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

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Chapter 6

General discussion

Recent decades have witnessed a rapid increase of studies in plant-soil interactions. A growing body of research from greenhouse and field has highlighted that plant-soil interactions have important consequences on not only plant growth, community dynamic, stress tolerance, but soil microbial assembly (van der Putten *et al.* 2016a; Bennett & Klironomos 2019). This is not surprising given the multiple functions of large soil biodiversity (Bardgett & van der Putten 2014). In this thesis, I aimed to study how plant-soil interactions influence the aboveground and belowground ecological processes and the community dynamics in a field experiment.

In my PhD project, I first explored if the soil community influenced the establishment of an ubiquitous microbial guild, arbuscular mycorrhizal fungi by using *P. lanceolata* as phytometer plant. I also investigated whether the productivity and functional traits of *P. lanceolata* were affected by the soil inoculation treatments and how they associate with the responses of soil AM fungi. Second, I investigated whether the soil community influence plant community resistance and recovery in response to a short-term drought event. Third, I tested the impacts of alterations in soil abiotic and biotic conditions on the plant community-level leaf and root traits, as well as their impacts on the functional linkage between aboveground and belowground. Fourth, I investigated the role of introduced soil community in driving the development of plant and soil microbial communities over time. Across all experimental chapters, I have observed that the soil community plays a critical role in determining ecosystem functions in both aboveground and belowground, while their impacts are highly dependent on the soil abiotic context, such as soil nutrients. Below, I will discuss the key findings of the previous chapters in a wider context, considering their implications for the understanding of plant-soil interactions.

6.1 The role of soil biota in determining ecosystem functioning in primary successional ecosystems

- *Adverse role of soil biota in influencing plant drought responses in early dune ecosystem*

Multiple interactions between plants and soil communities can enhance the capacity of plants to environmental stresses, such as drought and salinity (Classen *et al.* 2015; Fry *et al.* 2018; Yang *et al.* 2018). For example, plants associated with soil mutualists, such as AMF, have a stronger tolerance to the environmental disturbance in semi-grassland (Mariotte *et al.* 2017). Conversely, another important group of soil biota that can hamper plant performance are soil pathogens that play a vital role in plant species turnover and thereby driving succession in secondary successional ecosystems (Kardol *et al.* 2006, 2007; Mangan *et al.* 2010; Mordecai 2011). However, compared to the well-described patterns of plant-soil interactions in secondary succession, we found that soil biota function differently in a primary dune ecosystem. Also, their effects on plants relied on the context in which they were measured.

Unlike the general patterns in past studies, we observed that added soil biota from later-successional soil inocula play an adverse role in influencing plant community stability to drought stress (Chapter 3). This is in contrast to our expectations that soil biota from later successional stages could improve plant fitness to withstand drought due to the presence of soil symbionts, such as AM fungi (Augé 2001; Mariotte *et al.* 2017; Wu 2017). This can be attributed to the stronger nutrient competition between plant and soil communities due to low nutrient availability (Kuzyakov & Xu 2013) in our primary succession ecosystem. My study was conducted in an extremely nutrient-limited primary dune ecosystem (Table S5-1, Chapter 5), and strong competition between plant and soil communities for limited nutrients may outweigh the possible benefits from soil symbionts. Consequently, we observed no significant or even negative effects of added soil biota on the drought sensitivity of the plant community. The results from our experiment provide insight into the impacts of the complexity of soil biota on the ecosystem stability vary depending on the context and have implications for plant communities under climate change.

- *Limited role of soil biota in mediating plant community productivity and development.*

In Chapter 2 and Chapter 5, I observed that soil biota from different types of soil inocula did not influence plant growth and plant composition over time. Further, there were few responses of plant community biomass in response to soil inoculation treatments (Table 6.1). These results are in contrast to recent studies that demonstrate that soil biota plays a crucial role in determining the productivity and development of plant communities (Kardol *et al.* 2006; Wubs *et al.* 2016, 2019). This can be partly explained by the limited plant-soil microbial associations at the primary successional stage. It is known that in early successional stages, dominant plant species are generally annual, like ruderals which are assumed to have less linkage with soil biota (Koziol *et al.* 2015). Therefore, it is unlikely to have strong plant-soil interactions at the onset of primary succession (Castle *et al.* 2016). Importantly, our results show that changes in soil abiotic properties through soil inoculation significantly influenced plant community composition over time (Chapter 5), suggesting that soil biota might play a relatively subtle role than soil abiotic conditions in influencing the plant community.

