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

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Chapter 5

Soil microbes are more “passengers” of plant community dynamic in early ecosystem

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Submitted to Ecology letters

Abstract

A major question on plant-soil interactions is whether soil microorganisms are “drivers” or “passengers” of ecosystem dynamics. In an early successional dune ecosystem, we manipulated the soil community by adding living and sterile soil inocula, originating from natural ecosystems, and examined the responses of soil and plant communities. The experimental manipulations had a persistent effect on the soil microbial community with divergent impacts of living and sterilized soil inocula. 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. These results indicate that soil microorganisms are not “drivers” but “passengers”, i.e. soil microorganisms reflect plant community dynamics, but do not alter it. These results are critical for understanding the coexistence between plant and soil microbial communities, and are directly relevant for ecosystem management and restoration.

5.1 Introduction

Plants and soil microorganisms are core components of ecosystems and the plant-soil interactions can contribute to the coexistence of species and the maintenance of diversity (Kardol *et al.* 2006; Bever *et al.* 2015; Castle *et al.* 2016). Understanding how plant communities co-assemble with soil microbial communities over time and how dynamics of soil microbial composition influence this co-assembly provides insights into how aboveground and belowground 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 direction and magnitude of interactions between plants and soil organisms (i.e., causally determine each other) remain unclear.

Plant 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.* 2012a; Kardol *et al.* 2018; Heinen *et al.* 2020). 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; Wubs *et al.* 2016; Koziol & Bever 2017). 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 (Silvertown 2004). 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 (Urcelay & Díaz 2003; Scheublin *et al.* 2007; Bauer *et al.* 2020).

At the same time, increasing evidence indicates that plant species can shape the composition of the soil community via the quantity and quality of rhizodeposits, and litters (Bever *et al.* 1996; De Deyn *et al.* 2011; Leff *et al.* 2018; Zhalnina *et al.* 2018). For instance, Schmid et al (2021) found that the associations between plant and soil microbes are linked to the composition of plant species and functional groups. In an experiment conducted in a semi-natural grassland, grasses increased the fungal richness and evenness and legume plants increase fungal evenness, while bacteria were not influenced (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 (Wardle *et al.* 1999; Porazinska *et al.* 2003; De Deyn *et al.* 2011; Urbanová *et al.* 2015). These findings are supported by experiments under controlled conditions in greenhouse and in the field, showing that there

is an increasing association between plant and soil community assembly with increasing detection and discrimination of soil biota (Bezemer *et al.* 2010; Schmid *et al.* 2019, 2021; Wubs *et al.* 2019).

While the examples above illustrate that plant and soil communities are interrelated across different scales and circumstances, the question of the causality of these relationships, i.e. which group "drives" the assembly of the other group and in which situation remains open. Recently, several studies about the relationship between AMF and plant communities have been done in the context of the driver-passenger hypothesis (plants driving AM fungal communities or vice versa) (Horn *et al.* 2017; Kokkoris *et al.* 2020). For example, AMF symbiosis was demonstrated to play a driver role in determining the community assembly of plants (Neuenkamp *et al.* 2018). At the same time, a study investigating AMF communities in European semi-natural grassland yielded a different result: 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 being controlled by plant communities under field conditions or vice versa.

The reason for precluding understanding of the driver-or-passenger relationship between plant and soil microorganisms lies in the difficulty to manipulate the soil community composition under natural conditions (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 hypothesize that the introduction of soil biota persistently alters the plant community as well as soil microbial community development. Further, we hypothesize that the manipulation of soil microbial composition is the main driver of plant communities in the early successional stage. We introduced living soil inocula and sterilized soil inocula from different donor dune ecosystems, including primary dunes, dune grasslands and dune forests into a new experimentally created dune ecosystem. The sterilization setup allowed us to tease apart the effects of introduced soil biota vs. changes in soil abiotic properties on the plant and soil community assembly. During three years we annually analyzed plant, fungal and bacterial communities to examine the dynamics of their responses to the soil inoculation treatments.

5.2 Materials and methods

The experiment was carried out in the TERRA-Dunes (Meijendel Nature Reserve, Wassenaar, The Netherlands, 52°07'50.4"N; 4°20'27.6"E). A detailed outline of the experiment is provided in general introduction chapter of this thesis (Chapter 1).

- *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 each plot for each plant species. Soil samples were also collected annually, directly 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 then 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 weighted to measure abiotic parameters following protocols in supplementary information. The results for the soil chemical analysis is presented in Table S5-1.

