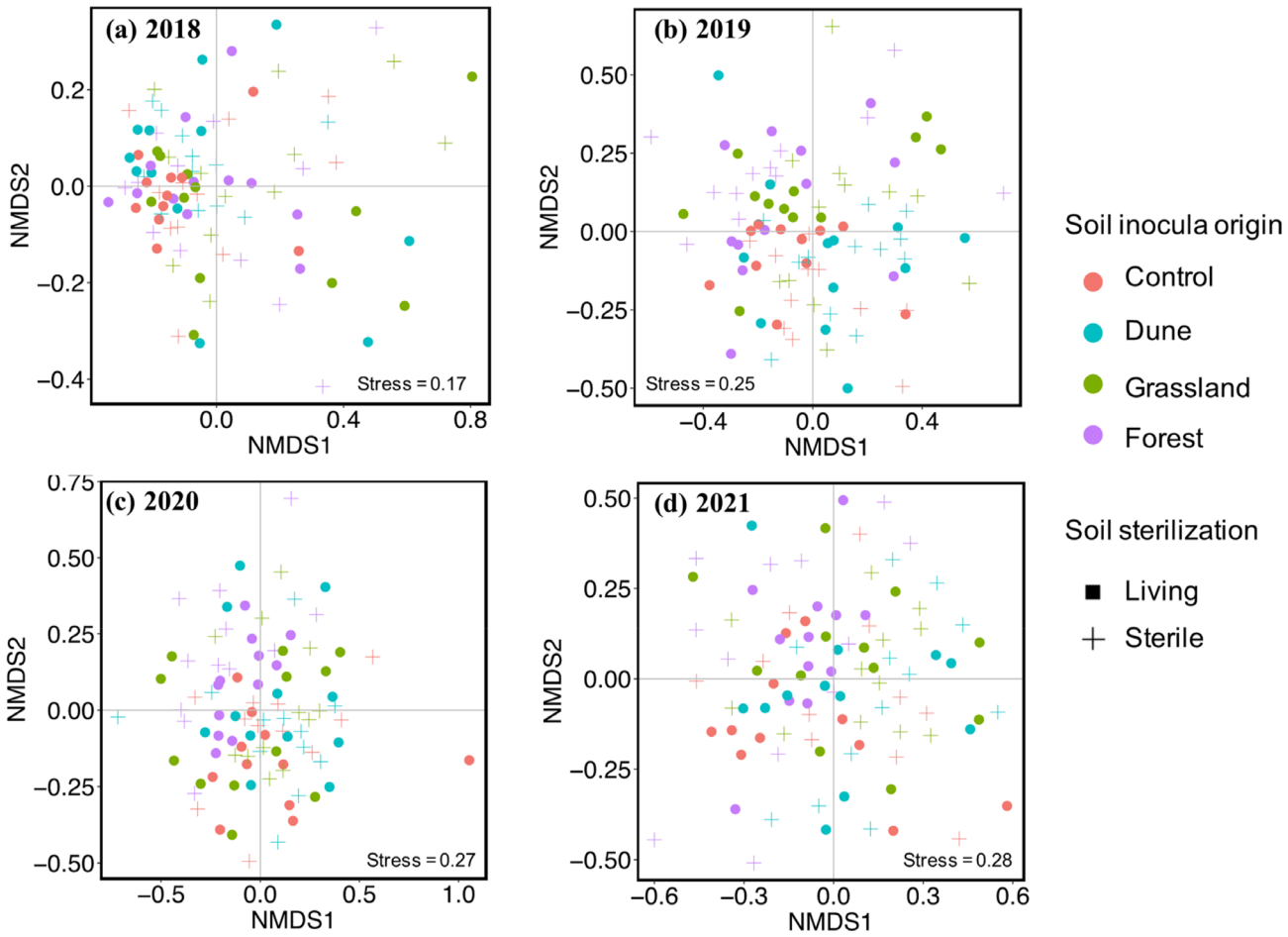

FIGURE 2 (a–d) Nonmetric multidimensional scaling (NMDS) ordination plots showing plant community composition in response to soil inocula origin and soil sterilization treatments over time.

TABLE 2 Summary statistics of a PERMANOVA testing the effects of different types of soil inoculation, soil sterilization and their interactions on the soil fungal and bacterial community composition (Inoculum origin, *I*. Sterilization, *S*).

