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

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Chapter 1

General introduction

1.1 Soil biodiversity

Soil communities are extremely complex and diverse, comprising an enormous number of organisms, ranging from microscopic bacteria and fungi, to larger organisms, such as nematodes, and earthworms (Bardgett 2010; Bardgett & van der Putten 2014; Wagg *et al.* 2019). Recent experiments and observational studies have shown that, consistent with observations of plant communities (Maestre *et al.* 2012), the ubiquitous and hidden soil diversity plays critical roles in driving a broad range of ecosystem functions, such as nutrient cycling, climate regulation, organic matter decomposition and primary production (Wardle *et al.* 2004; Crowther *et al.* 2019; Wagg *et al.* 2019). Accordingly, recent research, conducted both in primary succession ecosystems (e.g. glacial land, sand dunes) (Nemergut *et al.* 2007; Castle *et al.* 2016; Ortiz-Álvarez *et al.* 2018) and in secondary succession ecosystems (e.g. arable land, grassland) (Kardol *et al.* 2007; van der Putten *et al.* 2016a), have demonstrated that soil biodiversity is of high importance for the maintenance of multifunctionality in terrestrial ecosystems (Wagg *et al.* 2014; Manning *et al.* 2018; Guerra *et al.* 2020).

1.2 Soil biodiversity influences ecosystem functioning

- *Soil biodiversity directly influences soil nutrient cycling*

Decades of empirical and theoretical research have shown that biological activities in soils directly drive widespread key ecosystem functions, especially nutrient cycling (Figure 1.1) (Philippot *et al.* 2013; Fierer 2017; Kuypers *et al.* 2018). For example, soil biota contributes to soil formation through nutrient cycling and organic matter production. The activities of soil bacteria, archaea and fungi are involved in weathering of primary minerals, such as dissolution and crystallization (Uroz *et al.* 2009), thereby mobilizing nutrients from insoluble minerals to support plant growth (Aislabie *et al.* 2013). Soil microbes are also major determinants of the carbon storage capacity of soils (Allison *et al.* 2010; Soudzilovskaia *et al.* 2019; Zhu *et al.* 2020). Decomposition via microbes results in carbon loss from the soil due to microbial respiration (Trivedi *et al.* 2013). Further, denitrifying bacteria and fungi and methane-producing and consuming bacteria regulate nitrous oxide (N_2O) and methane (CH_4) emissions from soils (Schimel 2000; Baggs 2011; Shan *et al.* 2021).

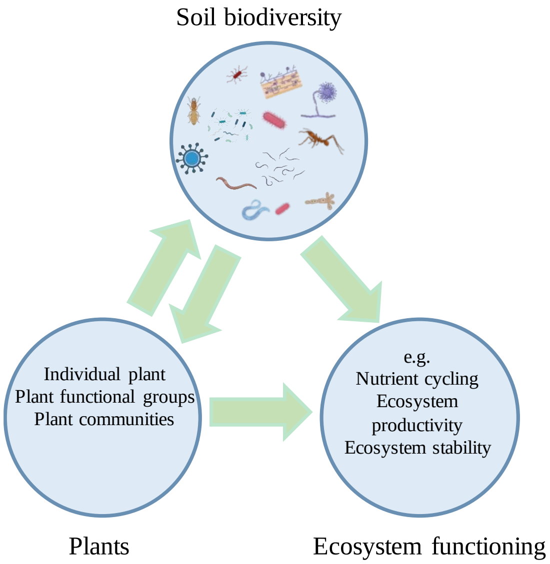

Figure 1.1 Conceptual scheme illustrating how soil biodiversity regulates ecosystem functioning directly, and indirectly through interactions with plants.

1.3 Soil biodiversity indirectly influences ecosystem functioning through interactions with plants

- *Soil biodiversity and primary production*

Primary productivity is generally recognized as an integrative indicator of ecosystem functioning. There is mounting evidence that soil biodiversity has crucial effects on regulating plant community structure and productivity:

1) Interactions between soil community and plants may result in antagonistic or synergistic influences on plant performance (van der Putten *et al.* 2016b; Wubs *et al.* 2018). On the one hand, beneficial soil organisms (e.g. mycorrhizal fungi, plant growth promoting bacteria, and earthworms) can promote plant nutrient acquisition, which often leads to positive impacts on plant growth (Compant *et al.* 2010; Wu *et al.* 2012; Zhang *et al.* 2019). On the other hand, the accumulation of non-beneficial soil biota organisms (e.g. soil pathogens, root herbivores) can hamper plant primary production by negatively affecting plant growth and decreasing root uptake capacity (Bever *et al.* 2010, 2015; Mendes *et al.* 2013).