- *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'- GTGARTCATCGARTCTTTG -3' (Ihrmark *et al.* 2012); ITS4: 5'-TCCTCCGCTTATTGATATGC -3' (White *et al.* 1990)) and a bacterial primer pair 515F/806R (515F: 5'-GTGCCAGCMGCCGCGGTAA -3'; 806R: 5'-GGACTACHVHHHTWTCTAAT -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 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 an Illumina MiSeq 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). A total of 13,846 high-quality-filtered sequences were obtained from all soil samples, of which 2,692 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. Analysis of plant community dynamics was conducted on the data of abundances of each taxon. The abundance data were Hellinger pre-transformed as the data included many zeros and we wanted 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 R package "vegan" (Oksanen *et al.* 2013). A principal component analysis (PCA) was used to visualize differences in plant community composition.

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 R package "vegan". Principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarities was used to visualize the effects of soil inoculation treatments on both fungal and bacterial community composition with the function "cmdscale".

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 (Van Den Brink & Ter Braak 1999; Moser *et al.* 2007). 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 inocula were treated as 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).

Table 5.1 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, 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.

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

Table 5.2 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, 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.

Year	Treatments	df1,df2	Fungal community			Bacterial community		
			F-value	R^2	p-value	F-value	R^2	p-value
	Inoculum	3,86	**6.84**	**0.18**	**0.001**	**4.39**	**0.13**	**0.001**
2018	Sterilization	1,86	**3.53**	**0.03**	**0.001**	**1.49**	**0.01**	**0.022**
	I x S	3,86	**2.33**	**0.06**	**0.001**	1.15	0.01	0.095
	Inoculum	3,86	**4.28**	**0.12**	**0.001**	**3.49**	**0.10**	**0.001**
2019	Sterilization	1,86	**3.07**	**0.03**	**0.001**	**1.84**	**0.02**	**0.012**
	I x S	3,86	**1.87**	**0.05**	**0.001**	**1.34**	**0.04**	**0.022**
	Inoculum	3,86	**2.79**	**0.08**	**0.001**	**2.43**	**0.07**	**0.001**
2020	Sterilization	1,86	**1.63**	**0.02**	**0.007**	1.29	0.01	0.062
	I x S	3,86	**1.30**	**0.04**	**0.005**	1.11	0.03	0.152

Table 5.3 Plant community composition Pearson's correlations between soil fungal and bacterial community composition. Significant effects (p<0.05) are presented in bold.

Comparison	Year	r	p-value
Plant community and soil fungal community	**2018**	**0.19**	**0.001**
	2019	**0.21**	**0.003**
	2020	**0.26**	**0.001**
Plant community and soil bacterial community	**2018**	**0.16**	**0.018**
	2019	**0.25**	**0.001**
	2020	**0.21**	**0.015**

5.3 Results

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

During the study, the plant and soil microbial composition showed strong changes, which depended on the origin of the soil inoculum (Figure 5.1). In the first year of the experiment, plant communities that developed in plots with soil inocula originating from dune grassland and dune forest had a larger divergence in plant composition compared to control (Figure 5.1, Figure S5-1). However, the effects of soil inoculum origin on plant communities were similar for sterile and living inocula ($P_{df=3,86} > 0.05$ for Inoculum x Sterilization interaction at all years, Table 5.1), 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) than the living biota added. The highest divergence was observed in the second and third year of the experiment (Figure 5.1). As time passed, the plant communities under different soil inoculation treatments started to converge (Figure 5.1).

In contrast with the plant community, the soil microbial community was significantly influenced by the sterilization treatment (Table 5.2, Figure 5.2). Added living soil biota (i.e. inoculation in absence of the sterilization treatment) significantly influenced the initial soil fungal composition in plots with dune forest soil inocula, although, similarly to the plant community, this pattern declined over time (Figure 5.2, Figure S5-2). The added living soil biota had little impacts on the fungal community in plots with soil inocula originating from primary dunes and dune grasslands. In contrast to the fungal community, the sterilization treatment had weaker effects on the soil bacterial composition, and this trend persisted over time (Table 5.2, Figure 5.2). In addition, compared to 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. The divergence of soil fungi and bacteria decreased over time (Figure 5.2). Particularly fungal communities developed with dune forest inoculum showed a major difference between sterile and living inoculum with microbial communities from living inoculum, showing larger initial divergence to control plots (Figure 5.2).

- *Correlation between plant and soil microbial community composition*

There were significant correlations between plant and soil fungal and bacterial communities over time (Table 5.3). Especially the association between soil fungi and plant community persisted over time and even increased in strength. The association between the soil bacterial community and the plant community was strong, and, in contrast to fungi, did not show a pattern of increasing strength over time (Table 5.3).

