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SOIL ECOLOGY

Root traits and their effect in plant-soil interactions

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Foreword

This is a cumulative dissertation based on manuscripts selected from my publication list.

The references cited throughout all chapters of the dissertation are listed together in alphabetical order after chapter 7.

Publication list:

Aguilar-Trigueros CA, Hempel S, Powell JR, Anderson IC, Antonovics J, **Bergmann J**, Cavagnaro TR, Chen B, Hart MM, Klironomos J, et al. 2015. Branching out: Towards a trait-based understanding of fungal ecology. *Fungal Biology Reviews* 29: 34–41.

Antonovics J, **Bergmann J**, Hempel S, Verbruggen E, Veresoglou S, Rillig M. 2015. The evolution of mutualism from reciprocal parasitism: more ecological clothes for the Prisoner's Dilemma. *Evolutionary Ecology* 29: 627–641.

Heinze J, **Bergmann J**, Rillig MC, Joshi J. 2015. Negative biotic soil-effects enhance biodiversity by restricting potentially dominant plant species in grasslands. *Perspectives in Plant Ecology, Evolution and Systematics* 17: 227–235. **(Chapter 2)**

Rillig MC, Aguilar-Trigueros CA, **Bergmann J**, Verbruggen E, Veresoglou SD, Lehmann A. 2015. Plant root and mycorrhizal fungal traits for understanding soil aggregation. *New Phytologist* 205: 1385–1388. **(Chapter 3)**

Bergmann J, Verbruggen E, Heinze J, Xiang D, Chen B, Joshi J, Rillig CM. 2016. The interplay between soil structure, roots, and microbiota as a determinant of plant–soil feedback. *Ecology and Evolution* 6: 7633–7644. **(Chapter 4)**

Vályi K, **Bergmann J**, Ryo M, Prati D, Hempel S. The influence of host traits and environment on the phylogenetic structure of intraradical communities of arbuscular mycorrhizal fungi. *To be submitted* **(Chapter 5)**

Bergmann J, Ryo M, Prati D, Hempel S, Rillig MC. 2017. Roots traits are more than analogues of leaf traits: the case for diaspore mass. *New Phytologist* 216: 1130–1139. **(Chapter 6)**

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Chapter 1: General introduction

Plant ecology aims to study individual plants and their interactions within populations, communities and ecosystems scaling up to global patterns. Detailed knowledge about plant species characteristics and their interactions with the environment is thereby pivotal to predict consequences for higher organizational levels. During the last decades the focus of studies has been mainly on aboveground plant characteristics mostly ignoring belowground parts and interactions. The aim of this thesis is the assessment and the further implementation of root traits for enabling a mechanistic understanding of plant-soil interactions as drivers of plant community structure and biodiversity.

Trait based ecology

Ecology as first mentioned by Ernst Haeckel in 1866 (Haeckel, 1866) was predominantly a descriptive science. It was Charles Darwin, who proposed to use species specific proxies not only to describe but to predict organismal performance (Darwin, 1859). Since then ecology has become gradually more functional, focusing on underlying mechanisms and potential impacts of patterns and aiming to find common principles at different scales. One major step on that way was the establishment of a “trait” concept and its implementation into ecological research within the last four decades (Grime, 1974; Chapin *et al.*, 1993; Lavorel & Garnier, 2002). A trait is “any morphological, physiological or phenological feature measurable at the individual level” (Violle *et al.*, 2007). It can have an impact on the fitness or may change directly or indirectly in response to environmental conditions or vice versa (Westoby & Wright, 2006; Violle *et al.*, 2007). Trait based approaches have become more and more important and are common tools in recent plant ecology (Wright *et al.*, 2004; Westoby & Wright, 2006; Violle *et al.*, 2007; Schroeder-Georgi *et al.*, 2016). A major advantage of analyzing communities and ecosystems by traits rather than species is the direct reference to the function. As functional traits can increase our knowledge of community assembly (Roscher *et al.*, 2013; Laughlin & Laughlin, 2013), ecosystem processes and services such as primary productivity and nutrient cycling (Díaz *et al.*, 2007a, 2016; Lavorel, 2013) as well as biotic responses to climate change (Díaz *et al.*, 2007b; Valladares *et al.*, 2015) they are useful as a way to make ecology a more predictive science (Webb *et al.*, 2010). Traditionally, species specific traits are defined on the basis of mean values within a common dataset and used to analyze the functioning and effects of existing or predict possible future plant communities (Laughlin & Laughlin, 2013). Furthermore, trait variability and plasticity can give insights into species-, community- or ecosystem performance under different conditions (Osmont *et al.*, 2007; Albert *et al.*, 2011; Violle *et al.*, 2012; Freschet *et al.*, 2013) and help to understand patterns of environmental change (Ostonen *et al.*, 2007; Freschet *et al.*, 2013; Laughlin & Laughlin, 2013). Standardized measurement of traits and combining and curating datasets and metadata into databases is therefore pivotal for reliable predictions in plant ecology (Cornelissen *et al.*, 2003; Kattge *et al.*, 2011; Pérez-Harguindeguy & Díaz, 2013). During the last decades, large amounts of data have been accumulated and analyzed mainly with a focus on plant reproduction and leafs (Wright *et al.*, 2004; Laliberté, 2016).

Root traits

It is widely known that roots and their morphology and physiology are of major interest for several fields in ecology and recent evidence suggests that root traits are underestimated tools for trait based ecology (Roumet *et al.*, 2015; Laliberté, 2016). We know that in grassland ecosystems, which cover more than 40 % (Suttie *et al.*, 2005) of the Earth's terrestrial surface, roots contribute up to 3/4 of the total biomass (Poorter *et al.*, 2012). Roots and their interaction with soil influence the cycles of carbon (C) (White *et al.*, 2013)and the acquisition of several mineral nutrients like phosphorus (P) (Brown *et al.*, 2013), nitrogen (N) (Lynch, 2013) or sulfur (S) (Zhao *et al.*, 2008). They also play a major role in the physical stabilization of soil (Burylo *et al.*, 2012), the aggregation process (Six *et al.*, 2004; Rillig & Mummey, 2006; Graf & Frei, 2013), and water retention and uptake. Moreover, they are the gateway through which plants and belowground microbes and invertebrates interact. Therefore working with root traits can offer insights into belowground plant-microbe interactions (Bardgett & Wardle, 2010; Friesen *et al.*, 2011; Powell *et al.*, 2013), which are emerging as a primary driver of community composition (Mangan *et al.*, 2010; Ke *et al.*, 2014) as well as mechanisms in evolutionary ecology (Kembel & Cahill, 2011).

In 2011, Kembel and Cahill pointed out, that "knowledge of root traits and their ecological and evolutionary relationships with leaf traits is limited compared to our understanding of aboveground traits". Unfortunately, databases as well as existing studies are still heavily biased against belowground plant traits - a fact that did not change during the last years, despite the common agreement on their importance for trait based ecology. Consulting the TRY database as a representative summary of plant trait datasets, in August 2013 the number of trait observations referring to belowground traits was only 3 % of all traits excluding whole-plant, life history and generative traits (Figure 1). Until today, the total number of traits in the database more than doubled, but belowground traits still constitute only 3.8 % of the observations (Figure 1). Moreover, the majority of aboveground observations in the TRY database is on morphological traits, whereas most belowground observations are on environmental traits like mycorrhizal type or plant tolerance to soil pH, which can actually be argued to be a "plant trait" rather than a "belowground trait". The recently launched database on fine root ecology (FRED) (Iversen *et al.*, 2017) is a huge step towards the integration of roots into trait based ecology. Referring to the authors' own calculation, implementing those data into TRY will double the observations on root traits in the database. Nevertheless, roots would still be underrepresented compared to aboveground traits. Furthermore, larger datasets on traits measured under common controlled conditions are widely missing. This is problematic, because the root system is known to be very plastic and most of the traits concerned are therefore strongly context dependent (Osmont *et al.*, 2007; Padilla *et al.*, 2013; de Vries *et al.*, 2016).

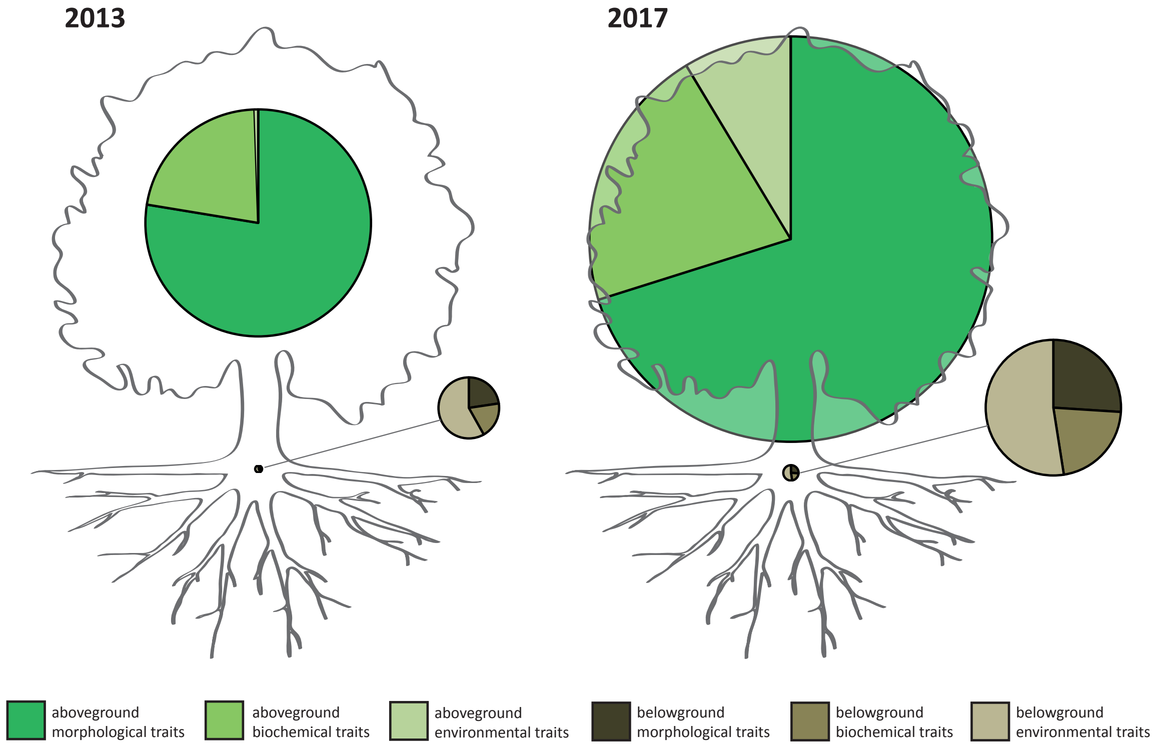

Figure 1 Distribution of trait categories in the TRY database (Kattge *et al.*, 2011). The database was searched for numbers of trait observations on 8/15/2013 and 2/23/2017. Sizes of the pie-charts in the tree are proportional to each other, enlarged belowground-charts are proportional to each other. Generative and whole plant traits were excluded from the analysis. Morphological traits include anatomical and architectural traits. Biochemical traits include all chemical compounds and ratios, as well as pH. Environmental traits include all traits directly describing the plants' interaction with the environment being biotic or abiotic, like tolerances to environmental conditions, microbial interactions, nutrient uptake or respiration and photosynthesis.