2) Plant interactions with pathogens, decomposers and mutualists affect plant diversity and composition through the modification of ecological niches and soil legacy effects (Eisenhauer *et al.* 2012a; Kardol *et al.* 2018; Heinen *et al.* 2020). For example, the accumulation of specific soil pathogens has stronger negative effects on the growth of early-

successional plants than on the growth of other co-existing later-successional plant species, thus contributing to the vegetation succession (Kardol *et al.* 2007; Maron *et al.* 2011).

3) Moreover, recent studies on plant diversity indicate that soil biota can indirectly influence primary production by mediating the relationship between species diversity and productivity (Schnitzer *et al.* 2011; Eisenhauer *et al.* 2012b). The soil-borne pathogens play a more pronounced role in determining plant diversity-productivity relationships than other soil organisms such as mycorrhizal fungi (Schnitzer *et al.* 2011).

4) Lastly, it has been shown that alternation in the modes of plant-soil interactions through techniques like soil inoculation can alter the direction of the development of vegetation, and this effect can last more than decades in nature (Wubs *et al.* 2016, 2019).

- *Soil biodiversity and ecosystem resilience to climate change*

Climate is a major factor in determining organism growth and composition. Thus climate change including temperature increase, altered precipitation patterns and other climate-related factors are likely to influence the activity and composition of plant and soil communities and the interactions between them (O'Brien *et al.* 2018; Pugnaire *et al.* 2019). Soil biota can exert important influences on ecosystem stability and consequently buffer the effects of global change in terrestrial ecosystems (Lau & Lennon 2012; Delgado-Baquerizo *et al.* 2017; Yang *et al.* 2018; Liu *et al.* 2022a). A growing body of studies suggests that the diversity and structure of soil communities have substantial impacts on plant-plant interactions, plant primary production, plant community composition and plant tolerance to stress factors (De Deyn *et al.* 2003; Maron *et al.* 2011; Wagg *et al.* 2014; Yang *et al.* 2021). These aspects of plant performance are closely associated with ecosystem stability (Loreau & de Mazancourt 2013; Liu *et al.* 2018), suggesting that the presence of soil biota helps stabilizing the plant production under stress by enhancing growth and by inducing community compensatory dynamics among plants (Pellkofer *et al.* 2016; Rubin *et al.* 2017). For instance, plants associated with soil mutualists, such as arbuscular mycorrhizal fungi, can facilitate the resistance of plant productivity to drought and the recovery of plant community structure back to its prior-drought state (Jia *et al.* 2021). In addition, fungal-based soil food webs and soil microbial diversity can reduce carbon and nitrogen losses under drought (De Vries *et al.* 2012), thereby indirectly enhancing plant performance during a drought event. Collectively, these studies suggest that soil biota could promote ecosystem stability by directly improving plant tolerance to stresses and by indirectly regulating the nutrient supply that plants depend upon during recovery (Yang *et al.* 2018).

1.4 Knowledge gaps in plant-soil interactions

The examples above illustrate that plant and soil communities are inextricably linked through a range of interactions, including the exchange of carbon and nutrients but also herbivory and pathogenic effects. The examples above also illustrate that plant-soil interactions have multiple facets, including plants and specific groups of soil organisms, such as soil mutualists (mycorrhizal fungi), plant-growth-promoting-rhizobacteria, soil decomposers (soil invertebrates and saprotrophic microorganisms), and soil pathogens (Smith & Read 2008; Van der Heijden *et al.* 2008; Lugtenberg & Kamilova 2009; Maron *et al.* 2011; Rousk & Bååth 2011).

These multiple interactions between plant and soil biota have the potential to mechanistically explain not only individual plant performance but also interspecific plant interactions and community assembly across ecosystem succession (Bever *et al.* 2010; Fukami & Nakajima 2013; Wubs *et al.* 2016). Therefore understanding the widespread interactions between plant and soil biota can advance our understanding to better predict the impacts of soil biodiversity on ecosystem functions (Figure 1.1). However, due to the complexity of interactions between plants and soil organisms (Semchenko *et al.* 2022), the outcome of these interactions across different abiotic (e.g. soil pH, nutrient resources, and water availability) environments and their variations across temporal scales is still in its infancy (Bennett & Klironomos 2019) and mostly based on observational studies.