Table 6.1 Effects of soil inocula origin (Inoculum, I), soil sterilization (Sterilization, S) and their interaction (I × S) on plant aboveground and belowground. 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.

Year		Plant community aboveground biomass			Plant community belowground biomass		
		F	*p*	η^2	F	*p*	η^2
2018	**Inoculum**	**3.02**	**0.03**	**0.09**	**10.94**	**<0.01**	**0.271**
	Sterilization	0.26	0.61	0.003	0.02	0.89	<0.001
	I × S	0.87	0.46	0.027	0.75	0.52	0.019
2019	**Inoculum**	0.17	0.92	0.005	**8.52**	**<0.01**	**0.203**
	Sterilization	0.03	0.86	<0.001	1.85	0.18	0.015
	I × S	1.88	0.14	0.061	**4.16**	**<0.01**	**0.099**
2020	Inoculum	0.30	0.83	0.010	1.41	0.25	0.046
	Sterilization	1.66	0.20	0.018	0.01	0.95	<0.001
	I × S	0.78	0.51	0.026	0.39	0.76	0.013
2021	Inoculum	0.64	0.59	0.021	0.98	0.41	0.032
	Sterilization	1.56	0.22	0.017	0.31	0.58	0.003
	I × S	0.09	0.97	0.003	0.32	0.81	0.010

- *Differences in soil biotic functions between primary and secondary succession*

The direction and strength of plant-soil interactions at the primary stage are different from the observed patterns under secondary succession. These differences can be explained from the aboveground and belowground perspectives. From the perspective of plant life strategies, often, early plants such as ruderals are dominant in the primary stage. These early plants are assumed as "fast-growing" plants, characterized by thin and relatively smooth roots and are often less dependent on associations with soil biota, particularly mycorrhizal fungi (Koziol *et al.* 2015; Ma *et al.* 2018; Williams & de Vries 2020). Therefore, strong interactions between plants and soil abiotic factors may overwhelm the outcomes of plant-soil-biotic interactions during primary succession.

From the view of belowground communities, the structure and functions of soil biota are dependent on the soil conditions, particularly on soil nutrients (Tedersoo *et al.* 2014; Lekberg & Waller 2016; Laliberté *et al.* 2017). For example, an increase in soil nutrient availability results in a decline in soil microbial biomass (Treseder 2008; Ramirez *et al.* 2012), and then directly modifies soil fungal, bacterial and archaeal composition (Ramirez *et al.* 2010; Leff *et al.* 2015). Additionally, several studies indicated that the functions of AM fungal symbionts are regulated by the availability of resources, including carbon, nitrogen and phosphorus (Johnson 2010; Johnson *et al.* 2010). The relationship between plants and mycorrhizal fungi may change during succession towards fewer mutualistic or even antagonistic interactions under the influence of increasing soil resources, like N and phosphorus (Johnson *et al.* 2003; Treseder 2004; Johnson & Graham 2013). Due to the substantial differences in soil environmental conditions at primary and secondary successional stages, there are strong differences in soil biotic composition and functioning with succession. Such changes are expected to interact with plants and consequently modify their interactions with plants as driven by aboveground and belowground processes (De Deyn *et al.* 2004b; Kardol *et al.* 2013; in 't Zandt *et al.* 2019). Taken together, my study provides valuable insights into the mechanistic understanding of the context-dependency of plant-soil interactions in primary succession, showing that the strength and direction rely on the environmental context, such as variations in soil resources, water availability and temperature (Kardol *et al.* 2013; Png *et al.* 2019).

6.2 Alterations in soil biota by soil inoculation in a primary succession ecosystem

- *Legacy effects of soil abiotic conditions*

Based on the experimental results, this study shows that soil inoculation treatments influence the performance and composition of both plant and soil microbial communities. Although we could not quantify the success of the establishment of added soil biota, we observed that the added soil biota significantly changed soil fungal and bacterial composition (Chapter 5) and even plant-mycorrhizal symbionts after the soil inoculation treatments (Chapter 2). This suggests that small scale inoculation is effective in driving both aboveground and belowground processes in the field (Wubs *et al.* 2016, 2019). However, in our case, the key "players" of soil inoculation in driving plant community development were soil abiotic properties instead of soil biotic factors, as the addition of living soil biota had little impacts on plant community growth and development (Chapter 5, Table 6.1). This is inconsistent with previous studies which assume that the changes of soil abiotic conditions are minor and tend to neglect the potential effects of soil abiotic conditions because 1) recipient sites were not nutrient-limited; 2) limited amount of soil inocula, especially in the field experiment; 3) low concentration of nutrients (Wubs *et al.* 2016, 2018).