			Fungal community			Bacterial community		
Year	**Treatments**	**df1, df2**	***F*-value**	**R^2**	***p*-value**	***F*-value**	**R^2**	***p*-value**
2018	Inoculum origin	3, 86	**6.84**	**0.18**	**0.001**	**4.39**	**0.13**	**0.001**
	Sterilization	1, 86	**3.53**	**0.03**	**0.001**	**1.49**	**0.01**	**0.022**
	$I \times S$	3, 86	**2.33**	**0.06**	**0.001**	1.15	0.01	0.095
2019	Inoculum origin	3, 86	**4.28**	**0.12**	**0.001**	**3.49**	**0.10**	**0.001**
	Sterilization	1, 86	**3.07**	**0.03**	**0.001**	**1.84**	**0.02**	**0.012**
	$I \times S$	3, 86	**1.87**	**0.05**	**0.001**	**1.34**	**0.04**	**0.022**
2020	Inoculum origin	3, 86	**2.79**	**0.08**	**0.001**	**2.43**	**0.07**	**0.001**
	Sterilization	1, 86	**1.63**	**0.02**	**0.007**	1.29	0.01	0.058
	$I \times S$	3, 86	**1.30**	**0.04**	**0.005**	1.11	0.03	0.152

Note: Presented are degrees of freedom, variance explained (R^2), *F*-values and *p*-values. Significant effects ($p < 0.05$) are presented in bold.

our expectation, the plant community assembly showed only limited responses to the addition of living soil biota, despite soil microbial communities being significantly affected by the experimental treatments. Instead, changes in soil abiotic properties, through soil inoculation, significantly influenced plant community composition over time, even though all plots started from the same diverse seed mixture. These results contradicted Hypothesis H_1