- *Plant-soil interactions under climate change*

Recent empirical studies offer some insights into plant-soil interactions in view of climate change (van der Putten 2012; Bardgett *et al.* 2013; Classen *et al.* 2015). For instance, drought has legacy effects on soil microorganisms that can influence plant competition with consequences for the direction and magnitude of plant-soil interactions (Kaisermann *et al.* 2017). The presence of some soil symbionts can buffer the negative effects of drought on seed germination and plant growth so that the host plant is more resistant and less affected by drought than other species (Preece & Peñuelas 2016; Jia *et al.* 2021). Rewetting of soils is expected to promote the abundance and activity of soil organisms and results in a decrease of fungal biomass (Bapiri *et al.* 2010). These drying-rewetting cycles can affect the growth of plants in a species-specific way, thereby shifting the competition among plans and hence community turnover (Meisner *et al.* 2013). There is also evidence that the increasing concentration of atmospheric CO_2 may change the nature of plant-soil interactions by regulating the activity of rhizosphere soil food webs in response to mediation in plant root carbon allocation (van der Putten *et al.* 2016a). Accordingly, the enhanced belowground carbon input could influence the nutrient cycling in ecosystems (Drake *et al.* 2011). Although there is increasing knowledge of how plant-soil interactions influence ecosystem functions, we lack an explicit understanding of how these effects might mediate the impacts of the ongoing climate change in terrestrial ecosystems, which weakens our capacity to quantify and predict the contribution of plant-soil interactions in the potential trajectories of ecosystem functions under global climate change (van der Putten *et al.* 2016b; Pugnaire *et* *al.* 2019).

- *Plant-soil interactions in the community organization*

Compared to the well-studied knowledge about the role of soil biota in mediating plant community organization, the question of how the assembly of soil organism communities is affected by aboveground plant features remains relatively less known. Several recent studies have shown that the interactions between plants and soil communities can shape the composition and diversity of soil communities (Bever *et al.* 1996; De Deyn *et al.* 2011; Leff *et al.* 2018; Zhalnina *et al.* 2018). It is becoming clear that plants detect, discriminate and associate with particular soil communities during growth, based on observational and experimental evidence (Bezemer *et al.* 2010; Schmid *et al.* 2019, 2021; Wubs *et al.* 2019). For instance, 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; Venturi & Keel 2016). Schmid *et al.* found that grasses increased the fungal richness and evenness and that legumes increased fungal evenness, while bacteria were not influenced, suggesting that soil microbes are linked with the composition of plant species and functional groups (Schmid *et al.* 2021). Combined with the well-studied knowledge about the influence of soil biota on plant composition, these findings indicate that plant and soil communities are closely interrelated across different scales and circumstances. There is, however, an important gap in knowledge of the causality in these relationships, i.e. which group "drives" the assembly of the other group and in which situation. This gap is owing to the difficulty in manipulating the soil community composition under realistic conditions (Zobel & Öpik 2014).

- *Plant-soil interactions in the primary succession*

Studies of plant-soil interactions during secondary succession have demonstrated that the strength and direction of plant-soil interactions effects are highly dependent on a plant's ability to form an association with soil biota and on the direction of those interactions (e.g. mutualistic vs antagonistic) (Kardol *et al.* 2006; van der Putten *et al.* 2016b; Zhou *et al.* 2017). For example, in early successional ecosystems, colonizing plant species generally suffer negative impacts of plant-soil interactions due to their limited defense against soil pathogens (Kardol *et al.* 2006, 2007; Middleton & Bever 2012). By contrast, during the later stages of secondary succession, there are positive impacts of plant-soil interactions on the late-successional species that can form strong mutualistic associations with symbionts like arbuscular mycorrhizal (AM) fungi (Kardol *et al.* 2006; Cheeke *et al.* 2019; Koziol & Bever 2019).