In my study, the resident sandy soils were extremely nutrient-limited (Table 6.2) and the environmental conditions were harsh, such as low water availabilities. The collected soil inocula originated from later successional dunes systems, including primary dunes, dune grasslands and dune forests. These sites are generally covered by litter and have better nutrient conditions than the experimental site (Table 6.2). Given the harsh abiotic conditions in the bare experimental site, the legacy effects of soil abiotic conditions on plants are most likely to overcome the effects derived from the interactions between plant and soil communities. Further, changes in soil abiotic conditions may indirectly influence plants through their impacts on the structure and composition of soil communities (Laliberté *et al.* 2017; Bennett & Klironomos 2019; Png *et al.* 2019).

We also noticed that the legacy effects of living inoculation on soil community composition decreased over time and tended to be similar as the one with sterile inoculation (Chapter 5), suggesting that the possible effects of added soil biota on plants are likely to disappear in a long term. Therefore, management of the soil community by soil inoculation may fail to promote the restoration process in primary succession due to the relatively subtle role of soil biota at this succession stage. Instead, improvement of soil abiotic conditions, such as soil nutrient availability, may provide a potential shortcut for a successful restoration process in primary successional ecosystems. In summary, for better implementation of soil inoculation in the future, (1) the timeline of the expected impacts should be seriously considered, as soil inoculations seems to impose changes in the soil biota for a limited time period of a few months, with the effects fading away over time; (2) and the impacts of changes in soil abiotic conditions via soil inoculation should be considered, especially in nutrient-poor sites, such as primary dunes.

Table 6.2 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 in 2018.

	Donor Dune site	Donor Grassland site	Donor Forest site	Experimental site
Fe (mg/kg)	0.17 ± 0.050	0.72 ± 0.280	2.06 ± 0.196	0.02 ± 0.002
P (mg/kg)	10.50 ± 0.706	56.00 ± 19.50	53.60 ± 12.70	12.9 ± 0.648
Zn (mg/kg)	0.05 ± 0.032	1.82 ± 0.769	1.68 ± 0.275	0.04 ± 0.003
S (mg/kg)	2.09 ± 0.201	5.34 ± 1.130	5.06 ± 0.992	0.89 ± 0.052
K (mg/kg)	10.50 ± 0.706	56.00 ± 19.50	53.60 ± 12.70	12.90 ± 0.648
Mg (mg/kg)	11.80 ± 2.74	65.60 ± 17.10	59.80 ± 12.60	7.26 ± 0.305
Nitrogen (NO_2^-+NO_3^-) (mg/kg)	1.46 ± 0.252	1.24 ± 0.662	1.90 ± 1.480	0.93 ± 0.127
Nitrogen (NH_4^+) (mg/kg)	1.12 ± 0.159	4.05 ± 1.55	19.40 ± 1.550	0.72 ± 0.171

- *Decoupling aboveground and belowground dynamics in response to soil inoculation*

The trait-based approach of this thesis advanced our understanding of the consequences of plant-soil interactions on plant community dynamics over time (Baxendale *et al.* 2014; Cortois *et al.* 2016; Xi *et al.* 2021). In Chapter 4, I showed that shifts in soil abiotic and biotic conditions resulted in responses in various plant community traits. Notably, I found that manipulation of the soil community by soil sterilization influenced only the root traits but not the leaf traits, suggesting decoupled effects of soil inoculation treatments on the plant community. These results suggest that the responses of plant communities to soil inoculation are different for aboveground and belowground parts. Given the close associations between plant roots and soil communities, the different responses may mainly derive from the involvement of soil biota (e.g. mycorrhizal fungi, plant-growth-promoting-rhizobacteria) as they can mediate plant root nutrient acquisition and consequently induce much more impacts on plant belowground processes than on aboveground processes (Ma *et al.* 2018; Bergmann *et al.* 2020). This empirical work is important because it indicates that on a community level, the frequent coincidental leaf-root relationships which arise from common drivers (e.g. climate, soil nutrients) can decouple at plant community level. Absence of consistent leaf-root trait correlations in response to alterations in soil communities highlights that key role of the soil community in mediating the multidimensionality of plant trait relationships (Bergmann *et al.* 2017; Laliberté 2017).