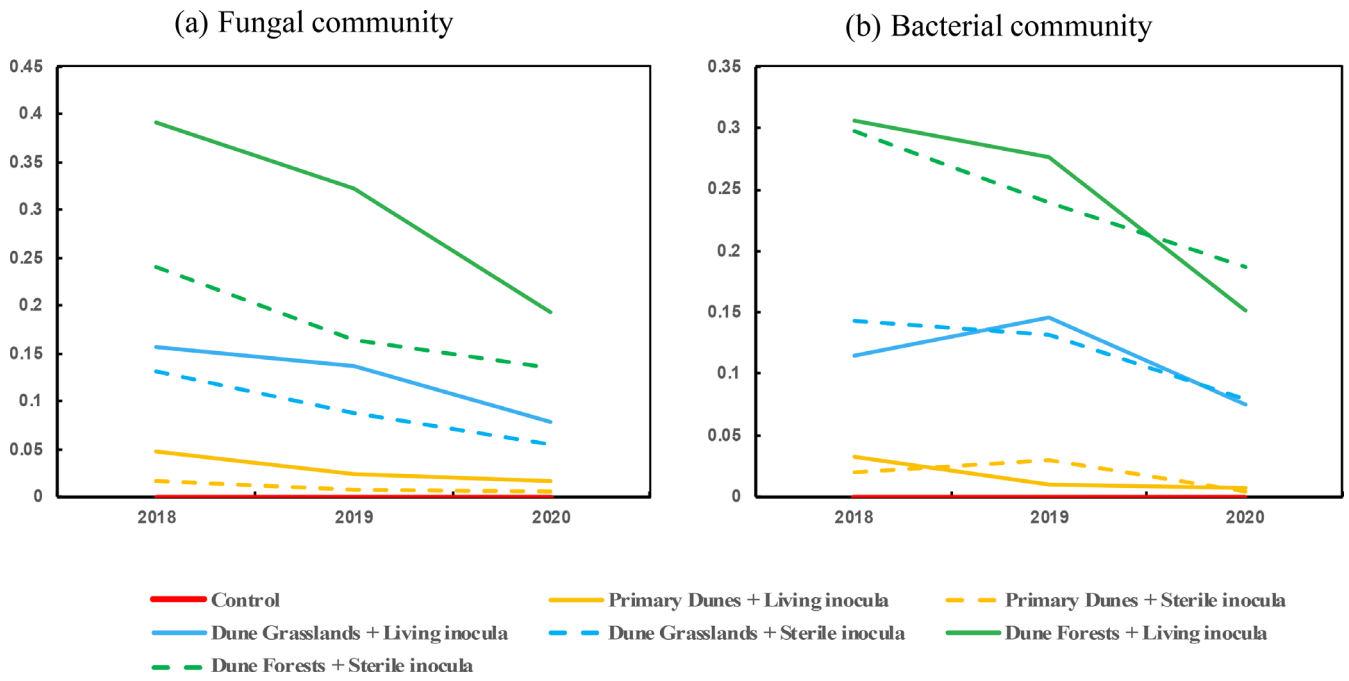

FIGURE 3 The first component of the principal response curves (PRC) shows the soil fungal (a) and bacterial (b) community composition over 3 years in response to soil inoculation treatments. The control treatment (no soil inocula) was used as an internal reference. The PRC analysis showed that 6.46% and 7.58% of the total variation in soil fungal and bacterial composition were explained by the soil treatments, respectively, whereas the year effects accounted for 16.55% and 14.68%. The first canonical axis of the PRC captured a significant part (43.00% and 40.29%) of the variance induced by inoculation and year (Monte Carlo permutation test, 999 permutations, $p = 0.01$; Appendix S1: Table S3).

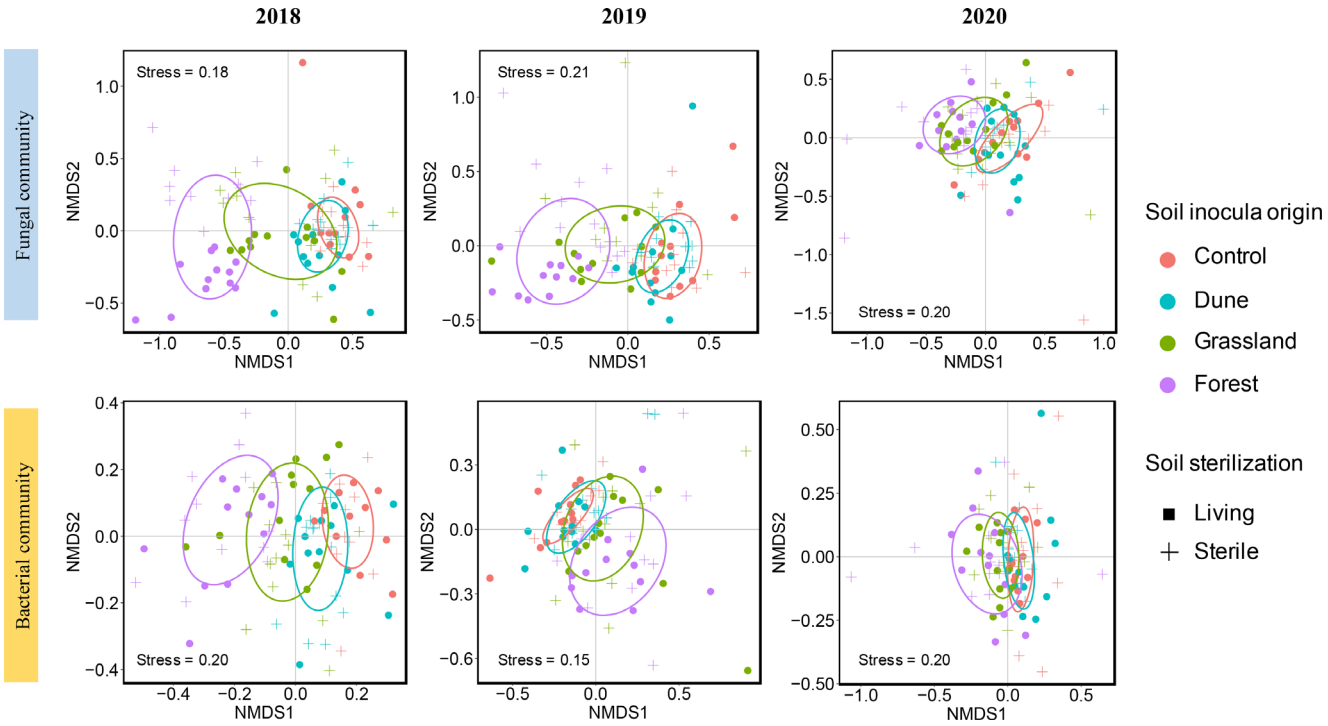

FIGURE 4 Nonmetric multidimensional scaling (NMDS) ordination plots showing soil fungal and bacterial community composition in response to soil inocula origin and soil sterilization treatments over time. Ellipses show the soil microbial community structure in plots with different types of soil inocula.

TABLE 3 Plant community composition Pearson's correlations between soil fungal and bacterial community composition in plots with living soil inocula and sterile soil inocula.