The conceptual foundation for much of what we currently know about plant-soil interactions mainly lies in secondary succession (Van der Heijden *et al.* 2008; van der Putten *et al.* 2009, 2013). However, compared to secondary succession ecosystems, patterns of plant-soil interactions may be fundamentally different during primary succession (Castle *et al.* 2016). First, the early stages of succession are often characterized by limited soil nutrient availability, harsh physiological properties and sparse vegetation cover of ruderal plant species (Lovett-Doust 2002; Walker & del Moral 2003). Similar to plant communities, soil food webs are also very simple and are composed of heterotrophic microorganisms (Bardgett *et al.* 2007). Further, soil organisms as early colonizers of primary succession may undergo rapid succession (Brown & Jumpponen 2014). There might be less association between plants and soil organisms, including mutualists or pathogens, before significant plant colonization occurs (De Deyn *et al.* 2004a). Additionally, over time, the accumulation of soil organisms is likely to result in different effects on plant dynamics. For instance, during primary succession, obligate mutualists between plants and specific soil microbial groups including N-fixing bacteria and mycorrhizal under conditions of low nitrogen and phosphorus availability positively affect plant growth (Chapin *et al.* 1994; Nara & Hogetsu 2004; Knelman *et al.* 2012), thereby facilitating the establishment of plant species and slowing down species turnover (Kardol *et al.* 2013; Castle *et al.* 2016). On the other hand, early successional plants of barren substrates are generally characterized by non-mycorrhizal associations or have a weak association with soil organisms (Cázares *et al.* 2005; van der Putten *et al.* 2009). Their establishment and growth may increase the abundance of soil pathogens more than of mutualists (van der Putten *et al.* 2013). Studies of primary succession have suggested the association between plants and these soil pathogens may lead to negative effects on plants and promote subsequent species turnover (Putten *et al.* 1988; van der Putten *et al.* 2013). Whether positive or negative plant-soil interactions are dominant in primary succession and the role of plant-soil interactions in driving early ecosystem functioning remains mostly unknown.

1.5 Soil inoculation method

In natural ecosystems, soil organisms are found within complex food webs, and there are complicated interactions among specific guilds, such as selection or complementation (Loreau & Hector 2001), making it difficult to predict their total effect on ecosystem functioning. Studies that used pairs of plants and soil organisms failed to fully capture the effects of soil organisms on plants (Wubs 2017). The great variation within soil biota with distinct organisms playing different roles in observed plant-soil interactions (e.g. symbionts, antagonists and decomposers) (van der Putten *et al.* 2016a; Wubs *et al.* 2018), makes it challenging to disentangle the various roles of soil biota in this process (van de Voorde *et al.* 2012). There is an urgent need to treat the soil communities as a whole to explore their influences.

Soil inoculation is a novel technique aimed to experimentally investigate how changes in the entire soil community composition affect plants, by adding soil inocula obtained from natural donor soils to target soil ecosystems, and therefore featuring the entire complexity of soil communities (Carbajo *et al.* 2011; van de Voorde *et al.* 2012). Earlier field experiments demonstrated that inoculation with soil communities originating from distinct ecosystems can change the soil microbial composition (Wubs *et al.* 2016, 2019), and suggested that this technique is a powerful tool to empirically examine the effects of the entire soil community on plant growth and composition (Middleton & Bever 2012; van de Voorde *et al.* 2012; Wubs *et al.* 2016).

In my PhD I conducted my studies based on a field experiment of Leiden university, Terra-dunes, in a Dutch coastal dune ecosystem (Meijendel Nature Reserve, Wassenaar, The Netherlands, 52°07'50.4"N; 4°20'27.6"E). To establish this field experiment, soil inocula were collected from three types of donor ecosystems in Meijendel Nature Reserve: primary dune vegetation, dune grassland and dune forest (Figure 1.2). This selection of donor ecosystem types enabled using in the experiment the inocula of soil communities developed under highly contrasting conditions, and therefore featuring large differences in community composition. The "Independent Soil Sampling" (ISS) approach (Gundale *et al.* 2017) was employed, to enable replication of inocula origin, i.e. for each donor ecosystem type, four distinct donor sites were selected for sampling (Figure 1.2).