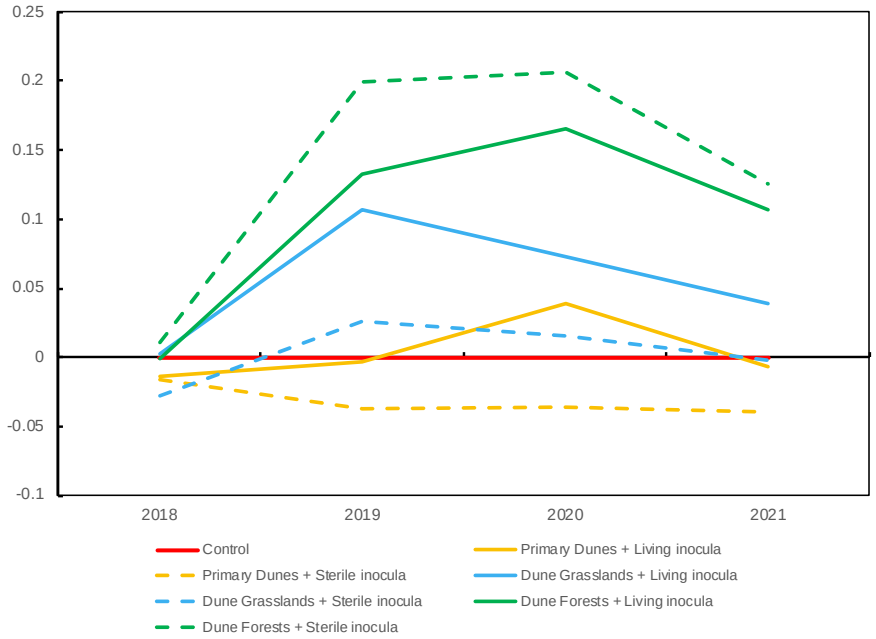

Figure 5.1 First component of the Principal response curves (PRC) showing the dynamics of plant community composition over 4 years in response to soil inoculation treatments. The colored lines connect different sample points in the figure. The PRC analysis showed that 6.04% of the total variation in 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)(Table S5-2). The control treatment (no soil inocula) was used as an internal reference. Taxon weights in the ordinations are shown in Figure S5-3 on the same axis.

Control: plots with no soil inocula; Primary dunes+Living soil ioncula: Plots with living soil inocula originating from primary dunes; Primary dunes+Sterile soil ioncula: Plots with sterile soil inocula originating from primary dunes; Dune grasslands+Living soil ioncula: Plots with living soil inocula originating from dune grasslands; Dune grasslands +Sterile soil ioncula: Plots with sterile soil inocula originating from dune grasslands; Dune forests+Living soil ioncula: Plots with living soil inocula originating from dune forests; Dune forests+Sterile soil ioncula: Plots with sterile soil inocula originating from dune forests.