The high plasticity might be one reason why scientists hesitate to measure root traits. Another obvious reason is the effort needed to extract roots from soil and clean them for proper trait measurement. The third and probably most important reason why ecologists often refuse to measure root traits is their disbelief in gaining additional information compared to leaf traits. There is evidence that some root traits are to a certain amount correlated to leaf or stem traits (Craine *et al.*, 2005; Freschet *et al.*, 2012; Fort *et al.*, 2013). The most prominent example of such a case is the relation of root fineness and leaf surface - namely the specific root length (SRL) and the specific leaf area (SLA) - as traits related to either nutrient or light uptake. The well established concept of the "plant economics spectrum" assumes that slow growing, persistent species invest in tissue quality, while fast growing, short lived species invest in surface for light and nutrient acquisition optimization (Freschet *et al.*, 2010; Reich, 2014). A very strong correlation would indicate that root traits would not contain much additional information to leaf traits, raising the question if the effort to collect them is worth it. However, there is evidence that belowground traits hold additional information to aboveground traits especially in a soil ecological context. Kembel and Cahill (2011) found low correlations between root and leaf traits in a phylogenetic comparison suggesting that different evolutionary pressures shape morphology of above- and belowground organs. Tissue structure and longevity of fine roots contrasts markedly with those of leaves in temperate tree species (Withington & Reich, 2006) leading to the conclusion that different environmental pressures above- and belowground may cause them to be uncoupled. Schroeder-Georgi *et al.* (2016) emphasize that in grassland ecosystems aboveground-belowground trait correlation is uncertain and Fort *et al.* (2013) found the relationship of SRL and SLA to be strongly mediated by plant specific drought tolerance. Valverde-Barrantes & Blackwood (2016) state that most likely there are factors driving belowground trait variation that have not been identified yet.

Altogether, there is evidence that the root economics spectrum cannot always directly be extrapolated from the “leaf economics spectrum” (Mommer & Weemstra, 2012; Chen *et al.*, 2013; Weemstra *et al.*, 2016). Weemstra *et al.* (2016) argue that opposed to the leaf economics spectrum, the root economics spectrum is multidimensional and strongly influenced by belowground interactions unrelated to nutrient uptake. Those can be biotic plant-soil interactions as well as abiotic effects of soil structure or chemistry. Legay *et al.* (2014) found that belowground traits affected soil microbial community structure while aboveground traits did not. Thus, if root traits contain unique information by being only loosely correlated with other plant traits, especially many soil ecological questions can only be answered using root functional traits. Given the plasticity of roots in adjustment to environmental conditions, it is unavoidable to use traits measured under common controlled conditions to address questions about interspecific root trait variability. To close identified gaps in trait data this thesis comprises the largest known root morphological trait dataset measured under common conditions. The implementation of root traits into trait based ecology for enabling a mechanistic understanding of plant-soil interactions is the main focus of this thesis.

Plant-soil interactions

Apart from species specific traits, interactions of plants and soil biota are known to affect plant fitness and community structure (Mangan *et al.*, 2010) and ecosystem productivity (Schnitzer *et al.*, 2011). They are well studied in plant-soil feedback research with a focus on microbial effects on plant biomass production as a proxy for plant fitness (Klironomos, 2002;

Kulmatiski *et al.*, 2008; van der Putten *et al.*, 2013; Bennett *et al.*, 2017). The basic idea of the plant-soil feedback (PSF) concept is that plants alter the biotic and abiotic soil conditions in a local area with an effect on conspecific (direct feedback) as well as heterospecific plants (indirect feedback) (van der Putten *et al.*, 2013). Beside abiotic effects like input of chemical compounds or organic matter, specific soil biota associated with a plant get enriched in the local area around their roots. This affects plants of the same and other species and hence species abundance and plant community structure (Klironomos, 2002). Experiments to determine PSF mostly consist of (i) a training phase, where a soil community is trained by a specific plant species resulting in accumulation of both species specific antagonists and mutualists and (ii) a feedback phase where plant biomass production on “home” soil (derived from training by the same species) is compared to those on “away” soil (derived from training by another species). The net feedback effect can be positive, neutral or negative and calculates the effect of “home/conspecific” versus “away/heterospecific” soil by different equations depending on the experimental design (Brinkman *et al.*, 2010). This thesis comprises two PSF studies evaluating the effect of root traits in mediating biotic and abiotic plant-soil interactions.

Biotic plant-soil interactions

Biotic plant-soil interactions are suggested to mediate plant coexistence and hence biodiversity and ecosystem productivity (Bever *et al.*, 1997; Klironomos, 2002; Bever, 2003). Bacteria, protozoa and soil fungi interact with plant roots in various ways. Most plant-microbe interactions can be determined to be either pathogenic, neutral or mutualistic, although there is also evidence for a mutualism-parasitism continuum depending on various biotic and abiotic factors (Johnson *et al.*, 1997; Klironomos, 2003; Hoeksema *et al.*, 2010). Several microbial taxa are known to play specific roles in PSF in grassland ecosystems. Arbuscular mycorrhizal fungi (AMF) are soil inhabiting, root colonizing fungi that form specific structures for the exchange of nutrients within the plants root - the arbuscules. AMF are obligate biotrophic symbionts associated with about 90% of all terrestrial plant species (Smith & Read, 2008). The mycorrhizal symbiosis is classically considered to be mutualistic with the fungus providing water and soil nutrients like phosphorus and sulfur in exchange for organic carbon from the plant partner. AMF are known to protect plants from colonization of pathogenic fungi by induced systemic resistance (Newsham *et al.*, 1995a,b; Cameron *et al.*, 2013) and different AMF species form multispecies symbiont communities within a plant’s root.

Knowledge about general mechanistic patterns of plant-soil interactions is still lacking in many cases because of species specific effects that are not easy to generalize. When trying to investigate the effects of soil biota on plant performance, root traits have one major edge over aboveground traits. They are able to reflect underlying mechanisms because of their direct involvement in this interaction. The microbial mediation of root architecture is well studied especially in mycorrhizal symbioses (Hetrick, 1991; Newsham *et al.*, 1995b; Friesen *et al.*, 2011). An overall relationship can be observed between root “fineness” and association with different endophytic fungi (Newsham *et al.*, 1995b) with finer roots being more susceptible to pathogenic fungal colonization. Mycorrhizal plants have been found to generally have coarser roots (Peat & Fitter, 1993) and many morphological traits like SRL, root average diameter or root tissue density change in response to ectomycorrhizal or arbuscular mycorrhizal colonization (Hetrick, 1991; Ostonen *et al.*, 2007; Zangaro *et al.*, 2008). Altogether it is well known that soil biota influence root traits and vice versa, but most specifics of these interactions still remain unclear. It therefore seems a promising approach to use root traits to answer ecological questions related to plant-soil interactions. This thesis assesses the effect of different root traits on AMF community structure and discusses the general impact of root fineness on various biotic and abiotic plant-soil interactions.

Abiotic plant-soil interactions

If we want to predict plant performance, community structure and ecosystem processes in a natural context, abiotic soil conditions are no less important than biotic interactions. Soil is a highly complex multidimensional habitat with a fine scale spatial structure influencing all colonizing biota as well as the distribution of nutrients and water (Six *et al.*, 2004). Soil aggregates are formed and stabilized as well as disintegrated by several biotic and abiotic factors and their interaction (Six *et al.*, 2004; Rillig & Mummey, 2006; Lehmann *et al.*, 2017). Disentangling causes and effects is thereby not trivial nor easy. Plant roots as well as soil microbes mediate soil aggregation and disintegration and are affected by the soil structure at the same time. To enhance and maintain soil stability is a major goal in agriculture as well as for restoration or prevention of erosion (Duchicela *et al.*, 2013; Erktan *et al.*, 2015). Furthermore, there is evidence that soil aggregation status changes with land-use intensity as well as successional stage (Jastrow *et al.*, 1998; Barto *et al.*, 2010). Hence, studying soil aggregation can offer insights into the mechanism underlying shifts of community composition along land-use or successional gradients. This thesis comprises different approaches integrating root traits as explanatory functional traits in explaining soil aggregation.

Study site

This thesis comprises studies from field sampling as well as greenhouse experiments. Those experiments all took place in the framework of the German Biodiversity Exploratories program (Fischer *et al.*, 2010). This is a long term project financed by the DFG studying grassland and forest biodiversity in Germany with a focus on land-use intensity since 2006. 300 experimental plots locate in three regions (exploratories) of Germany ranging from North (Schorfheide-Chorin) over Central (Hainich-Dün) to South Germany (Schwäbische Alb). The plots are equally divided between grassland and forest and along a land-use gradient from intensively to extensively managed areas within each exploratory (Blüthgen *et al.*, 2012). Studies are carried out on numerous taxa of plants, animals, bacteria and fungi as well as their interaction on the community and ecosystem level. Extensive vegetation records as well as long-term monitoring of environmental parameters enable the cooperating scientists to link greenhouse and field data to answer questions related to land-use, biodiversity and ecosystem processes. This thesis focuses on grassland ecosystems using plots and data from all three exploratories with a plant biodiversity of about 300 species.

Outline of the thesis

This thesis aims to study the importance of root traits for plant ecology by investigating different aspects of plant-soil interactions:

Chapter 2 tests the hypothesis if plants that have a competitive advantage due to their size are held in check by negative biotic soil effects and if these vary with land-use intensity mediated by differences in root fineness.

Chapter 3 proposes a framework for studying soil aggregation by integrating plant root and mycorrhizal fungal traits.

Chapter 4 addresses the question if specific mechanisms of plant soil feedback - namely the plants' and microbial communities' interaction with soil structure - is mediated by root traits.

Chapter 5 assesses the predictive power of root traits for phylogenetic structure of AMF communities along a land use gradient.

Chapter 6 presents the largest known root morphological trait dataset elaborating their unique value in explaining and predicting different ecological concepts and mechanisms in grassland ecosystems.

Chapter 7 combines the results of the previous chapters as a general discussion elaborating the relevance of root traits for trait based ecology.

Chapter 2

Negative biotic soil-effects enhance biodiversity by restricting potentially dominant plant species in grasslands

Heinze J, **Bergmann J**, Rillig MC, Joshi J. 2015. Negative biotic soil-effects enhance biodiversity by restricting potentially dominant plant species in grasslands. *Perspectives in Plant Ecology, Evolution and Systematics* 17: 227–235.