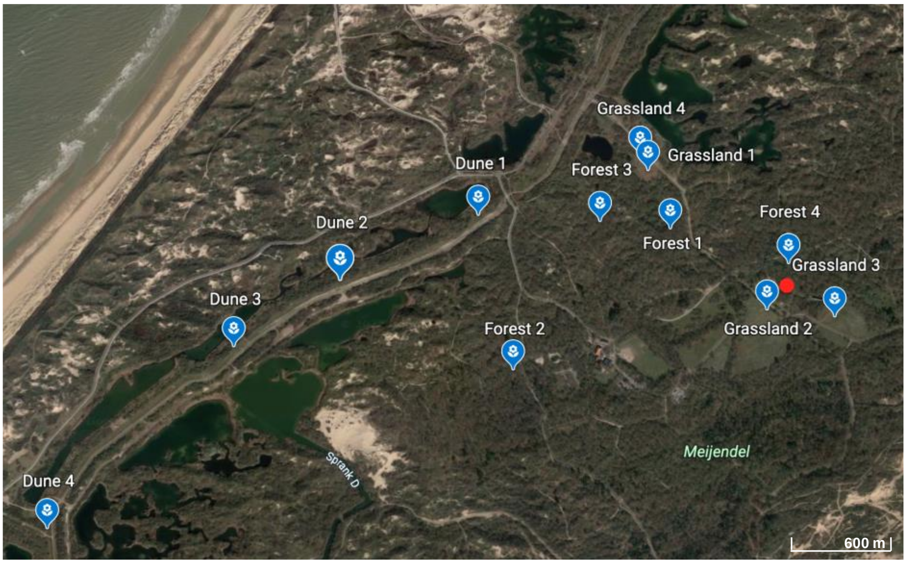

Figure 1.2 The positions of donor soil sites in Meijendel Nature Reserve. The location of the experimental site is indicated as the red point.

The Terra-dunes experiment was established in May 2018. In summary, in this experiment the soil community was manipulated via the addition of soil inocula in a newly established sandy dune site. In order to speed up vegetation development in the experimental plots and to enable initially similar soil seed bank in all plots, seeds of 30 plants typical for European coastal dune ecosystems were sown into the experimental plots simultaneously with soil inocula additions. Bellow I provide a detailed description of each step of the experimental setup. Figure 1.3 provides a graphical illustration of the set of treatments employed in the experiment.

Preparation of the experimental area. An area of 45 m × 50 m which initially was occupied by an old building and its yard, was thoroughly cleaned from building material and vegetation. Hundred and four plots of 2 m x 2 m were established in rows with a distance of 2 m between plots.

Assignment of plots to inoculation treatments. The plots were randomly assigned to be inoculated with soil inocula originating from different successional stages of dune ecosystems or to be used as control plots. Twenty four plots were assigned to be inoculated with soil inocula originating from primary dune vegetation, 24 plots were assigned to be inoculated with soil inocula originating from dune grassland and 24 plots were assigned to be inoculated with soil inocula originating from dune forest. Thirty two plots were assigned as control plots.

Assignment of plots to inocula sterilization treatments. Half of the experimental plots assigned to receive soil inocula were prescribed to receive a sterilized soil inocula where the resident soil community was eliminated through gamma radiation (>25 KGray gamma radiation, Isotron, Ede, the Netherlands), and the other half on the plots assigned to be inoculated was prescribed to receive living soil inocula. The sterilization setup allowed us to tease apart the effects of introduced soil biota vs. changes in soil abiotic properties on development of plant and communities in the plots. This setup also makes this field experiment unique and novel because it allows us to explicitly account for the role of added ecologically realistic suits of soil organisms and contrast the effects of these additions to additions of sterilized inocula. Each combination of soil inoculum origin and sterilization treatment was replicated 12 times (Figure 1.3).

Control plots. The 32 control plots were divided into two groups. In the control plots of the second group (22 plots) no soil inocula were added, but plant seeds were sown. The control plots of the second group entailed no inocula and no seed additions. These plots were used for overall monitoring purposes and were not included into the analyses presented in the this thesis.

Assignment of plots to a treatment with ectomycorrhizal mycelium. The assignment to mycorrhizal treatment was done in a full-factorial mode to the two other treatments, inoculation of living and sterile inocula originated from three different types of ecosystems

(Figure 1.3). In the plots assigned to received ectomycorrhizal treatment, mycelium of five ectomycorrhizal fungi (*Pisolithus arrhizus*; *Cenococcum geophilum*; *Amanita muscaria*; *Hebeloma crustuliniforme*; *Scleroderma sp.*) was added. With this treatment we aimed to test the role of ecotomycorrhiza, in the development of plant-soil interactions. Ectomycorrhiza is a typical type of mycorrhiza in late successional dune forest surrounding or bordering the young dune patches, like the one established in our experiment. Commercial ectomycorrhizal inoculum for this treatment was purchased from the INOQ company in Germany (http://inoq.de). However, subsequent assessments of presence or absence of ectomycorrhizal fungi in the plots revealed absence of the added fungal strains. Also no effects of the ectomycorrhizal treatment on aboveground neither belowground communities was observed. Details of this assessment are provided in the Chapter 3. Therefore the EMF addition treatment was concluded to have failed in Terra-Dunes (Chapter 3). Hence in all studies reported in this thesis the EMF-treated plots were used as additional replicates of other treatments.