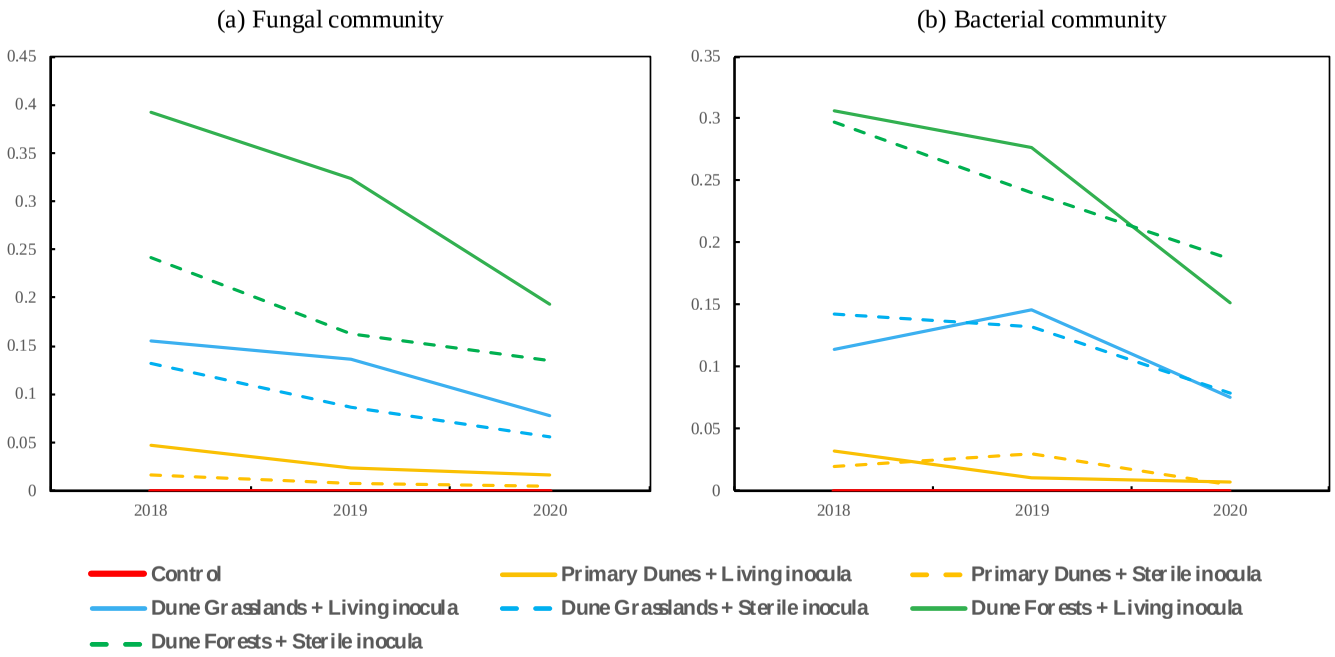

Figure 5.2 First component of the PRC showed the soil fungal and bacterial community composition over 3 years in response to soil inoculation treatments. The control treatment (no soil inocula) was used as an internal reference. The PRC analysis showed that 6.46% and 7.58% of the total variation in soil fungal and bacterial composition was 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

5.4 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. Plant and soil microbial communities became increasingly associated, and the divergence of plant and soil microbial communities in response to soil inoculation treatments tended to decrease over time. These results suggest that the soil biota acted more as "passenger" (soil microbial community dynamics followed changes in the plant community) rather than as "driver" (soil microbial community patterns drive host plant community).

- *Soil biota are not the driver of plant community dynamic*

In contrast to our expectation, the plant community assembly showed only limited responses to the presence of added living soil biota, despite soil biota communities being significantly affected by the experimental treatments. Instead, changes in soil abiotic properties through soil inoculation significantly influenced plant community composition over time. This suggests that plant community composition changes due to soil inoculation were not driven by the inocula-induced shifts in soil microbial community over time. Thus, soil biota was 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.* 2004a). For example, at the beginning of the experiment, the dominant plants were generally seedlings and early-successional plants (Table S5-3) which are assumed to be less strongly linked with soil biota, like arbuscular mycorrhizal fungi (Koziol *et al.* 2015). Plants might need a longer time to develop relationships with particular soil taxa. In addition, under the naturally low nutrient conditions of primary dune soils, soil microbes might play a subtle role in influencing the plant community through limitations in nutrient availability (Castle *et al.* 2016).

- *Soil biota are passengers of plant community dynamics in early-successional ecosystems*

Initially, the soil inoculation treatments induced a major divergence in the soil microbial community (Figure 5.2). This is in line with our expectation that the introduction of soil biota would result in shifts in soil microbial composition. The divergence 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 5.1). This convergence of both communities seems to explain

the increasing associations between plant and soil microbial community composition over time (Table 5.3), suggesting that the covariation in plant and soil community composition reflected direct interactions between plant and soil microbial communities rather than a common response to soil inoculation treatments.

Importantly, we observed that the soil sterilization treatment affected the plant composition less than the soil microbial community, and especially soil fungi (Figure 5.1, Figure 5.2). These differences due to soil sterilization disappeared quickly in both plant and soil microbial communities, highlighting that soil microbes did not act as "drivers" of plant community composition. Together these results suggest that the soil microbial community might follow the plant community dynamics and that they play a "passenger" role rather than a "driver" one during our study. Plants may select for a specific suite of soil microorganisms (Bezemer *et al.* 2010; Wubs & Bezemer 2018; Schmid *et al.* 2019), 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 (Hausmann & Hawkes 2009; Schmid *et al.* 2021).

Last but not least, the direction and degree of plant-soil microbial community assembly might vary over time, especially with succession (Zobel & Öpik 2014; Neuenkamp *et al.* 2018). The "passenger" role of soil microbes could, therefore, switch to a "driver" role in determining plant community composition over time. Moreover, many soil microbes are assumed to be cosmopolitan and can have associations with a broad range of plant species. It may take years to observe evident effects of aboveground vegetation on the composition of soil microbial communities (Crowther *et al.* 2014). 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).