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Summary

Interactions between soil microorganisms and plants can play a vital role for plant fitness and therefore also for plant-community composition and biodiversity. However, little is known about how biotic plant-soil interactions influence the local dominance and abundance of plant species and whether specific taxonomic or functional groups of plants are differentially affected by such biotic soil-effects. In two greenhouse experiments, we tested the biotic soil-effects of 33 grassland species differing in individual size and local abundance. We hypothesized that large plants that are not locally dominant (despite their size-related competitive advantage enabling them to potentially outshade competitors) are most strongly limited by negative biotic soil-effects. We sampled soils at the opposite ends of a gradient in land-use intensity in temperate grasslands to account for putative modulating effects of land-use intensity on biotic soil-effects.

As hypothesized, large, but non-dominant species (especially grasses) experienced more negative biotic soil-effects compared with small and abundant plant species. Land-use intensity had contrasting effects on grasses and herbs resulting in more negative biotic soil-effects for grasses in less intensively managed grasslands. We conclude that biotic soil-effects contribute to the control of potentially dominant plants and hence enable species coexistence and biodiversity especially in species-rich less intensively managed grasslands.

Introduction

Interactions between plants and soil microorganisms can have a major impact on species coexistence and thus on plant-community composition and diversity by directly or indirectly affecting the fitness and competitive ability of plant individuals (Bever *et al.*, 1997; van der Heijden *et al.*, 2008a; Bever *et al.*, 2010; van der Putten *et al.*, 2013). Plant-soil microbe interactions can either result in negative effects on individual plant growth (mediated by pathogens and/or parasites; e.g., nematodes, pathogenic fungi and bacteria; Bever *et al.*, 2010), or positive effects (mediated by symbiotic microorganisms; e.g., arbuscular mycorrhizal fungi and N-fixing bacteria; van der Heijden *et al.*, 2008b; van der Putten *et al.*, 2013). Previous studies have shown that such biotic plant-soil interactions play an important role for plant productivity (Bartelt-Ryser *et al.*, 2005; Maron *et al.*, 2011; Schnitzer *et al.*, 2011), succession (van der Putten *et al.*, 1993; de Deyn *et al.*, 2003; Kardol *et al.*, 2006; Brandt *et al.*, 2013), invasion processes (Reinhart & Callaway, 2006; Zuppinger-Dingley *et al.*, 2011), and plant-species abundances (Klironomos, 2002; Petermann *et al.*, 2008; Mangan *et al.*, 2010). In addition to biotic soil-effects, the abundance of a plant species within an ecosystem also depends on many other biotic and abiotic factors such as interspecific competition for light and nutrients (e.g., Hautier *et al.*, 2009) and on species-specific adaptations to abiotic soil and environmental conditions (e.g., Grime, 1977). The individual size of each plant species is crucial for its strength of competition for light, which is the most important factor affecting plant-species diversity in various ecosystems (especially in grasslands; Lepš, 1999; Hautier *et al.*, 2009).

Theoretical models suggest that negative biotic soil-effects prevent plant species' dominance resulting in the maintenance of species coexistence and therefore enhance plant diversity (Bever *et al.*, 1997; Bonanomi *et al.*, 2005). However, only a few studies have experimentally investigated the net effect of soil biota on the relative abundance of plant species; with relative abundance expressed either as percent cover of plant species per site (Reinhart, 2012) or based on presence-absence per site (Klironomos, 2002). Studies in a Canadian old field (Klironomos 2002) and in a tropical forest (Mangan *et al.* 2010) provided evidence for a positive relationship between plant relative abundance and plant-soil feedbacks with the regionally rarest species experiencing the most negative feedbacks. However, no such relationship was found in semiarid grasslands (Reinhart, 2012).

In addition to biotic soil-effects and aboveground competition for light, land use is one of the most dominant factors affecting biodiversity (Sala *et al.*, 2000, Foley *et al.* 2005). In grasslands, land use, especially via fertilization, affects the productivity and therefore competition for light in plant communities and may cause a decrease in plant-species richness (Lepš, 1999; Zechmeister *et al.*, 2003; Hautier *et al.*, 2009). In addition, land use includes different types of disturbances (e.g., mowing, grazing, trampling) that are differentially tolerated by plant species, resulting in plant communities typical for the specific land-use management. Land-use also affects interactions between plants and soil microbes. For example, with increasing land-use intensity, total plant-community root mass decreased whereas extraradical arbuscular mycorrhizal hyphae increased in a study by Barto *et al.* (2010), indicating that mutualistic interactions are affected by land-use intensity.

Our objective was to examine the connection between a plant's local abundance and individual size (defined here as the individual biomass produced in sterile soil x the average height of the plant species - as surrogate for competitive ability for light) in grasslands. We tested the hypothesis that plants with large individual size that are, however, not locally dominant are most strongly restricted and therefore held in check by negative soil-biota effects. Furthermore, we tested whether any putative biotic soil-effect on plant growth is modified by land-use intensity. Therefore, we investigated the net effect of the soil-biota community originating from field soils sampled along a gradient in land-use intensity (frequent and intensive vs. infrequent and less intensive agricultural management). There are different methods to investigate plant-soil interactions, each (by differently affecting biotic and abiotic soil properties) having advantages and disadvantages for the ecological interpretation of the results (described in Brinkmann *et al.*, 2010). Here, we used an approach where plants were grown in sterile soil vs. sterile soil to which 5% of a non-sterilized, field-soil inoculum was added (Bartelt-Ryser *et al.*, 2005, Zuppinger-Dingley *et al.*, 2011) to ensure comparability of the 33 species and to avoid confounding effects of nutrients.

Material and Methods

Study sites

This study was conducted within the framework of a large-scale and long-term project on biodiversity and land-use intensity in Germany, the "Biodiversity Exploratories" (Fischer *et al.*, 2010). Within this interdisciplinary project, there are three distinct study regions that are approximately 300-600 km apart and that span a latitudinal gradient of 4 degrees (Figure S2.1 in Supporting Information): (1) the UNESCO Biosphere Area Schwäbische Alb in the low mountain ranges of south-western Germany, (2) the National Park Hainich and surroundings situated in the hilly areas of Central Germany, and (3) the UNESCO Biosphere Reserve Schorfheide-Chorin embedded in a glacial landscape in the lowlands of north-eastern Germany (see Fischer *et al.*, 2010). Within each region, there are 50 grassland sites that capture a gradient of land-use intensity from less intensive to highly intensive agricultural management.

Plant local abundances and frequency distributions

To estimate the mean local abundance (A) per site and frequency of every plant species across all grassland sites studied, vegetation was recorded on 150 grassland sites (50 sites per region) in May and June 2011. At every site, percentage cover was estimated for every vascular plant species in four 1 m^2 subplots (located north, east, south and west within each grassland site). In this study, mean local abundance (A) is expressed as the mean percent cover of every plant species at each grassland site where the species was detected; whereas frequency of a plant species was defined as the total number of grassland sites where that species was present.

Species and site selection

Plant species selection for this experiment was based on five criteria: All selected species i) occurred within the three regions, ii) differed in individual size and local abundance, iii) were mycorrhizal (extracted from Wang & Qui, 2006), iv) were not symbiotic with rhizobia, and v) originated from different phylogenetic clades (see Table 2.1). Seeds of all species (except *Veronica teucrium* L. – seeds ordered from a commercial provider of local genotypes: Rieger Hofmann; Blaufelden-Raboldshausen) were collected at the grassland sites where the vegetation was recorded within all three regions from spring – autumn 2011 (Table 2.1).

Sites for soil sampling were chosen according to soil type (cambisols and luvisols), weather conditions within regions and land-use intensity: We used a land-use index (LUI) especially designed to quantify land-use intensity within the "Biodiversity-Exploratories" project (Blüthgen *et al.*, 2012) to select one extreme pair of grassland sites within each region (Figure S2.1). The land-use index ($LUI = [(F_S / F_R) + (M_S / M_R) + (C_S / C_R)]^{1/2}$) incorporates the ratios of mean fertilization (F), mowing (M) and cattle (C), respectively, per site (S) to the mean per region (R) (Blüthgen *et al.*, 2012). In this study, the land-use index varied between 0.5 (unmown pasture without application of chemical fertilisers) and 2.51 (intensively managed grasslands that are frequently grazed and mown and artificially fertilised; (see Table S2.1). In April 2011, soil that served as substrate and as inoculum in both experiments was collected from one less intensively and one intensively managed grassland site per region. Bulked soil samples (top 10 cm) were collected randomly from 6–10 different positions within each field site to account for the heterogeneity and spatial variation within sites.

Seed and soil preparation

Seeds were cleaned and afterwards surface-sterilized to avoid microbial contamination (3' in a 7% sodium hypochlorite solution; Bartelt-Ryser *et al.*, 2005), washed five times with autoclaved-sterilized water (20’, 121°C) and subsequently dried for one day under sterile conditions. Seeds of *Ranunculus acris* L., *R. bulbosus* L. and *Agrimonia eupatoria* L. were stratified (3 months, -20 °C) prior to the experiment to enhance germination rate.

Soils were sieved (mesh size 7 mm) to remove stones, large roots, and macrofauna, whereas small roots, that act as a source of mycorrhizal and other fungal and bacterial inoculum, remained in the soils. A large portion of the soils was autoclaved twice (20’, 121°C), whereas a small portion of the soils was left untreated and was used as non-sterile inoculum for both experiments. In addition to the soils, we autoclaved (20’, 121°C) sand (grain size: 2 mm; Brun & Böhm; Potsdam, Germany) twice for the establishment of soil-sand mixtures (see below). Afterwards, the sterilized soils and sand were stored in plastic bags at room temperature under sterile conditions to stabilize for five months. All instruments were sterilized with 70% ethanol before and between all working steps.

Experimental setup

We conducted two experiments with two different sets of grassland species grown in single pots (experiments 1 and 2 with 22 species each). Two separate experiments were ran due to glasshouse-space restrictions; see Table 2.1. Eleven species of experiment 1 were also used in experiment 2 and therefore served as internal controls (Table 2.1). Plants were grown in sterile soil vs. sterile soil to which a non-sterilized, field-soil inoculum was added (Bartelt-Ryser *et al.*, 2005, Zuppinger-Dingley *et al.*, 2011). Although at the start of the experiments the natural densities of the soil community were diluted with this approach, we avoided confounding abiotic effects (mainly nutrient effects) with biotic soil-effects (Bartelt-Ryser *et al.*, 2005). Sterilized soil and sand (40/60; vol./vol.) were mixed for each field site separately: For the non-sterile treatment, each sterile soil-sand mixture was inoculated with 5% (vol.) non-sterilised soil of the appropriate field site. For experiment 1 and 2, 0.4 l pots (8 x 8 x 8 cm; MR-pots Meyer; Germany) and cones (depth: 25 cm, diameter: 5 cm; Stuewe & sons Inc.; USA) were used, respectively. Five surface-sterilized seeds of every species were sown in pots and subsequently placed in a randomized block design. After germination, if pots contained more than one seedling, additional seedlings were randomly removed resulting in one seedling per pot. Before and between all working steps, all instruments were sterilized with 70% ethanol.