Selection of plants to be sown into the plots. In total 30 plant species typical for the dune area were selected, including 26 herbaceous species and two woody perennial shrubs that were purchased from Cruydt Hoeck, a company selling seeds of wilds plants (http: //www.cruydthoeck.nl). Seeds of two tree species *Betula pubescens* and *Quercus cerris* were purchased at TreeSeeds company (www.treeseeds.com). The complete list of sown plants was presented in Table S3-2 (Chapter 3).

Protocol of experimental plots establishment. All plots were surrounded by a plastic sheet, dug into the soil to a depth of 40 cm to minimize the interaction between added soil biota and soil biota naturally present in the surrounding area. Plots were prepared according to the following procedure. First, in each plot, 10 cm of soil was removed. Then, ectomycorrhizal inoculum was added and about 8 cm of the soil previously removed from the same plot was put back into the plot and a seed mixture of 30 plant species was sown in the plot. Subsequently, in the plots subjected to a sterile soil inoculum treatment, 2 cm of sterilized soil was spread on the surface of each plot. In non-sterile plots, a layer of sterile soil (about 1.5 cm per plot) was added first and an additional layer of live soil (about 0.5 cm per plot) was spread on top. In treatments without any soil inoculum (control), 2 cm of the originally removed soil was put back on the surface (Figure 1.3).