- *Different Responses of soil fungi and bacteria to the introduction of soil biota*

Compared to soil bacteria, there were much bigger differences in soil fungi between plots with living and sterile soil inocula, especially in plots with forest-originated soil inocula. Furthermore, the soil sterilization-induced divergence within the soil fungal community persisted during the study, whereas the effects of soil sterilization treatments on the soil bacterial community declined over time. These results suggest that the impacts of added soil biota on soil fungi remained, while the effects diminished for soil bacteria. These findings can be explained by the different life history strategies of soil fungi and bacteria. Generally, soil fungi are slow-growing while soil bacteria are more dynamic (Rousk & Bååth 2007; Allison & Martiny 2008). Therefore, soil fungi are less affected than bacteria by temporal

variability in the habitat (Barnard *et al.* 2013; Hannula *et al.* 2021). Because of this, the legacy effects of living forest soil inocula on soil fungi were persistent. This is supported by the results from the cluster analysis (Figure S5-2). At the beginning of the experiment, there were larger differences among soil fungi and soil bacteria compared to control plots because of the different compositions at the donor sites (Figure S5-4). In our study, soil bacterial communities in plots tended to become more similar over time, while the soil fungi in plots with soil inocula, especially with forest inocula, showed persistent differences in comparison to control plots.

5.5 Conclusions

In a field experiment, we show that changes in soil community exerted 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 of a soil microbial passenger role hypothesis in early successional ecosystems. Our evidence consists of 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 microbial communities increased over time. These findings give valuable insight into the further understanding of the community assembly of plant and soil microorganism under natural conditions and enable us for better ecosystem management and restoration.

5.6 Acknowledgements

We are grateful to Dunea Duin & Water company for the help with establishment of the experiment. We thank Clémentine Lepinay for analyzing soil samples. Funding was provided by the Netherlands Organization for Scientific research (NWO; VIDI Grant No. 016.161.318 issued to NAS; VICI grant 865.14.006 issued to TMB) and the China Scholarship Council (Grant No. 201804910632) issued to C.G.

5.7 Author contributions

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

5.8 Supporting information

Table S5-1 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. (Samples collected in 2018)

	Dune		Grassland		Forest		Control
	Living	Sterile	Living	Sterile	Living	Sterile	No inocula
Fe (mg/kg)	0.03 ± 0.003	0.04 ±0.006	0.06 ± 0.004	0.06 ± 0.005	0.09 ± 0.006	0.10 ± 0.011	0.02 ± 0.002
P (mg/kg)	0.51 ± 0.032	0.51 ± 0.036	0.71 ± 0.038	0.68 ± 0.044	0.88 ± 0.053	0.83 ± 0.058	0.49 ± 0.038
Zn (mg/kg)	0.05 ± 0.005	0.05 ± 0.004	0.12 ± 0.036	0.07 ± 0.011	0.11 ± 0.024	0.09 ± 0.009	0.04 ± 0.003
S (mg/kg)	1.30 ± 0.094	1.08 ± 0.093	1.97 ± 0.141	1.90 ± 0.164	2.44 ± 0.167	2.33 ± 0.189	0.89 ± 0.052
K (mg/kg)	13.80 ± 1.08	12.70 ± 0.781	16.30 ± 1.44	15.70 ± 1.60	18.00 ± 1.290	18.90 ± 1.590	12.90 ± 0.648
Mg (mg/kg)	10.10 ± 0.944	9.13 ± 0.440	16.20 ± 1.250	18.30 ± 2.320	20.90 ± 1.610	20.50 ± 1.600	7.26 ± 0.305
Nitrogen (NO_2^- +NO_3^-) (mg/kg)	1.54 ± 0.251	1.02 ± 0.252	2.42 ± 0.401	2.04 ± 0.385	2.84 ± 0.461	2.93 ± 0.424	0.933 ± 0.127

Table S5-2 Statistics of the Principal Response Curve (PRC) analysis. The PRC-Statistics show Eigenvalue, F-ratio and p-value of the Monte Carlo permutation test (999 permutations) on significance of the 1st canonical axis of the PRC and the explanatory content. Furthermore, the part of the total variance explained by time and by treatment and the part of the variance explained by treatment that is captured by the 1st axis of the PRC is given.

Community	Monte Carlo permutation test on significance of the 1st canonical axis of the PRC				% of the total variance explained by		% of the variance explained treatment captured by the 1st canonical axis of the PRC
					Time	Treatment	
Plant community	Eigenvalue	0.009	*p*-value	0.001	51.23	6.04	27.21
	F-Ratio	13.38	Explanatory content %	51.23			
Soil Fungal community	Eigenvalue	0.044	*p*-value	0.01	16.55	6.46	43.00
	F-Ratio	24.13	Explanatory content %	16.55			
Soil Bacterial community	Eigenvalue	0.023	*p*-value	0.01	14.68	7.58	40.29
	F-Ratio	19.86	Explanatory content %	14.68			

Table S5-3 Classification of present plant species into functional groups. Early-successional plants (Early), Mid-successional plants (Mid), Late-successional plants (Late), Arbuscular mycorrhizal (AM), ectomycorrhizal (EM), nonmycorrhizal (NM), nonmycorrhizal but AM habit may exist (NM-AM).