Each species x soil treatment x land-use intensity x region was replicated ten times (Figure S2.1),resulting in a total of 5280 pots / cones for 44 species (including 11 species, which were part of both experiments). To reduce the probability of contamination, each pot /cone was placed in a sterile plastic cup. Both experiments were carried out under greenhouse conditions with supplemental light (140 µmol * s^{-1} * m^{-2}; 12 / 12 h light / dark).

Table 2.1 Plant species used in the experiments. Data on mean height of each species were extracted from Jäger *et al.* (2009). Biomass classifies the mean species biomass based on the median of species' biomass in the experiments resulting in species with large (+) or low (–) individual biomass. Frequency represents the occurrence (presence or absence) of each species on the 150 grassland experimental sites in the three regions sampled, whereas mean local abundance denotes the mean cover of the species m^{-2}. Experiment represents the occurrence of the species in the two experiments: (1) = experiment 1; (2) = experiment 2 and (1+2) = both experiments. Species were part of two different functional types: grasses (Poaceae) and non-leguminous herbs (remaining families). With the exception of the biennials *Daucus carota* and *Carum carvi*, all plant species were perennials.

Family	Species	Height (m)	Biomass	Frequency (X / 150)	Mean local abundance (% / m²)	Experi-ment
Poaceae	*Alopecurus pratensis* L.	0.30 - 1.00	+	50	15.0	1
Poaceae	*Anthoxanthum odoratum* L.	0.15 - 0.45	+	23	3.4	2
Poaceae	*Arrhenatherum elatius* (L.) J. Presl. et C. Presl.	0.60 - 1.20	+	66	15.2	1
Poaceae	*Briza media* L.	0.20 - 0.60	-	16	1.5	2
Poaceae	*Bromus erectus* Huds.	0.30 - 0.90	-	17	10.2	1+2
Poaceae	*Cynosurus cristatus* L.	0.30 - 0.90	-	37	1.8	1+2
Poaceae	*Dactylis glomerata* L.	0.50 - 1.20	+	118	9.9	1+2
Poaceae	*Festuca pratensis* Huds.	0.40 - 1.00	+	80	11.1	1
Poaceae	*Helictotrichon pratense* (Huds.) Pilg.	0.30 - 0.80	-	1	0.7	2
Poaceae	*Helictotrichon pubescens* (L.) Besser	0.30 - 1.00	+	25	1.8	1+2
Poaceae	*Holcus lanatus* L.	0.30 - 1.00	+	31	5.8	2
Poaceae	*Koeleria pyramidata* (Lam.) P.Beauv.	0.30 - 1.00	+	11	0.9	1
Poaceae	*Lolium perenne* L.	0.10 - 0.60	-	80	10.8	1
Poaceae	*Poa pratensis* L.	0.20 - 0.90	-	108	6.7	2
Poaceae	*Trisetum flavescens* (L.) P.Beauv.	0.30 - 0.70	-	54	4.9	1
Ranunculaceae	*Ranunculus acris* L.	0.30 - 1.00	+	46	2.2	2
Ranunculaceae	*Ranunculus bulbosus* L.	0.15 - 0.35	-	24	2.1	2
Rosaceae	*Agrimonia eupatoria* L.	0.30 - 1.00	+	35	2.1	2
Rosaceae	*Sanguisorba minor* Scop.	0.15 - 0.40	+	23	1.7	2
Plantaginaceae	*Plantago lanceolata* L.	0.15 - 0.50	+	99	5.8	1+2
Plantaginaceae	*Plantago major* L.	0.05 - 0.40	-	38	3.3	1+2
Plantaginaceae	*Plantago media* L.	0.10 - 0.45	+	31	3.4	1+2
Plantaginaceae	*Veronica chamaedrys* L.	0.15 - 0.40	+	57	4.5	1
Plantaginaceae	*Veronica serpyllifolia* L.	0.05 - 0.25	-	12	0.7	1
Plantaginaceae	*Veronica teucrium* L.	0.20 - 0.80	+	4	0.2	1
Apiaceae	*Carum carvi* L.	0.30 - 0.80	-	35	2.0	2
Apiaceae	*Daucus carota* L.	0.30 - 1.00	+	26	2.3	2
Asteraceae	*Achillea millefolium* L.	0.20 - 1.20	+	74	8.2	1
Asteraceae	*Bellis perennis* L.	0.05 - 0.15	-	20	2.7	1+2
Asteraceae	*Centaurea jacea* L.	0.20 - 1.50	+	12	2.9	1+2
Asteraceae	*Leucanthemum vulgare* (Vaill.) Lam.	0.20 - 0.70	+	29	1.3	1
Asteraceae	*Taraxacum officinale* Kirschner *et al.*	0.05 - 0.40	-	124	10.5	1+2
Asteraceae	*Tragopogon pratensis* L.	0.30 - 0.60	-	27	0.9	1+2

Experiment 1 was set up in November 2011 (Min./Max: temperature – 10°C / 20°C; rel. humidity – 40% / 96%) and experiment 2 in June 2012 (Min./Max: temperature – 10°C / 40°C; rel. humidity – 30% / 96%). Both experiments lasted for 18 weeks. Plants were watered 2–3 times (with approx. 15 ml) a week with autoclaved water (20’, 121°C). After 18 weeks, aboveground biomass of all plants of each experiment were harvested, dried (48 h, 80°C) and weighed. Given the amount of plants used in the experiments the determination of root biomass and root colonization was not possible, even for a subsample. However, we were interested in the effect of soil biota on aboveground plant biomass, which is a very prominent factor influencing plant abundance in the field (van der Putten *et al.* 2007).

Statistical analysis

All statistical analyses were conducted using R version 2.15.1 (R Development Core Team 2011). For the classification of plant species into two different categories, field data of mean local abundance and the individual aboveground biomass produced in sterile soil in the greenhouse x average height of each plant species in German grasslands were used (see below). Individual size of each plant species was estimated according to the following equation: $S_i = B_i \times H_i$; where S (estimated size of each species) is the product of mean biomass (B; measured from greenhouse dry weight under sterile soil conditions) and mean height (H; extracted from Jäger *et al.* 2009) of species i. Using the median as separator, mean local abundance (A) and estimated size (S) were used to classify the species into four categories: A+/ S+; A–/ S+; A+/S–; A–/S– (+ = high / large; – small / low).

Due to their morphological differences, the classification was conducted separately for grasses and herbs. For species that were part of both experiments, biomasses were averaged and classified using data from both experiments. The calculation of biotic soil-effects was modified from Petermann *et al.* (2008):

$$\text{Biotic soil-effect}_i = \log [\text{biomass (non-sterile)}_i / \text{biomass (sterile)}_i];$$

where biomass (non-sterile) = biomass of species A under non-sterile conditions and biomass (sterile) = biomass of species A under sterile soil conditions. Positive values correspond to positive biotic soil-effects and vice versa.

To test for differences between categories and to investigate the effect of land-use intensity on biotic soil-effects, analyses of variance (ANOVA – hierarchical; type I) were performed for both experiments. The model used for both experiments contained the factors: block (1-10); category (A+/ S+; A–/ S+; A+/S–; A–/S–); functional type (grasses vs. herbs); species; LUI (high vs. low); region (Schwäbische-Alb, Hainich, Schorfheide), as well as all interactions. Because of partly unbalanced conditions, Scheffé tests (post hoc test) were used to detect differences in biotic soil-effects for categories and land-use intensity.

To check if relative and mean local abundances of plant species are related to biotic soil-effects correlation analyses were performed.

Results

Relationship between land-use intensity and plant-species richness

Over all 150 experimental grassland sites across all three regions, land-use intensification had a significant negative effect on total plant-species richness ($F_{1,148}$ = 17.33; $P < 0.001$; $R^2 = 0.16$). With increasing land-use intensity, the number of herbs decreased rapidly ($F_{1,40}$ = 42.15; $P < 0.001$; $R^2 = 0.5$), whereas the number of grass species was only slightly reduced in intensive vs. less intensively managed grasslands ($F_{1,40}$ = 18.24; $P < 0.001$; $R^2 = 0.29$). Thus, significant differences in the proportion of herbs/grasses were found depending on land-use intensity resulting in a high proportion of herbs/grasses at less intensively and a low proportion at intensively used grassland sites (Figure 2.1).

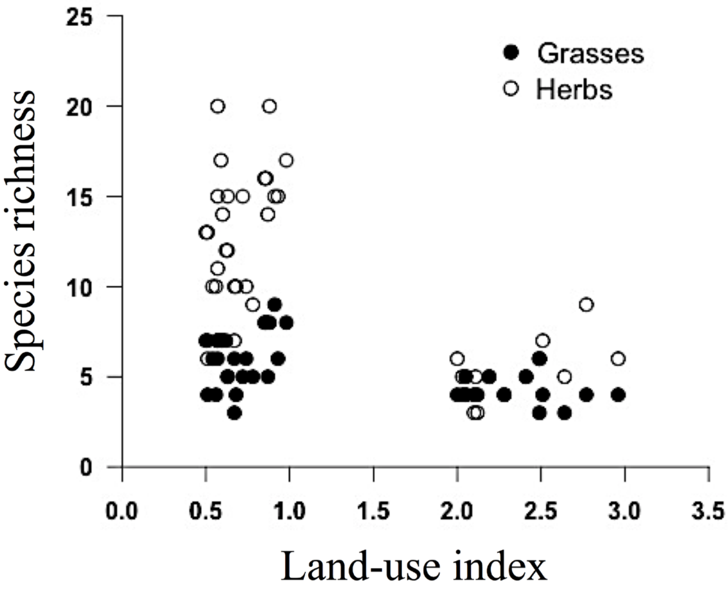


Figure 2.1 Relationship between land-use intensity [represented by the land-use index (LUI)] and mean species richness (based on species richness data) on intensively and less intensively managed grassland sites. Data represent mean species richness of grasses (filled circles) and herbs (open circles) in 4 x 1 m^2 plots per grassland site in two land-use intensities: intensive land-use (mown pastures; LUI > 2; n = 16) and less intensive (pastures; LUI < 1; n = 26).

Effect of soil biota on categories of plant size and local abundance

Plant species categorised according to plant size and local abundance differed in response to the presence/absence of soil biota in both experiments (experiment 1: $F_{3,1031} = 6.78$; $P < 0.001$; experiment 2: $F_{3,1120} = 7.14$; $P < 0.001$; Table 2.2, Figure 2.2a) resulting in different biotic soil-effects. In both experiments, plant growth for species with large size, but low local abundance (S+/A-) was negatively affected by the soil biota resulting in more negative biotic soil-effects for species of this category compared with species in other categories (Figure 2.2a). In addition, species with low local abundance (A-) in the field experienced more negative biotic soil-effects than locally abundant species (A+; Figure 2.2a).

The net effect of soil biota on growth differed for grasses vs. herbs. In both experiments, herbs experienced predominantly more positive effects from soil biota, whereas grasses exhibited negative biotic soil-effects overall (experiment 1: $F_{1,1031} = 266.01$; $P < 0.001$; experiment 2: $F_{1,1120} = 293.94$; $P < 0.001$; Table 2.2; Figure 2.2b, 2.2c).