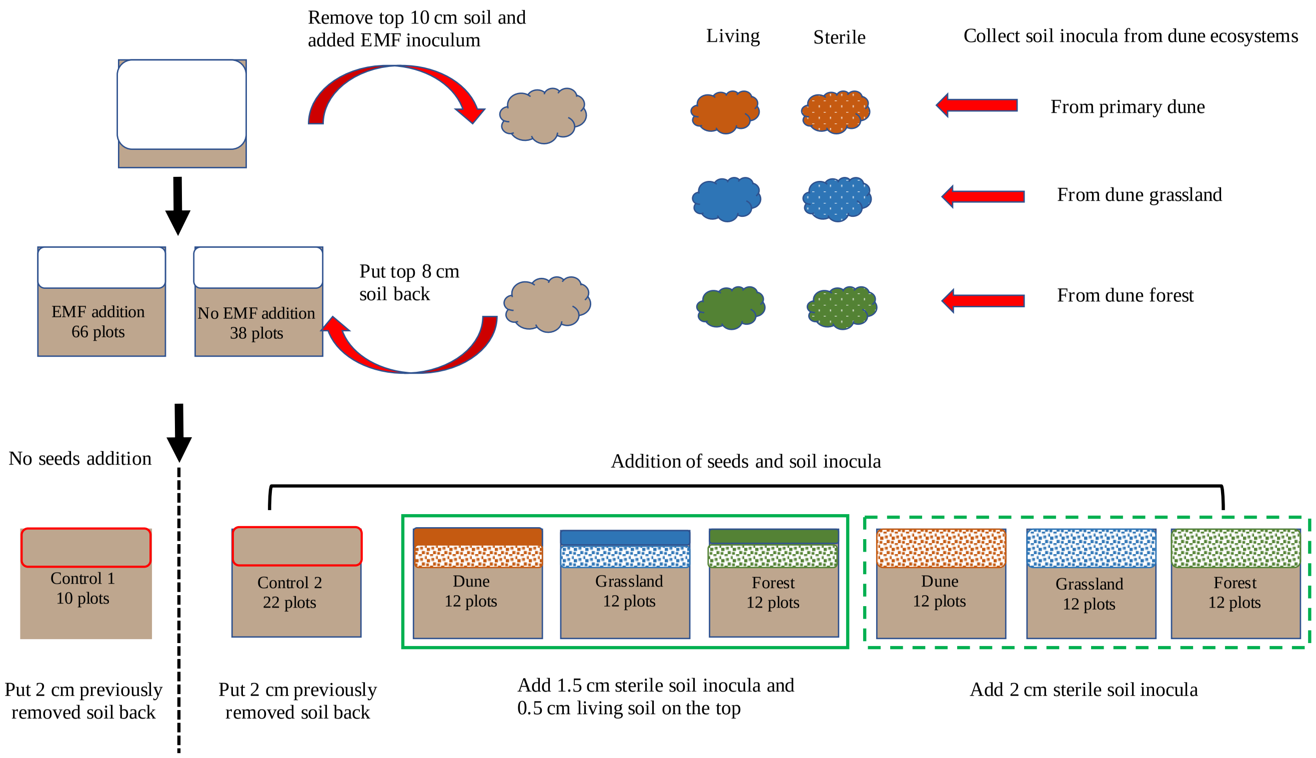

Figure 1.3 Experimental design of TERRA-Dunes

1.6 Aims and questions

The interplay between plant and soil biota can have important consequences for the ecosystem processes of both aboveground and belowground communities, such as community assembly, nutrient cycling and responses to stresses (terHorst & Zee 2016). However, the insights about the combined impact of various plant-soil interactions have less been empirically examined under natural conditions. The overarching aim of this thesis is to assess how the changes in plant-soil interactions influence the aboveground and belowground processes. In this Ph.D. thesis, I explore plant-soil interactions at different angles and scales, aboveground and belowground, from individual plant to the plant community, single year to multiple years, using the unique setup provided by the Terra-dunes experiment. I aim to examine 1) how the plant-soil interactions influence plant community functioning; 2) how the plant-soil interactions influence the assembly of aboveground and belowground communities over time. Table 1.1 gives an overview of the research questions. More details are further provided in each chapter.

Table 1.1 A brief overview of the main research questions

Chapter 2	Q1: Does the manipulation of an entire soil community influence the composition of AM fungi in soils and roots? Q2: Does the manipulation of an entire soil community influence the colonization intensity of host roots and plant performance?
Chapter 3	Q1: How do distinct soil communities influence the drought sensitivity of plants?
Chapter 4	Q1: How does a manipulation of both soil abiotic and biotic conditions influence plant community traits? Q2: How do these responses influence the relationship between community-level leaf and root traits?
Chapter 5	Q1: How does the introduction of soil biota influence the community dynamics of plant and soil microbes? Q2: Are soil biota the "driver" of plant communities at early successional stages?

1.7 Thesis outline

First, I used *Plantago lanceolata* as phytometer plant to examine whether the changes in soil communities could influence the establishment of arbuscular mycorrhizal fungi (Chapter 2). Then, taking advantage of a severe drought event in the summer of 2020, I tested the effects of soil biota on plant drought sensitivity (Chapter 3). Next, I investigated how above and belowground plant community functional traits were affected by the changes in soil conditions (Chapter 4). Finally, I used annually collected plant and soil microbial composition data to examine the role of added soil microorganisms in the development of the plant community (Chapter 5). Figure 1.4 provides the conceptual scheme of this thesis.