	Plant and fungal communities				Plant and bacterial communities			
	Living soil inocula		Sterile soil inocula		Living soil inocula		Sterile soil inocula	
Year	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value
2018	**0.165**	**0.014**	**0.227**	**0.003**	0.055	0.262	**0.246**	**0.003**
2019	0.124	0.065	**0.355**	**0.001**	0.106	0.128	**0.372**	**0.001**
2020	**0.344**	**0.001**	**0.261**	**0.006**	0.145	0.080	**0.292**	**0.009**

Note: Significant effects ($p < 0.05$) are presented in bold.

and supported Hypothesis H_2. Other soil inoculation studies have also shown that inoculation can lead to rapid changes in the plant community (Han et al., 2022; Heinen et al., 2020; Wubs et al., 2016). The absence of effects of adding living soil biota suggests that plant community composition changes due to soil inoculation were not driven by the inocula-induced shifts in the soil microbial community over time. Thus, soil biota were not the driver of soil inoculation effects on plant community development in this early successional ecosystem. This result contrasts with studies in other systems showing that soil biota from soil inoculation play a crucial role in affecting plant composition (Middleton & Bever, 2012; Wubs et al., 2016, 2019). This may be explained by the limited association between plant and soil biota (e.g., plant-relevant mutualists/pathogens) in the early successional stage (De Deyn et al., 2004). For example, at the beginning of the experiment, the dominant plants were generally seedlings and early successional plants (Appendix S1: Table S5) which are assumed to be less strongly linked with soil biota, like AMF (Koziol et al., 2015). Plants might need a longer time to develop associations with particular soil taxa. In addition, the microcosm and mesocosm experiments showing strong patterns of soil microbial diversity driving plant community structure and composition may overestimate the role of the soil community, because of the lower dispersal limitation compared with natural conditions. Finally, although soil microbes might play a role in influencing the plant community composition (Castle et al., 2016), strong interactions between plants and soil abiotic factors likely override the outcomes of plant–soil biotic interactions during primary succession.

Soil fungi are "passengers" of plant community dynamics in early successional ecosystems

The soil inoculation treatments induced a major divergence in the soil microbial community (Figure 3). This is in line with our expectation that the introduction of soil biota would result in shifts in soil microbial composition. The divergence in soil communities under different treatments tended to decrease with time. Also, the plant community tended to converge over time, despite differences at the beginning of the experiment, although more slowly than the microbial community (Figure 1). This convergence of both communities explains the increasing associations between plant and soil fungal community composition over time (Table 3), suggesting that the covariation in plant and soil community composition could reflect direct interactions between plant and soil microbial communities rather than a common response to soil inoculation treatments.

We observed that the soil sterilization treatment affected the plant composition less than the soil microbial community, especially soil fungi (Figures 1 and 3). These differences due to soil sterilization disappeared quickly in both plant and soil microbial communities, highlighting the idea that soil microbes did not act as "drivers" of plant community composition. While increasing associations between plant and soil fungi suggest that the soil fungal community might follow the plant community dynamics and that they might play a "passenger" role rather than a "driver" role during our study (Table 3) (Appendix S1: Table S4). Importantly, we also observed that the addition of seeds influenced the soil fungal composition in control plots over time (Appendix S1: Figure S7). This further supports the idea that soil fungi might be more "passengers" in plant community dynamics. Recent experimental evidence has shown that plants may select for a specific suite of soil microorganisms (Bezemer et al., 2010; Schmid et al., 2019; Wubs & Bezemer, 2018), like rewarding the best AM fungal partners with more carbohydrates (Bever et al., 2009; Kiers et al., 2011). Therefore, particular plant communities may facilitate or "drive" the development of specific soil microbial communities (Hannula et al., 2019; Hausmann & Hawkes, 2009; Schmid et al., 2021). Nevertheless, in the current study, there is limited proof

for a "driver" role of the plant community in shaping the fungal community. Instead, the fast convergence and the increasing association between plant and soil fungal communities over time, as well as the effects of seed addition on soil fungal composition, all provide indirect support for the "passenger" role of soil fungi (H_2).