Table 2.2 Analyses of variance (ANOVA) of biotic soil-effects for 33 Central European grassland plant-species for experiments 1 and 2. Biotic soil-effect was calculated according to Petermann *et al.* 2008: biotic soil-effect (species A) = log ratio (biomass species A (grown on non-sterile soil) / biomass species A (grown on sterile soil)). The factor Category tests for differences in biotic soil-effects for four categories separating plant species according to individual size and mean local abundance (full factorial). Functional type distinguishes between grasses (Poaceae) and non-leguminous herbs (remaining families). LUI (land-use index) tests for differences of intensively vs. less intensively managed grassland soils. The factor region comprises the three regions investigated in Germany: Schwäbische Alb, Hainich – Dün, and Schorfheide – Chorin.

	Experiment 1			Experiment 2		
Effects	*D*f	*F*	*P*	*D*f	*F*	*P*
Block	9	1.03	0.413	9	1.28	0.242
Category	3	6.78	**< 0.001**	3	7.14	**< 0.001**
Functional type	1	266.01	**< 0.001**	1	293.94	**< 0.001**
Functional type x Category	3	3.26	**0.021**	3	10.92	**< 0.001**
Species	14	3.58	**< 0.001**	14	12.55	**< 0.001**
LUI	1	2.46	0.117	1	137.98	**< 0.001**
LUI x Category	3	5.23	**0.001**	3	0.54	0.658
LUI x Functional type	1	44.58	**< 0.001**	1	125.93	**< 0.001**
LUI x Species	17	5.68	**< 0.001**	17	4.20	**< 0.001**
Region	2	24.61	**< 0.001**	2	37.09	**< 0.001**
Region x Category	6	1.62	**< 0.001**	6	4.17	**< 0.001**
Region x Functional type	2	34.20	**< 0.001**	2	65.79	**< 0.001**
Region x Species	33	3.13	**< 0.001**	34	3.36	**< 0.001**
Region x LUI	2	0.02	0.980	2	46.98	**< 0.001**
Region x LUI x Category	6	1.72	0.113	6	1.97	0.067
Region x LUI x Functional type	2	11.64	**< 0.001**	2	40.95	**< 0.001**
Region x LUI x Species	33	2.81	**< 0.001**	34	4.69	**< 0.001**
Residuals	1031			1120		
$R^2_{adj.}$	0.39			0.53		

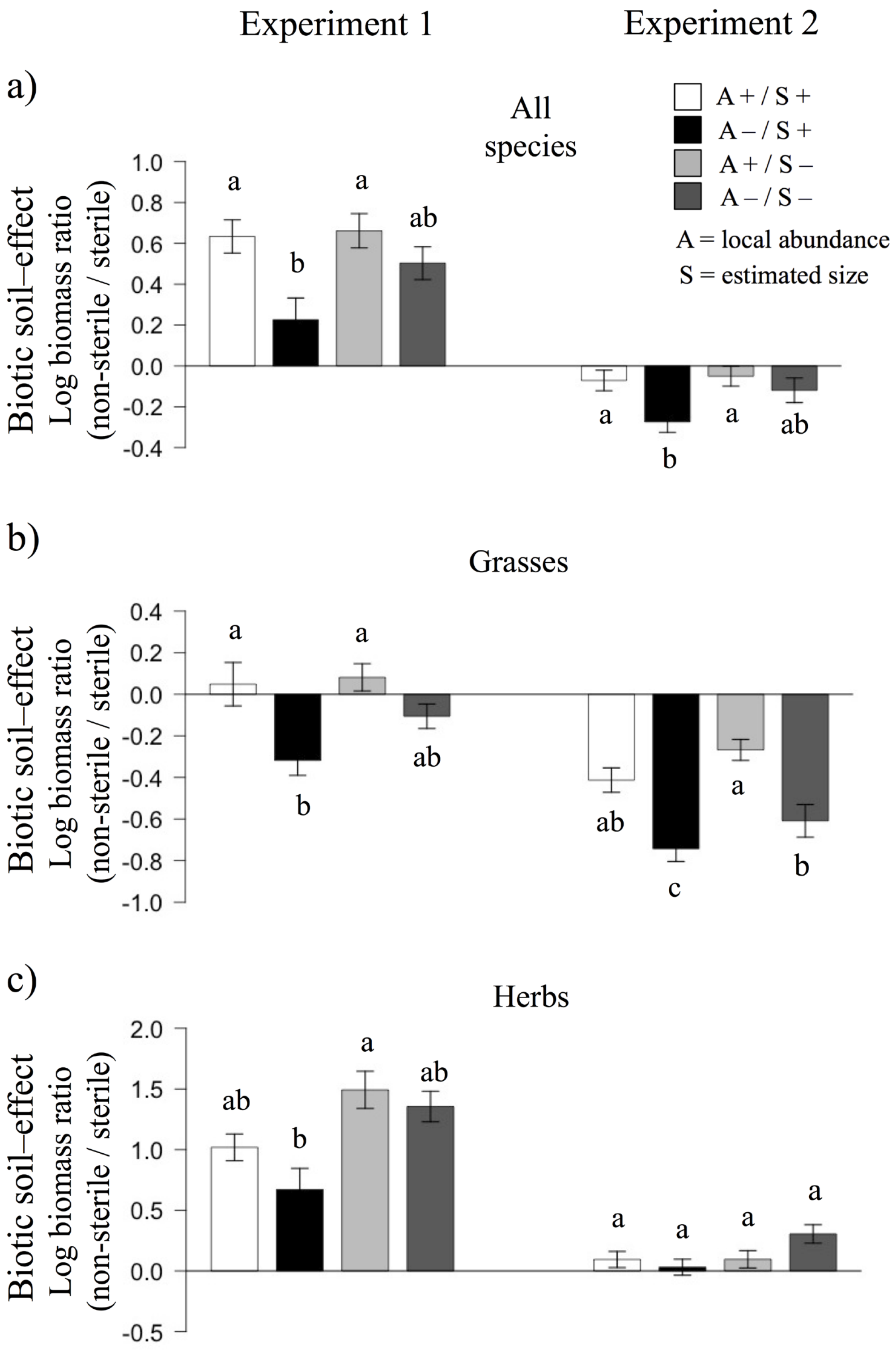


Figure 2.2 Biotic soil-effects for (a) 33 European grassland plant-species, (b) grasses and (c) herbs in experiment 1 (left) and experiment 2 (right). Species were classified into four different categories [A + / S+ (white bars); A – / S + (black bars); A + / S – (light grey bars); A – / S – (dark grey bars)]: A = local abundance; S = estimated size; + = high / large; – small / low. Biotic soil-effect values were calculated as: biotic soil-effect (species A) = log ratio (biomass species A (gown on non-sterile soil) / biomass species A (grown on sterile soil); modified from Petermann *et al.* (2008)). Negative values denote negative biotic soil-effect, i.e., a lower biomass production on non-sterile than on sterile soil. Data represent means ± SE (for all three regions). In all graphs (a – c) post-hoc tests (Scheffé test) were performed for each experiment: bars with the same letters are not significantly different at Alpha = 0.05.

Differences between categories were most pronounced in grasses (both experiments), with grasses with large size, but low local abundance (S+/A-) experiencing the most negative biotic soil-effects compared with the remaining categories (Figure 2.2b). In addition, the net effect of soil biota on growth of locally dominant grass species (A+) was more positive than for grasses with low local abundance (A-) (Figure 2.2b). Herbs in experiment 1 showed similar patterns in biotic soil-effects between categories, whereas no differences in biotic plant-soil interactions among categories were detected in experiment 2 for herbs (Figure 2.2c).

Land-use intensity effects on biotic soil-effects

In general, plant species experienced more negative biotic soil-effects in soils from intensively used grasslands compared to less intensively used grasslands (Figure 2.3a); this effect was especially pronounced in experiment 2 ($F_{1,1120}$ = 137.98; $P < 0.001$; Table 2.2; Figure 2.3a). However, biotic soil-effects for grasses and herbs showed contrasting responses to land-use intensity (experiment 1: $F_{1,1031}$ = 44.58; $P < 0.001$; experiment 2: $F_{1,1120}$ = 125.93; $P < 0.001$; Table 2.2; Figure 2.3b, c). Grasses in contrast to herbs either experienced positive or less negative biotic soil-effects in intensively used grasslands (Figure 2.3b), whereas herbs experienced more positive biotic soil-effects in less intensively managed grasslands (Figure 2.3c).

Relationship of biotic soil-effects and relative and local abundance

The mean local abundance of grasses in the field was positively correlated with biotic soil-effects for both experiments (experiment 1: $F_{1,9}$ = 3.65; $P < 0.1$; R^2 = 0.23; experiment 2: $F_{1,8}$ = 9.05; $P < 0.05$; R^2 = 0.5; Figure S2.2), but no relationship between the frequency of the investigated plant species (i.e., the total number of grassland sites where the species was present) and biotic soil-effects was detected.

Overall, biotic soil-effects differed between both experiments, resulting in significantly higher biotic soil-effect values for experiment 1 compared with experiment 2 (Figure 2.2). Temperature differences in the greenhouse between experiment 1 (winter) and experiment 2 (that experienced higher temperatures in summer) most likely influenced the activity and community composition of soil microorganisms causing the different magnitudes of biotic plant-soil interactions between the two experiments (see also Ehrenfeld *et al.*, 2005; van der Putten *et al.*, 2013; whereas no effect on plant-soil interactions were found for small temperature differences in van Grunsven *et al.*, 2010). Although the magnitude of biotic soil-effects differed between the two experiments (Figure 2.2), for the species that acted as replicates categories (estimated size/ mean local abundance) as well as the interaction of categories and functional type (grasses vs. herbs) between the two experiments showed no difference in biotic soil-effects (Experiment x Category: $F_{3,1092} = 1.02$; $P \geq 0.5$; Experiment x Category x Functional type: $F_{4,1092} = 0.88$; $P \geq 0.5$; see also Figure S2.3).