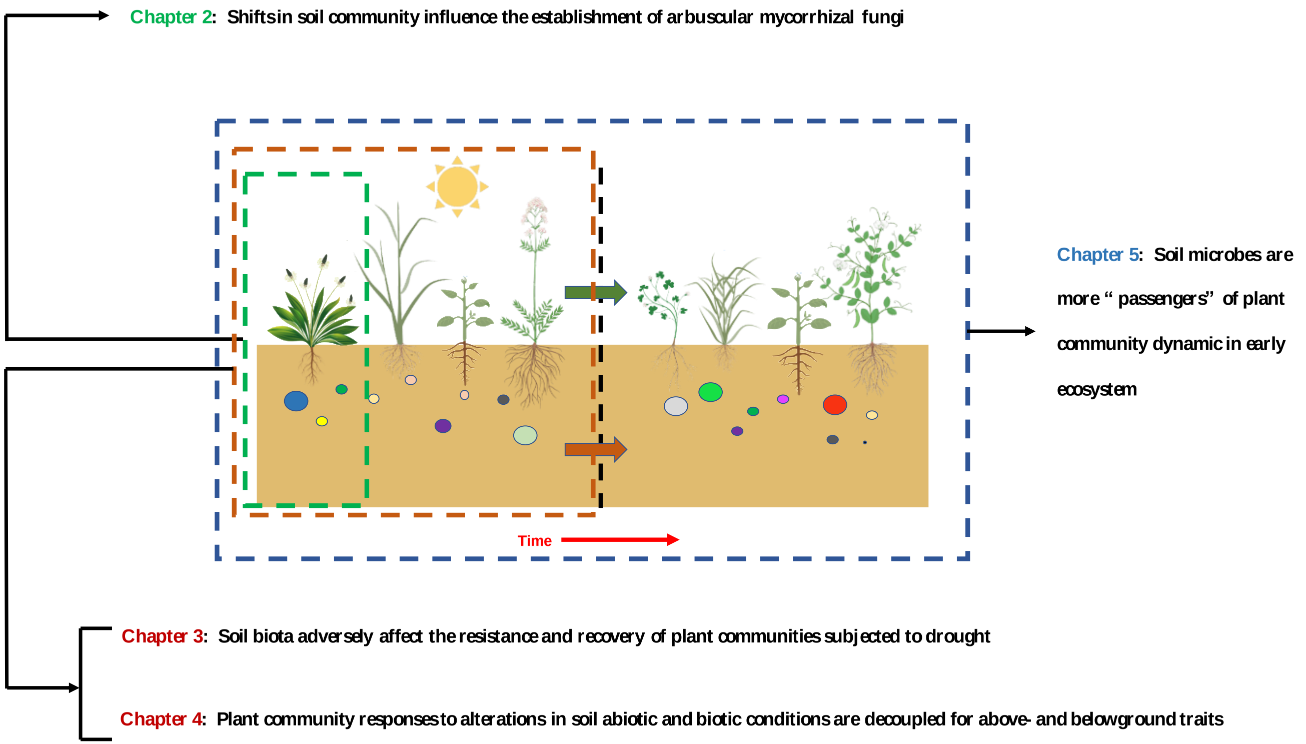

Figure 1.4 Conceptual scheme of the topics of Chapters 2, 3, 4, and 5. The colorful circles in the figure denote soil communities.

Chapter 2: Shifts in soil community influence the establishment of arbuscular mycorrhizal fungi

This chapter explores how added soil communities via soil inoculation treatments influence AM fungal composition, root AM fungal association and growth of a phytometer plant species *Plantago lanceolata*. We found that one year after inoculation the composition of AM fungi in the roots was still modified by the treatments, but an inoculation impact on AM fungal communities in soil was not detectable anymore, which suggests that the effects of inoculation on AM fungi disappear in soil but can be conserved in the roots of the host plant.

Chapter 3: Soil biota adversely affect the resistance and recovery of plant communities subjected to drought

Climate change predictions indicate that summer droughts will become more severe and frequent. Yet, the importance of soil communities in modulating the response of plant communities to drought remains unclear. In this chapter I assess if and how added soil biota from later-successional ecosystems influences the sensitivity (resistance and recovery) of plant communities to drought. The field experimental evidence suggests an adverse role of soil biota on plant community responses to drought. These outcomes indicate that impacts of soil biota on the sensitivity of plant communities subjected to drought are highly context-dependent and suggest that in some cases the soil biota activity can even destabilize plant community biomass responses to drought.

Chapter 4: Plant community responses to alterations in soil abiotic and biotic conditions are decoupled for above- and belowground traits

Plant functional traits are increasingly recognized as being impacted by soil abiotic and biotic factors. However, how the interactions between soil abiotic factors and soil community influence plant functional traits and how this influences the correlation between aboveground and belowground traits remains poorly understood. Based on the TERRA-Dunes field experiment, this chapter reveals that altered soil biotic and abiotic conditions resulted in strong decoupling in responses of plant community-level leaf and root traits. Moreover, changes in soil nutrients are the major driver of the orthogonality relationship by affecting both community leaf and root traits, whereas added soil biota played a minor role.

Chapter 5: Soil microbes are more "passengers" in the community development of early successional dune ecosystems

Understanding how plant communities co-assemble with soil microbial communities over time and how changes in soil microbial composition influence this coexistence provides valuable insights of how aboveground and belowground biodiversity affect ecosystem processes (Van der Heijden *et al.* 2008; Wagg *et al.* 2019). However, the nature and direction of the interactions between plants and soil organisms (i.e., causally determine each other) remains unclear. This chapter uses data about the inter-annual dynamics of plant and soil microbial community composition, to explore the covariation of plant and soil microbial communities in response to soil inoculation. In line with the expectations, soil inoculation can lead to persistent impacts on the community composition of both plant and soil microorganisms over time. The experimental manipulations of soil community had a persistent impact on soil fungal and bacterial composition with a stronger impact on soil fungi than on bacteria, but not for plant community. We also found that there was an increasing correlation between plant and soil microbial composition over time. By combining results from aboveground and belowground, our work brings direct evidence that in the early successional ecosystem soil microorganisms are not “drivers” but rather “passengers” reflecting plant community dynamics, but not altering it.

Chapter 6: General discussion

In this chapter, I conclude with a general discussion of my findings. In addition, based on the findings of previous chapters, I provide an outline for further research questions.