- *Soil inoculation-induced legacy effects on soil microbial development*

Based on Chapter 5, I found that the addition of living soil inocula had significant influences on the soil fungal and bacterial composition. However, it is important to note that 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 are in line with a recent study which shows that the soil legacy effects resulted from soil inoculation are longer-lasting within soil fungal communities compared to bacterial communities (Heinen *et al.* 2020). The different responses in soil microbial composition may be attributed to the different life history strategies of soil fungi and bacteria. Soil fungi are generally assumed as be slow-growing while soil bacteria are more variable in their strategies (Rousk & Bååth 2007; Allison & Martiny 2008). In addition, recent findings indicate that soil fungi are mainly shaped by temporal changes in the plant community while soil bacteria are rather affected by temporal variability in the habitat (Barnard *et al.* 2013; Hannula *et al.* 2019, 2021).

In contrast to the soil microbial communities, I did not observe significant effects of soil inoculation on plant communities. This is contrast to other studies which show that soil inoculation-induced changes in soil microbial communities have knock-on effects on the subsequent plant communities (Kardol *et al.* 2006; Heinen *et al.* 2020). The harsh environmental conditions at the early stage of an ecosystem may limit the prevalence of plant-soil associations. Therefore, the effects of soil abiotic factors on plant communities may outweigh the effects derived from plant-soil microbial interactions. This finding supports that the context-dependency is a general rule in community ecology (Fukami 2015; Wubs 2017). Last but not least, my study shows that by using soil inoculation in the field, we can mediate the composition of soil microbial communities, and even possibly the ecological functions they provide. This can enhance our capacity for optimizing nature management practices (e.g. improve ecosystem resilience to climate change).

6.3 Future perspectives for holistic understanding the role of plant-soil interactions in ecosystem functioning

- *Soil abiotic factors need to be considered in plant-soil interactions*

Both soil biotic and abiotic factors are of importance in driving plant-soil interactions (Ehrenfeld *et al.* 2005; Manning *et al.* 2008; de Kroon *et al.* 2012; Castle *et al.* 2016). My study highlights the importance of soil abiotic factors, including climate and soil, in mediating the strength and direction of plant-soil interactions in primary succession. Elucidating how these interactions depend on shifts in abiotic conditions is fundamental and essential for understanding the effects of plant-soil interactions in determining ecosystem functioning in natural environments. However, so far, studies on whether and how changes in environmental conditions influence the current balance in the interactions between plant and soil biota are still in their infancy (van der Putten *et al.* 2009, 2016a). Furthermore, an increasing number of studies points out that plant-soil interactions become more variable under climate change compared to current environmental conditions (Duell *et al.* 2019; Pugnaire *et al.* 2019), making it very difficult to predict their effects on ecosystem functioning in experiments or modeling. There is an urgent need for empirical studies on refining how plant-soil interactions will change over time with changing soil and climate conditions and its consequent effects on aboveground and belowground ecosystem processes (Kardol *et al.* 2013).

- *Integrating plant functional traits advances in our understanding of plant-soil interactions*

Based on the Chapter 4, we found that the decoupled relationship between community-level leaf and root traits derived from the impacts of added soil biota on root traits. This finding is in agreement with recent findings that the involvement of soil community can change frequent coincidental trait-trait relationships between plant leaf and root functional traits, breaking the relationship between commonly co-varying drivers (Bergmann *et al.* 2020). This finding is important because it indicates that on a community level, the dynamics of the plant aboveground processes across a gradient of altered environmental conditions may not be informative of belowground processes as reflected through functional traits expression. In addition, given the important role of plant functional traits in driving multiple ecosystem functions (Faucon *et al.* 2017), this finding indicates that a comprehensive understanding of the multifunctionality of terrestrial ecosystems cannot be achieved without explicit analyses of the dynamics of belowground traits and community composition.