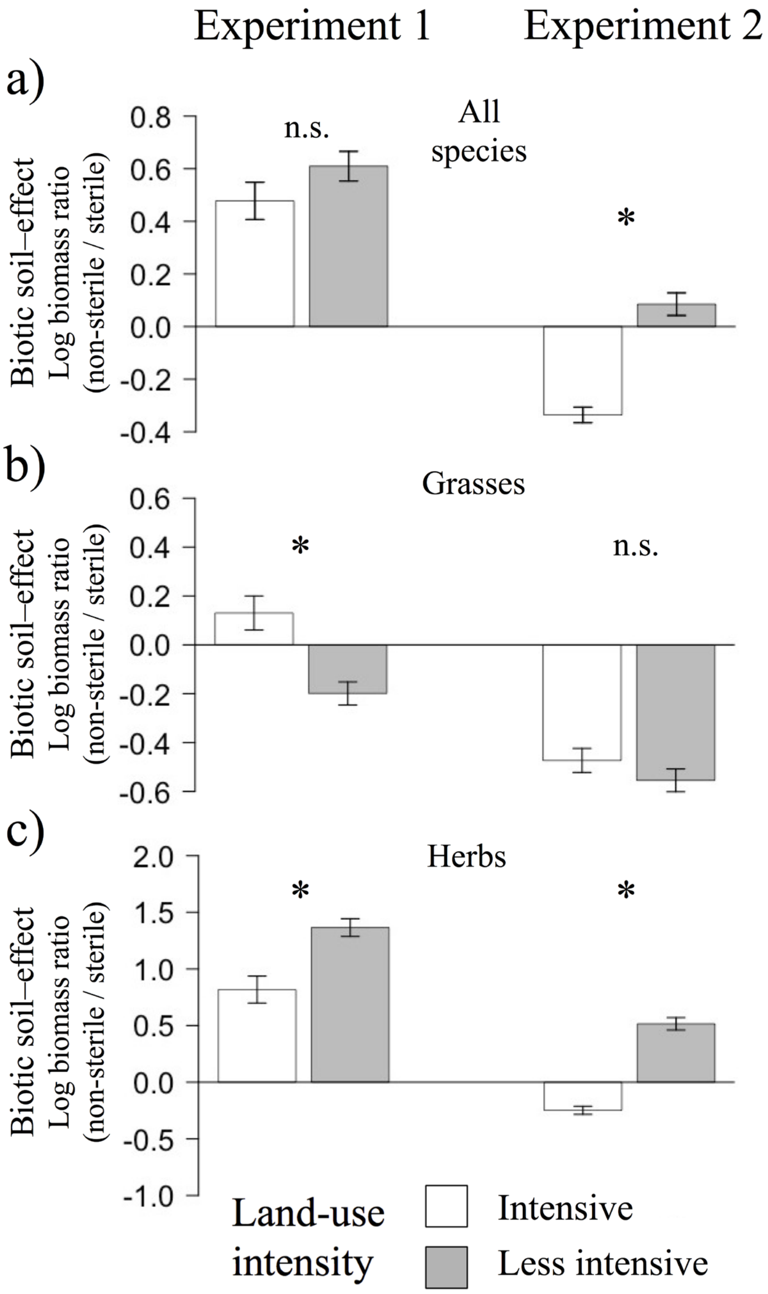


Figure 2.3 Mean biotic soil-effects for 33 European grassland plant-species (a – c) on intensively and less intensively managed grassland sites. In (a – c), mean biotic soil-effect is represented for (a) all 33 European grassland plant-species investigated, (b) grasses and (c) herbs grown in experiment 1 (left panel) and experiment 2 (right panel) in two different land-use categories (white bars = intensive land-use; grey bars = less intensive land-use). Data represent mean ± SE (for all three regions); for each experiment in grasses and herbs, a post-hoc test (Scheffé test) was conducted: signs above bars represent significant differences at Alpha = 0.05 (n.s. = not significant).

Discussion

Our observation that biotic soil-effects varied in strength depending on plant-size and local abundance of plant species support our hypothesis that plants with large individual size that are albeit not locally dominant are most strongly restricted by negative biotic soil-effects. This pattern was especially pronounced in the grass species investigated.

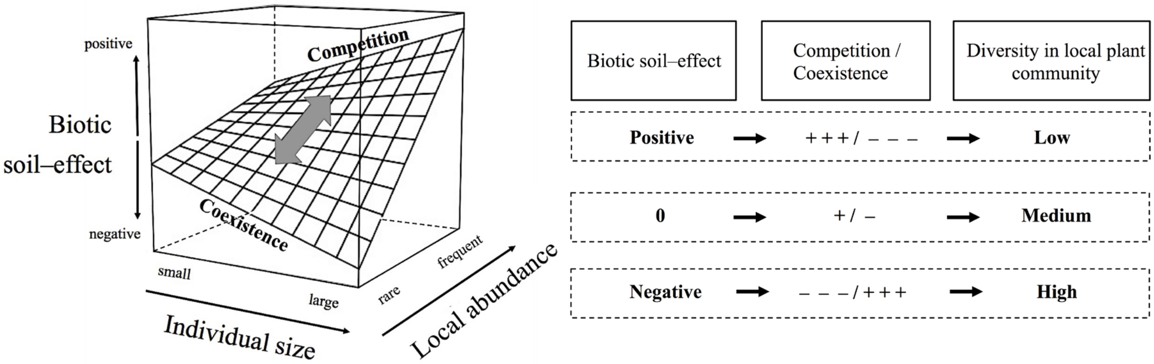
Locally abundant species experienced more direct positive net effects through the soil-microorganism community in our study. In contrast, locally rare species were negatively affected in biomass production by the presence of soil biota receiving more negative biotic soil-effects (Figure 2.2). Positive correlations between plant distribution, i.e. the regional frequency or relative plant abundance (Klironomos, 2002) and plant-soil interactions were found in various ecosystems such as tropical forests (Mangan *et al.*, 2010) and Canadian old fields (Klironomos, 2002), but not in semi-arid grasslands (Reinhart, 2012). In the central European grasslands investigated, we did not find a significant correlation with regional frequency, but with local population size, i.e., with local plant abundance (which is important for the dominance structure within local plant communities), which was positively related with biotic soil-effects (Figure 2.2).

In addition to local abundance, plant size as a functional trait was significantly related to biotic soil-effects: in the presence of soil biota, the potentially dominant plant species (species with large individual size but low local abundance) suffered the greatest losses of biomass and therefore experienced the most negative net soil biota response (Figure 2.2). Because the natural soil community was diluted at the beginning of the experiments (non-sterile soil inoculum added to sterilized soil), we assume that under natural field conditions effects of soil biota on plant species that differ in local abundance and individual size might be even more pronounced.

Typically, negative plant-soil interactions depend on density of pathogenic and parasitic microorganisms (e.g., nematodes, pathogenic fungi and bacteria; Bever *et al.*, 2010); whereas positive plant-soil interactions are attributed to symbiotic microorganisms (e.g. arbuscular mycorrhizal fungi (AMF) and N-fixing bacteria; van der Heijden *et al.*, 2008b; van der Putten *et al.*, 2013). However, beside general positive effects of AMF on plant growth, there is also evidence that AMF can mediate negative plant-soil interactions (Bever 2002), depending on nutrient status of the soil (Reynolds *et al.* 2005). As discussed by Klironomos (2002), plant species with a wide distribution that are locally abundant should either accumulate fewer pathogens (or accumulate them less rapidly) or profit more from symbionts compared with rare species. However, as shown in a dune ecosystem, the accumulation of pathogens by locally abundant plant species can lead to successional change (van der Putten *et al.*, 1993) indicating that the importance of negative biotic soil-effects may be ecosystem dependent. In our grassland systems studied, locally rare plant species that have the potential to become dominant because of their large individual size received the most negative biotic soil-effects. Soil borne pathogens may keep these potentially dominant species in check and therefore help maintain plant-species diversity in central European grasslands. This study was designed to (i) experimentally test the hypothesis that potentially dominant plant species are held in check by negative biotic soil-effects and to (ii) investigate putative effects of land use and therefore aimed to detect patterns rather than processes and explicit mechanisms of negative biotic soil-effects. In this study, we focussed on biotic soil-effects on aboveground plant biomass only, as this is one of the most relevant factors for plant abundances in the field (van der Putten 2007). Hence, further studies on the underlying mechanisms are needed to disentangle the role of specific agents of the soil community on plant performance (above- and belowground).

In general, grasses experienced more biomass loss in the presence of soil biota and therefore received more negative biotic soil-effects compared with herbs (Figure 2.2b and 2.2c). This has also been observed by Kulmatiski *et al.* (2008) and Pizano et a. (2014). By having large and often finely branched root systems with a large surface area, grasses may profit less from arbuscular mycorrhiza in terms of nutrition, but may be more susceptible to root pathogens (Newsham *et al.*, 1995a). In this study, the relationship between local-abundance and estimated species-size categories were more pronounced for grasses. In addition to the differences in root architecture between grasses and herbs, this may partly be attributed to the simplified method of categorization. Thus, due to their similar morphology, grasses are more comparable when using individual biomass and mean height to estimate plants' individual size, whereas the growth form of herbs varies more widely and therefore these are less directly comparable.

Based on our results obtained with grass species, we propose a relationship between biotic soil-effects, local abundance (as surrogate for local establishment success) and individual size (as surrogate for light competitive ability; depicted in Figure 2.4). Large plant species that are not locally dominant experienced a disproportionate amount of negative biotic soil-effect compared to other species. Therefore, we assume that coexistence (as a function of local abundance and species competitive ability (individual size)) is modulated by biotic soil-effects. Negative biotic soil-effects for potentially dominant plant species decrease competition between species and can therefore increase the probability of coexistence and enhance plant species diversity (Reynolds *et al.*, 2003). In contrast, positive biotic soil-effects may increase competition between plant species in grassland plant-communities and therefore decrease grassland diversity (Reynolds *et al.*, 2003). Using a modelling approach, Mack & Bever (2014) provided evidence that negative plant-soil interactions play an important role in structuring plant communities. Corroborating these theoretical predictions (Mack & Bever, 2014), our results provide empirical evidence that negative soil interactions for potentially dominant plant species enhance species coexistence. Furthermore, we suggest that taking individual size of a plant species into account improves the understanding of species assembly in plant communities. However, for a deeper understanding of how coexistence in grassland plant-communities is modulated by negative biotic soil-effects further studies should i) investigate plant communities for indirect biotic soil-effects (including interactive effects of plant-soil interactions and plant–plant competition), and ii) assess putative trade-offs between biotic soil-effects and dispersal limitation (see Janzen, 1970; Connell, 1971).



Biotic soil–effect	Competition / Coexistence	Diversity in local plant community
Positive →	+ + + / – – – →	**Low**
0 →	+ / – →	**Medium**
Negative →	– – – / + + + →	**High**

Figure 2.4 Conceptual model of the hypothetical relationship among individual size, local abundance and biotic soil-effects. Here, the extent of the plane represents the diversity within a local plant community. Biotic soil-effects are hypothesized to modulate competition and coexistence within grassland plant-communities and therefore having an effect on plant-species diversity (spatial extent of the plane). Without any biotic soil-effect, competition between strong and weak competitors is predicted to be slightly positive (+), which leads to medium values of plant-species diversity. Negative biotic soil-effects disproportionately affect potentially dominant (large) plant species and therefore increase coexistence and plant-species diversity within a grassland plant-community, whereas positive biotic soil-effects increase competition and therefore exert negative effects on plant-species diversity. Symbols (+ and –) indicate direction and magnitude of biotic soil-effects on competition and coexistence.