Soil bacteria show different community dynamics compared to soil fungi in early successional ecosystems

Compared with soil fungi, there were smaller differences in soil bacteria between plots with living and sterile soil inocula. The soil sterilization-induced divergence within the soil bacteria community did not persist during the study and the effects of soil sterilization treatments on the soil bacterial community declined over time. These results suggest that the impact of soil inoculation on soil fungi remained stable, while the effects decreased for soil bacteria. These findings may be explained by the different life history strategies of soil bacteria and fungi. Generally, soil bacteria are more dynamic, while soil fungi are slow-growing (Allison & Martiny, 2008; Rousk & Bååth, 2007). Therefore, soil fungi are less affected than bacteria by temporal variability in the habitat (Barnard et al., 2013; Hannula et al., 2021). We propose that this can explain why the legacy effects of living forest soil inoculum on soil fungi were persistent. This is supported by the results from the NMDS ordination (Figure 4). At the beginning of the experiment, there were larger differences between soil fungi and soil bacteria when compared with control plots because of the different microbial composition at the donor sites (Figure 4; Appendix S1: Table S6, Figure S8). In addition, differences in the soil fungal and bacterial dynamics may arise from their dispersal abilities that can substantially affect their establishment and dominance (Boynton et al., 2019; King & Bell, 2022). For instance, certain spore characteristics of bacterial species may allow them to establish well in unfavorable environmental conditions (King & Bell, 2022). These active dispersal strategies may accelerate the establishment of bacterial communities and enable them to adapt to the abiotic and biotic conditions in experimental plots.

We noticed that soil bacteria showed significant associations with plants only in plots with sterile soil inocula. This is different from the response of fungal communities, suggesting that soil bacteria may not reflect plant community dynamics but mainly respond to changes in soil conditions (H_3). This is in accordance with recent studies that showed that bacterial communities do not reflect changes in plant community composition, whereas soil fungal communities closely follow the alterations in vegetation (Emilia Hannula et al., 2019; Harantová et al., 2017). The weak link between plant and soil bacterial communities could be due to a lack of biotic interactions in the early successional stage (Cutler et al., 2014) as bacteria may be adapted to barren environments and can fix nitrogen and carbon from the atmosphere (Schmidt et al., 2014).

Last but not least, the direction and degree of plant–soil microbial community assembly might vary over time, especially with succession (Neuenkamp et al., 2018; Zobel & Öpik, 2014). The "passenger" role of soil microbes could, therefore, switch to a "driver" role in determining plant community composition over time. Many soil microbes are assumed to be cosmopolitan and can have associations with a broad range of plant species. Further longer term experimental work is required to establish how the co-assembly of natural plant and soil microbial composition changes over succession. This knowledge could improve our understanding of how the co-evolution between plant and soil communities affects the maintenance of biodiversity in ecosystems and the subsequent effects of biodiversity on ecosystem functioning (van Moorsel et al., 2021).

CONCLUSIONS

In a field experiment, we showed that the addition of soil communities exerted a limited impact on plant community composition over time. This result indicates a minor role of the added soil community in the assembly of plant communities and provides experimental evidence that soil microbes are not a "driver" of plant community dynamics in early successional ecosystems. We observed smaller differences between treatments with living vs. sterile inocula for soil microbial than for plant communities. Moreover, these differences tended to decrease over time and the correlation between plant and soil fungal communities increased over time, suggesting the "passenger" role of soil fungal community. Finally, there were more persistent effects of soil inoculation treatments on soil fungal communities than on soil bacterial communities, probably due to different life history strategies, and we suggest that soil bacteria were neither "drivers" nor "passengers" of plant community dynamics in early successional ecosystems. These findings give valuable insight into the further understanding of the community assembly of plant and soil microorganisms under natural conditions and can be used for better ecosystem management and restoration.

AUTHOR CONTRIBUTIONS

Chenguang Gao, Nadejda A. Soudzilovskaia, and Peter M. van Bodegom conceived the idea.

Nadejda A. Soudzilovskaia, T. Martijn Bezemer, Riccardo Mancinelli, and Harrie van der Hagen established the experiment of TERRA Dunes. Chenguang Gao and Riccardo Mancinelli collected the samples. Chenguang Gao, Riccardo Mancinelli, Petr Baldrian, and Petr Kohout processed the samples. Chenguang Gao analyzed the data with helpful input from Nadejda A. Soudzilovskaia, Peter M. van Bodegom, T. Martijn Bezemer, Petr Baldrian, and Petr Kohout. Chenguang Gao wrote the first draft, and all authors contributed to editing the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data and code (Gao et al., 2024) are available in Figshare at https://doi.org/10.6084/m9.figshare.25425601.v1.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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