- *Plant community level vs species level in the study of plant-soil interactions*

There is mounting evidence that plant monocultures may create distinct conditions which are not common in natural communities (Kulmatiski 2019) and that observed interactions between selected plant species and soil communities are not relevant to the interactions between plant and soil communities of natural systems (Smith-Ramesh & Reynolds 2017; Wubs & Bezemer 2018; Grenzer *et al.* 2021). In this thesis, I found that the influences of soil biota on plant drought responses were dependent on soil treatments and plant species and the functional groups these plants belong to (Chapter 3). Additionally, the sensitivity of individual species and functional groups to drought was idiosyncratic and inconsistent with the drought responses of whole plant community (Gao *et al.* 2022). These results suggest that the effects of observed plant-soil interactions for certain individual plant species may be counter- balanced by the effects of other plant species when they grow in natural communities (Heinen *et al.* 2020). Thus, the observational results based on individual species or different plant functional groups may not be representative to the patterns of entire plant community. Furthermore, I observed that the responses of plant community traits to soil inoculation treatments were inconsistent with previous observations based on individual species (Chapter 4). Although species-based-measured plant-soil interactions are important for conceptual models, to fully understand the role of plant-soil interactions in affecting ecosystem functioning, empirical work with realistic plant communities under natural settings is strongly required.

- *Bridging natural plant-soil interactions to agriculture science*

In agricultural ecosystems, many crops are characterized as early successional, fast-growing plant species. Moreover, similar to early successional natural ecosystems, the agricultural ecosystem is relatively simplified with a limited number of plant and soil species (Mariotte *et al.* 2018). Advanced understanding of plant-soil interactions in primary succession therefore offers an opportunity for improving the sustainability of crop ecosystems (van der Putten *et al.* 2009; Jing *et al.* 2022). As I show in this thesis, the manipulation of soil abiotic and biotic conditions can influence the aboveground and belowground processes and consequent ecosystem functions (i.e. stability, productivity, nutrient cycling). These concepts and methodology attained from natural ecosystems can also be implemented in agricultural systems (Mariotte *et al.* 2018; Liu *et al.* 2022b). For example, one might consider optimizing the direction and strength of plant-soil interactions through engineering the "unplanned" soil biota (Brussaard *et al.* 2007; Hoeksema *et al.* 2010; Jing *et al.* 2022) with soil inoculation. Research has indicated that isolates of beneficial soil microbial guilds (e.g. AM fungi and nitrogen-fixing bacteria) from wild plant species can promote plant defense against soil pathogens (Zachow *et al.* 2014). This suggests that soil inoculation from wild soil is likely to assist the control of crop pathogens. Moreover, the presence of these mutualistic soil biota have the potential to enhance plant nutrient uptake, thus reducing the use of fertilizers and preventing the accretion of N and P in agricultural soil (Yang *et al.* 2009, 2022). Lastly, in my study, I found that the effects of soil inoculation in an early ecosystem mainly relied on the soil abiotic context. The effects of hash environmental conditions may outweigh the soil-microbial-induced effects on plants in primary ecosystems. In contrast, the relatively high-nutrient conditions in agricultural soil are likely to increase the success of inoculation by supporting more variable soil microbial activities under better abiotic conditions.

6.4 Conclusions and outlook

In this thesis, using a field experiment in a primary dune ecosystem, I showed that changes in soil conditions cannot only alter plant performance and plant community development but also influence soil microbial communities (Chapter 2 & 5). In turn, the altered soil communities may affect the root traits of the community, but not necessarily the aboveground traits (Chapter 4). Furthermore, the soil inoculation-induced alteration in the soil community did not mediate plant community recovery to a drought event (Chapter 3). The added soil biota from soil inocula had little impact on plant community composition during the early-successional stage regardless of the significant impacts of soil inoculation treatments on both plant and soil microbial community composition (Chapter 5). In summary, my study demonstrates that the effects of plant-soil interactions on ecosystem functions are highly context-dependent and my work underlines the crucial role of abiotic factors in determining aboveground and belowground ecosystem processes in primary dune ecosystems. Lastly, my work suggests that managing the soil community through soil inoculation may not work in all environmental conditions. Moving forward, to fully understand the mechanisms of how plant-soil interactions affect ecosystem functioning, future work is required to test how abiotic conditions alter the strength and direction of plant-soil interactions in the long run under natural conditions.