Land-use effects on biotic soil-effects

In grasslands, species composition and diversity are affected by many biotic (e.g. herbivory, see Olff & Ritchie, 1998) and abiotic factors (Janssens *et al.*, 1998; Bennie *et al.*, 2006). By generating disturbances (grazing, mowing) and modifying nutrient content of the soil, land use is the most prominent factor influencing grassland species-diversity (Grace, 1999; Sala *et al.*, 2000). In addition to land use, species-specific competitive ability, adaptation to land use and abiotic environment as well as the individual growth form of a species are important factors shaping plant-community composition and therefore biodiversity. Nevertheless those biotic (e.g., competition) and abiotic (e.g., mowing) factors were excluded in the greenhouse experiment as direct effects to elucidate the contribution of the biotic part of the soil to the patterns observed.

Data describing land-use effects on biotic plant-soil interactions are scarce although interactions between soil microorganisms and plants have been investigated in many ecosystems: e.g., agricultural ecosystems (Ryan & Graham, 2002) and grasslands (Bezemer *et al.*, 2006; Kardol *et al.*, 2006; Casper & Castelli, 2007). Previous studies for instance investigated the effects of fertilization (Johnson, 1993) and land use (Oehl *et al.*, 2003) focussing on AMF. However, only few studies showed how interactions of the whole biotic soil community and plants change under natural environmental conditions of land use (e.g. Pizano *et al.* 2014).

In our study, herbs experienced more negative biotic soil-effects in intensively managed compared with less intensively managed grasslands, whereas grasses showed the opposite pattern in response to land-use intensity (Figure 2.3). This result is in accordance with results of Morris *et al.* (2013) who found a decrease of AMF-colonization and an increase of non-AMF colonization with increasing land-use intensity in the Exploratories project for the herbaceous plant species *Plantago lanceolata*. Additionally, it has been suggested that pathogen protection provided by AMF is weak under intensive land use (Morris *et al.*, 2013) and that the protection against fungal pathogens by AMF fungi is weaker for herbs than for grasses (Veresoglou & Rillig, 2012). In addition, with increasing N-fertilization severity of pathogen disease was found to be more pronounced for herbs than for grasses (Veresoglou *et al.*, 2013). Hence, the more negative biotic soil-effects for herbs in intensively managed compared to less intensively managed grasslands may be caused by weak colonization with AMF in herbaceous species in intensively managed grasslands, increased colonization with non-AMF, and/or increased severity of disease in these systems.

In microcosm experiments, biomass and abundance of grasses were negatively affected by AMF fungi (van der Heijden *et al.*, 2008b). In addition, by producing large root systems mycorrhizal dependency of grasses might be lower in comparison with herbs as large root systems result in large contact areas for root pathogens. Although in an earlier study, grasses were better protected from fungal pathogens by AMF than herbs (Veresoglou & Rillig, 2012) negative interactions with AMF, competition with bacteria and fungi for nutrients and high probability of pathogenic root infection may lead to negative biotic soil-effects for grasses at nutrient-poor less intensively managed sites.

The application of artificial fertilizer was the main difference between intensively and less intensively managed grassland in this study. Therefore, we assume that biotic plant-soil interactions changed depending on fertilization as shown by Manning *et al.* (2008). However, the different reactions of grasses and herbs might be caused by differences in AMF-protection from fungal pathogens, AMF- dependency and severity of pathogen effects (as outlined above). Beside the effects of fertilization on biotic soil-effects other environmental factors accompanied by land-use intensity might be responsible for the different reactions of grasses and herbs. Soil aggregation is known to be influenced by abiotic as well as biotic components and important for maintaining water infiltration, nutrient availability and soil porosity. Barto *et al.* (2010) found an overall effect of land-use intensity on mean weight diameter of the soil (a measure of soil aggregation status) with mowing being the only component of the LUI with an exclusive significant effect. Soil aggregation is also likely to have different effects on plants due to root architecture. As the soil mean weight diameter as well as the percentage of water stable aggregates differs between our low- and high-LUI sites in each region of the Exploratories project (see Table S2.2), this could be one possible environmental factor responsible for our findings. In addition to soil aggregation, plant-soil interactions were found to be influenced by legacy effects in the soil and therefore play an important role for plant community structure and composition (Kardol *et al.*, 2007; van der Voorde *et al.*, 2011). Therefore, we assume that additionally to nutrient enrichment and soil aggregation effects, legacy effects in the soil generated by land use (via disturbances like mowing and grazing) might play an important role for the different biotic soil-effects for grasses and herbs.

Land-use effects on plant species richness

Fertilization increases plant height and productivity and therefore exacerbates competition for light, which is the most prominent factor affecting plant diversity in grasslands (Lepš, 1999; Hautier *et al.*, 2009). In comparison to most herbs, in temperate European ecosystems many grass species tolerate land-use intensification (increased intensity of fertilization, mowing and grazing) and react e.g. with increased tillering and biomass production. Land use has various above- and belowground effects on plants. The observed decrease in proportion of herbs in comparison to grasses on intensively managed sites could be caused by the high stress tolerance (against mowing by meristems situated at the base of grass tillers) and the high competitive ability of many grasses (aboveground effects) but also by negative biotic soil-effects for herbs compared with less intensively managed grasslands (belowground effects) (Figure 2.1). In contrast, less intensively managed grasslands in this study showed a high proportion of herbs compared to grasses. Low concentrations of nutrients (N and P; Table S2.2) and therefore low productivity leads to low competition for light (aboveground effect) and, additionally, to negative biotic soil-effects for otherwise strongly competitive grasses (belowground effect) (Figure 2.1). This may lead to coexistence of weak and strong competitors.

We conclude that in addition to factors affecting plant-species coexistence in grasslands, there is evidence that negative biotic soil-effects play a role in maintaining plant-species diversity in central European grasslands. By negatively affecting potentially dominant plant species, especially competitive grasses, soil biota may prevent competitive exclusion among plant species that would lead to a loss of biodiversity especially in less intensively managed species-rich grasslands. Our study illustrates the usefulness of including plant-size related traits when testing for feedback effects in plant communities. This may help to uncover more general relationships and aid the mechanistic understanding of differential feedbacks.

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Chapter 3

Plant root and mycorrhizal fungal traits for understanding soil aggregation

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Introduction

Soil aggregation is a key ecosystem process resulting in the formation and stabilization of soil structure, consisting of soil aggregates and the resulting matrix of pore spaces. As such, it significantly alters the environment of plant roots and microbes in a multitude of ways; thus, soil structure provides the basic setting in which mycorrhizas operate and have evolved. Not surprisingly, soil aggregation is important for root growth and for a wide range of soil features and ecosystem process rates, such as carbon storage and resistance to erosion (e.g. Jastrow *et al.*, 1998; Six *et al.*, 2006). The aggregation of soil is a complex process, regulated by a range of abiotic factors (e.g. texture) and mediated by plants and multiple biota groups and their interactions; in spite of this complexity, plant roots and their mycorrhizal symbionts are consistently found to be a crucial force in driving soil aggregation (Six *et al.*, 2004; Rillig & Mummey, 2006; Leifheit *et al.*, 2014).

Plant and mycorrhizal fungal species, respectively, differ in their contribution to soil aggregation (Reid & Goss, 1981; Angers & Caron, 1998; Eviner & Chapin, 2002; Rillig *et al.*, 2002; Piotrowski *et al.*, 2004; Six *et al.*, 2004; Duchicela *et al.*, 2012; Graf & Frei, 2013; Pérès *et al.*, 2013), but from previous studies it has not yet become clear what specific root (and mycorrrhizal) traits contribute to this effect. This is likely because studies typically focus on only a limited suite of physiological and architectural characteristics, and the general research focus has traditionally been more on management practices and physico-chemical factors. Therefore, we lack a mechanistic understanding of the role of organisms in soil aggregation.

To this end, we argue for a shift from a classical species-based comparative approach, or the mere consideration of summary variables (e.g. root length) to a systematic trait-based approach. Summarizing ecological characteristics of species by means of traits has become an essential tool in plant ecology (Westoby & Wright, 2006), and is increasingly proposed for root–fungal associations (e.g. van der Heijden & Scheublin, 2007; Chagnon *et al.*, 2013; Aguilar-Trigueros *et al.*, 2014). We believe trait-approaches are a promising tool to achieve progress in our mechanistic understanding of soil aggregation from an organismic perspective, to enhance our predictive ability regarding effects of plant and fungal diversity, and to ultimately provide informed recommendations for, for example, agricultural management and environmental restoration. With such data we can then ultimately address questions such as: how can plant and mycorrhizal fungal community data enhance prediction of soil aggregation beyond the consideration of state variables such as soil type, climate and management? Is soil aggregation an intermediate driver of plant and mycorrhizal fungal community processes and thus also an integral part of plant–soil feedbacks? And, on the applied side, can we enhance ecosystem restoration by designing tailor-made mycorrhizal fungal inocula and seed mixes specifically optimized for complementarity in the ability to enhance soil aggregation?

In addition to general advantages, we see specific benefits of a trait-based approach applied to the understanding of mycorrhizal and plant contributions to soil aggregation: (1) it will allow testing for the extent of root and mycorrhizal fungal functional complementarity with regard to soil aggregation, and thus whether soil aggregation is another example of an ecosystem process that positively relates to biodiversity like other more well-studied processes such as primary productivity. This is very likely true because soil aggregation is the result of multiple interlocking component processes such as aggregate formation and stabilization (both of microaggregates and macroaggregates) which might be driven by different sets of traits represented in different species. Additionally, soil aggregation may be stimulated through diversity-fueled overyielding of fungi and roots through niche complementarity (also see Table 3.1); (2) if we can convincingly relate soil structure to traits of plant and fungal communities, this will allow predicting which ecosystems are under most risk of deterioration and also guide towards measures to counter this process.

Root and mycorrhizal fungal traits

An approach such as advocated here must begin with a selection of candidate traits related to soil aggregation. Of some traits we already know that they relate to soil aggregation propensity: using correlational approaches (path analysis) to exploit existing environmental gradients in landscapes, coarse-level indicators such as root biomass (or often root length) and mycorrhizal fungal hyphal length in soil have been identified as significant determinants (e.g. Miller & Jastrow, 1990; Rillig & Mummey, 2006). Thus, clearly productivity-related characteristics like root and hyphal density are traits to be considered.

Table 3.1 is divided into architectural/morphological (A) and physiological (B) traits of roots and mycorrhizal fungi, respectively. These traits have been formulated mostly with arbuscular mycorrhizas inmind, but can be directly extended in many cases to ectomycorrhiza or other mycorrhizal types. [a]Degens *et al.* (1996), Miller & Jastrow (1990); [b]Li *et al.* (2002), Ritz & Young (2004); [c]Friese & Allen (1991), Thonar *et al.* (2011); [d]Bronick & Lal (2005), Traore *et al.* (2000); [e]Chau *et al.* (2010), Mataix-Solera & Doerr (2004); [f]Rillig & Mummey (2006); [g]Caesar-TonThat & Cochran (2000); [h]de la Providencia *et al.* (2005), Klironomos & Kendrick (1996).

Table 3.1 Root and mycorrhizal fungal traits (and, when applicable, proposed measurable proxies) hypothesized to influence eitherprimarily formation or stabilization of soil aggregates.