Plant species	Functional groups	Successional stage*	Mycorrhizal type**
Agrostis capillaris	Grass	Mid	AM
Agrostis gigantea	Grass	Mid	AM
Aira praecox	Grass	Early	NM-AM
Amaranthus blitoides	Forb	Early	NM-AM
Amaranthus retroflexus	Forb	Early	NM-AM
Anagallis arvensis	Forb	Early	AM
Anchusa arvensis	Forb	Early	AM
Anchusa officinalis	Forb	Early	AM
Anthyllis vulneraria	Legume	Late	AM
Arabis hirsuta	Forb	Early	NM
Calamagrostis epigejos	Grass	Mid	AM
Cardamine hirsuta	Forb	Early	NM
Carex hirta	Grass/sedge	Early	NM-AM
Chenopodium album	Forb	Early	NM-AM
Chenopodium foliosum	Forb	Early	NM-AM
Cirsium arvense	Forb	Early	AM
Cirsium vulgare	Forb	Early	AM
Clinopodium acinos	Forb	Early	AM
Convolvulus arvensis	Forb	Early	AM
Conyza canadensis	Forb	Early	AM
Corispermum intermedium	Forb	Early	NM
Crepis capillaris	Forb	Early	AM
Cynoglossum officinale	Forb	Early	AM
Datura stramonium	Forb	Early	AM
Daucus carota	Forb	Early	AM

Digitaria ischaemum	Grass	Early	AM
Echinochloa crus-galli	Grass	Early	NM-AM
Echium vulgare	Forb	Early	NM-AM
Elytrigia repens	Grass	Early	AM
Equisetum arvense	Forb	Early	NM-AM
Erodium cicutarium	Forb	Early	AM
Euphorbia cyparissias	Forb	Early	AM
Fallopia convolvulus	Forb	Early	NM-AM
Fallopia dumetorum	Forb	Early	NM-AM
Festuca filiformis	Grass	Late	AM
Galium mollugo	Forb	Late	NM-AM
Galium verum	Forb	Late	NM-AM
Geranium pusillum	Forb	Early	AM
Glechoma hederacea	Forb	Mid	AM
Helianthemum nummularium	Forb	Mid	AM
Helictotrichon pubescens	Grass	Early	AM
Hieracium pilosella	Forb	Late	AM
Holcus lanatus	Grass	Mid	AM
Hypochaeris radicata	Forb	Mid	AM
Jasione montana	Forb	Early	NM-AM
Koeleria macrantha	Grass	Early	AM
Leontodon hispidus	Forb	Early	AM
Linaria vulgaris	Forb	Early	NM-AM
Lithospermum officinale	Forb	Early	NM-AM
Lotus corniculatus	Legume	Mid	AM
Moehringia trinervia	Forb	Early	NM
Myosotis arvensis	Forb	Early	NM-AM
Oenothera spec.	Forb	Early	AM
Ononis repens	Legume	Early	AM

Papaver dubium	Forb	Early	NM-AM
Persicaria amphibia	Forb	Early	NM-AM
Phragmites australis	Grass	Early	NM-AM
Picris hieracioides	Forb	Mid	AM
Plantago lanceolata	Forb	Mid	AM
Polygonum aviculare	Forb	Mid	NM-AM
Portulaca oleracea	Forb	Early	NM-AM
Quercus cerris	Tree	Late	EM
Robinia pseudoacacia	Tree	Late	AM
Rubus caesius	Small shrub	Late	AM
Rumex acetosella	Forb	Early	NM-AM
Rumex crispus	Forb	Early	NM-AM
Scrophularia nodosa	Forb	Early	NM-AM
Sedum acre	Forb	Early	NM-AM
Senecio inaequidens	Forb	Early	AM
Senecio jacobeae	Forb	Early	AM
Senecio vulgaris	Forb	Early	AM
Setaria viridis	Grass	Early	AM
Silene conica	Forb	Early	NM-AM
Silene dioica	Forb	Early	NM-AM
Silene nutans	Forb	Early	NM-AM
Solanum nigrum	Forb	Early	AM
Solanum triflorum	Forb	Early	AM
Sonchus arvensis	Forb	Early	AM
Sonchus asper	Forb	Early	AM
Sonchus oleraceus	Forb	Early	AM
Stellaria media	Forb	Early	NM-AM
Thymus pulegioides	Small shrub	Late	AM
Trifolium arvense	Legume	Early	AM

Trifolium dubium	Legume	Mid	AM
Urtica urens	Forb	Early	NM-AM
Verbascum nigrum	Forb	Early	AM
Viola canina	Forb	Early	AM

* Based on ref (Schaminée *et al.* 1996) and long-term vegetation survey in Dutch dune ecosystems.

** Based on ref (Soudzilovskaia *et al.* 2020, 2022)

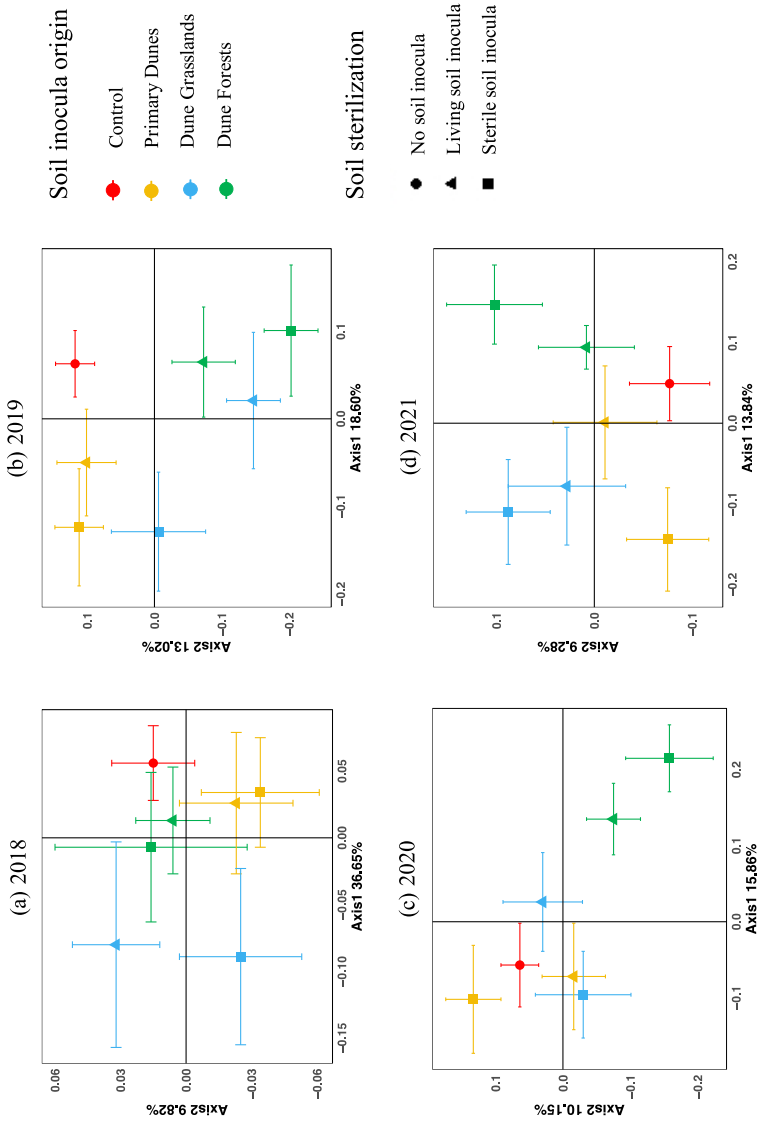

Figure S5-1 PCAs for plant community in the field experiment during study. The means (dots) and SEs (error bars) were calculated from the eigen values of the first and second PCA axis under different treatments in each year.

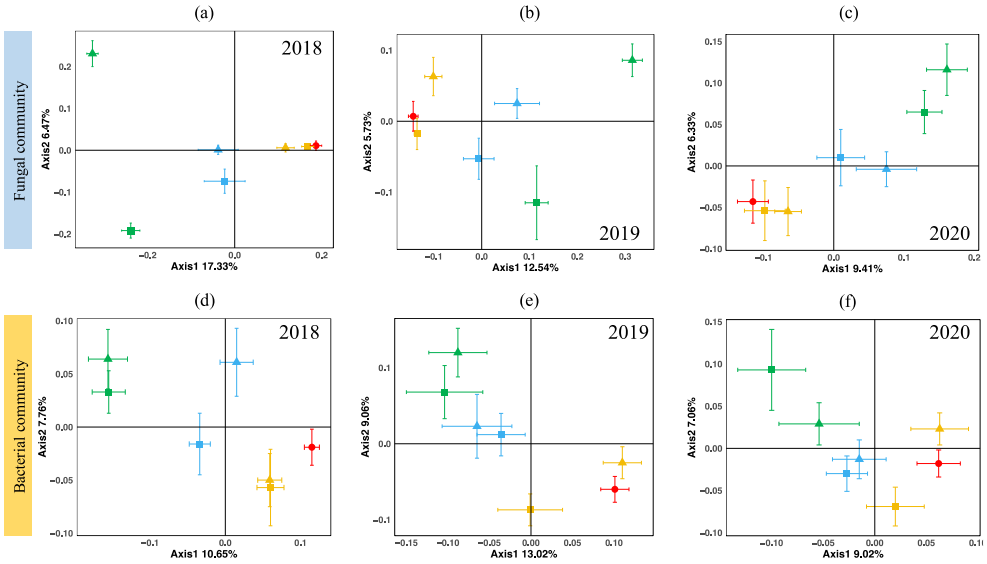

Figure S5-2 PCoAs for soil fungal (a, b, and c) and bacterial community (d, e, and f) in the field experiment over time. The means (dots) and SEs (error bars) were calculated from the eigen values of the first and second PCA axis under different treatments in each year.

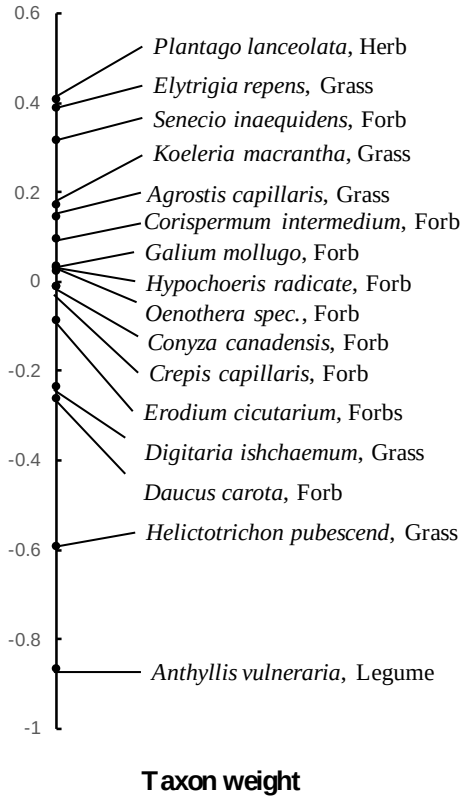

Figure S5-3 Taxon weights in the PRC ordinations of Figure 5.1 for plant communities on the same PRC axis as in Figure 5.1. The one-dimensional plot shows changes in the abundance of each taxon across the first axis. For clarity, only taxa with the best fit to the ordination axes are shown by name.

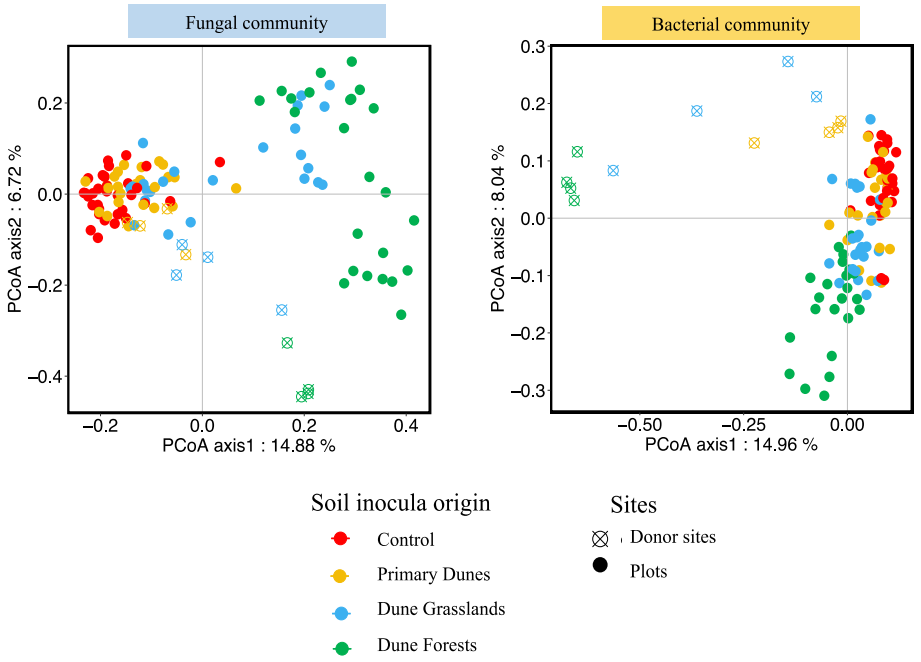

Figure S5-4 PCoAs for soil fungal and bacterial composition in experimental plots and donor sites in 2018.