Formation-related traits		**Stabilization-related traits**	
(A) Architectural/ morphological traits			
Specific length	*measure*: (root or hyphal length) * (root or hyphal biomass)$^{-1}$	**Tensile strength**	*measure:* force required to tear roots or to fragment mycelium
Extension ability	*measure:*(length and number of runner hyphae or fine roots with diameter < 1mm) * (growing space volume)$^{-1}$	**Density**	*measure:* (root or hyphal length) * (growing space volume)$^{-1}$
Relative growth	*measure:* [ln(root or hyphal length$_{t0}$) – ln(root or hyphal length$_{t1}$)] * ($t_0 - t_1$)$^{-1}$ *rationale:* these **3 traits** represent the growth ability of hyphae or roots to encounter building materials of aggregates in soil (primary particles, organic matter particles) and then form macroaggregates [a]	**Entangling ability**	*proxy*: (number of branches) * (root or hyphal length)$^{-1}$; root or hyphal branching number and angle; alternatively: directly measure ability to engulf an object *rationale:* these **3 traits** measure the capability of hyphae or roots to stabilize aggregates depending on their tearing-resistance (i.e. ability to hold material together without breaking apart) and enmeshment potential [b]
Root-to-hyphae distance (symbiotic trait)	*proxy:* average distance between hyphae and associated root surface *rationale:* larger distance between root and hyphae allows for increased probability of contact of aggregate building materials with either hyphae or roots [c]	**Intensity of root-hyphae-linkages (symbiotic trait)**	*proxy:* (entry points of hyphae or hyphal density close to root) * (root surface area)$^{-1}$ *rationale:* greater proximity of hyphae and roots increases probability of synergistic/ combined effects of roots and hyphae (e.g., cementing agents, entangling) contributing to the stability of engulfed aggregates [c]
(B) Physiological traits			
Exudate quality	*proxy*: root or hyphal exudate C:N ratio	**Water repellency**	*proxy:* intensity and persistence of root or hyphal water repellency
Exudate quantity	*measure:* (exudate amount) * (root or hyphal length)$^{-1}$	**Mediation of water repellency**	*proxy:* intensity and persistence of root or hyphal-mediated soil water repellency
Soil particle alignment	*measure:* (surface charge of roots or hyphae) * (root or hyphal length)$^{-1}$ *rationale:* quantity and quality of secreted exudates determine the degree of particles adhering to roots or hyphae and agglutination of particles and aggregates [d]	**Mediation of wet-dry-cycles**	*proxy*: root water uptake or hyphal water transport (out of root-exclusion compartments) *rationale:* these **three traits** evaluate directly and indirectly the fungal or root impact on soil rewetting capability affecting soil stability [e]
Mediation of soil compression	*proxy*: root or hyphal density in relation to soil porosity *rationale:* enmeshment of soil by roots or hyphae lead to soil compression supporting initial aggregate formation [f]	**Cementation capability of exudates**	*proxy*: stability of artificial aggregates (= soil particles + extracted root or hyphal exudates) *rationale:* exudates stabilize aggregates by filling up intra-aggregate pore necks and cracks [g]
Mediation of soil particle movement	*measure:* (observation of particles moved by roots or hyphae) * (time unit)$^{-1}$	**Life span**	*proxy*: production and mortality of fine roots or hyphae in defined growing space volume
	rationale: ability of roots or hyphae to bring soil particles together by moving them, leading to potential aggregation	**Palatability**	*proxy*: amount and availability of nutrients and carbon in roots or hyphae
		Repair capacity	*measure:* (root or hyphal growth [length and branching]) * (time unit after disturbance)$^{-1}$ *rationale:* these **three traits** represent aspects of root or hyphal longevity, resistance and resilience against biotic and abiotic disturbance affecting the potential aggregate stability mediated by root or hyphae [h]

However, when such measures of root and hyphal abundance were compared to soil aggregation at a finer scale, for example in small field plots (e.g. Rillig *et al.*, 2002) or pot experiments (e.g. Piotrowski *et al.*, 2004), there was generally a low match, prompting the consideration of more specific physiological or architectural traits.

In order to select these specific traits, we have first divided soil aggregation into the component processes soil aggregate formation and the stabilization of existing aggregates, and then considered measurable plant and fungal traits that are likely to be variable, and may mechanistically relate to either or both of these processes. For a full list see Table 3.1, and later we will further explain why these traits were selected and which difficulties may be encountered. Regarding aggregate formation and stabilization, even though these processes will occur simultaneously in ecosystems, these may be executed by different organisms expressing different traits. We define soil aggregate formation as the initial binding together of particles, whereas soil stabilization is the process that renders the aggregate increasingly resistant to the application of disintegrating forces, such as water penetrating into pores. If an aggregate is nonstable it will disintegrate; it has become clear that formation, stabilization and disintegration occur in a dynamic fashion in soils (e.g. Six *et al.*, 2004).

For the formation of soil aggregates, we consider traits to be important that are related to the likelihood to encounter soil particles (e.g. fineness of roots and hyphae, their extent and spatial distribution) and to bring soil material together (ability to move, entangle and engulf). In addition, exuded materials from roots and hyphae should play an important role in the initial formation of aggregates (Six *et al.*, 2004). For stabilization of existing aggregates, traits related to persistence are hypothesized to be more important, such as tensile strength or longevity of roots and hyphae. The ability to render surfaces hydrophobic could also be important, as well as the release of cementing agents (Oades & Waters, 1991). We emphasize that these are hypotheses at this stage; only controlled experiments employing a range of plant and fungal species will reveal which of these traits are in fact of explanatory value at all, and for which component process. We realize that some of these traits will be relatively easy to measure, and have also been proposed as general functional traits (see Van der Heijden & Scheublin, 2007); others will be quite a challenge, and for some we propose proxy traits that will be easier to capture (Table 3.1). Clearly, much innovation is still possible once this research effort is under way.

Another challenging aspect of linking single trait values to strains/species is trait variability (e.g. Cordlandwehr *et al.*, 2013), which can be caused either by genetic variability at the species level or by phenotypic plasticity within the same genotype. Intraspecific diversity can have a profound influence on trait expression in mycorrhizal fungi (Johnson *et al.*, 2012; Angelard *et al.*, 2014) as well as in plants (e.g. Kichenin *et al.*, 2013). Nevertheless, it remains yet to be seen if root and mycorrhizal fungal traits are more variable than other plant traits. Apart from this challenge, investigating this very plasticity could yield further insights; for example, some root traits have been proposed as highly relevant response traits in ecological studies due to their high plasticity (Ryser, 2006; Valverde-Barrantes *et al.*, 2013), and if these include traits responsible for soil aggregation we might better understand under which circumstances plants are more likely to contribute to soil aggregation.

Approaches and the way forward

For much of what we propose here, dedicated experiments are necessary, for example, for the separation of formation and stabilization of aggregates, or for comparing different root systems or fungal isolates under otherwise identical conditions. Nevertheless, several other approaches are possible to make inroads. For example, some trait data are already available because they have been collected for other purposes. Nevertheless, at present, plant trait databases (e.g. TRY, Kattge *et al.*, 2011) are relatively poor in terms of coverage of root traits, in particular root traits that would be important in our specific context (Table 3.1). So far, comparable mycorrhizal fungal trait databases do not yet exist. Thus one important research focus should be the collection of root and mycorrhizal fungal traits for a number of species and linking these with experimentally measured soil aggregation, ideally dissected into component processes. These could be complemented by observational approaches, consisting of measuring community-average root traits in the field, which are linked to soil aggregation (here it would not be possible to distinguish formation and stabilization). Such data could also be particularly valuable for verifying predictions derived from knowledge of species-level data.

Conclusion

Using traits can increase our fundamental understanding of the intricate relationship between plants, symbiotic fungi, and their immediate environment which is the soil aggregate. However, knowledge of root and mycorrhizal fungal traits could also have great applied significance. Such data could give rise to innovations such as tailored seed mixes (or fungal inoculum mixes) for grassland restoration which maximize trait coverage in terms of soil aggregation within the available plant species pool, as well as setting priorities for conservation efforts through predicting which ecosystems are most prone to degradation in light of invasive species and imminent global change. Likewise, better information on plant traits could be used to foster crop breeding for sustainable agriculture, or for agroecosystem management to enhance soil stability (e.g. by selecting cover crops also for complementary trait values).

Chapter 4

The interplay between soil structure, roots and microbiota as a determinant of plant-soil feedback

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Summary

Plant-soil feedbacks (PSF) can influence plant community structure via changes in the soil microbiome. However, how these feedbacks depend on the soil environment remains poorly understood. We hypothesized that disintegrating a naturally aggregated soil may influence the outcome of PSF by affecting microbial communities. Furthermore, we expected plants to differentially interact with soil structure and the microbial communities due to varying root morphology.

We carried out a feedback experiment with 9 plant-species (5 forbs and 4 grasses) where the "training phase" consisted of aggregated versus disintegrated soil. In the feedback phase, a uniform soil was inoculated in a fully factorial design with soil washings from conspecific versus heterospecific trained soil that had been either disintegrated or aggregated. This way, the effects of prior soil structure on plant performance in terms of biomass production and allocation were examined.

In the training phase soil structure did not affect plant biomass. But on disintegrated soil plants with lower specific root length (SRL) allocated more biomass aboveground. PSF in the feedback phase was negative overall. With training on disintegrated soil, conspecific feedback was positively correlated with SRL and significantly differed between grasses and forbs. Plants with higher SRL were likely able to easily explore the disintegrated soil with smaller pores, while plants with lower SRL invested in belowground biomass for soil exploration and seemed to be more susceptible to fungal pathogens. This suggests that plants with low SRL could be more limited by PSF on disintegrated soils of early successional stages.

Synthesis. This study is the first to examine the influence of soil structure on PSF. Our results suggest that soil structure determines the outcome of PSF mediated by SRL. We recommend to further explore the effects of soil structure and propose to include root performance when working with PSF.

Introduction

It has long been known that environmental context can have a profound influence on outcomes of biotic interactions, which can range from negative to positive. For instance, increasing facilitation among plants is found to correlate with environmental stress (He *et al.*, 2013), and soil fertility can change plant-mycorrhiza interactions from positive to negative (Johnson, 2010). One potentially important context for plant-soil biotic interactions is soil structure, as it can vary greatly depending on land-use history, plant-species composition and successional stage (Jastrow *et al.*, 1998; Barto *et al.*, 2010; Pérès *et al.*, 2013; Erktan *et al.*, 2015). Soil structure is often described by aggregate measurements (e.g., water stable aggregates = WSA, or mean weight diameter = MWD) as surrogates for the soil matrix. These aggregates have profound influences on nutrient cycling and soil organic matter dynamics through different physical processes (Six *et al.*, 2004). Furthermore, they determine the spatial structure of the environment in which soil microbial communities interact with plants. Studies examining the interaction of soil biota, plants and aggregation mostly focus on unidirectional effects of the biota on the formation or destabilization of aggregates (Rillig & Mummey, 2006) rather than investigating the role of soil structure as a microhabitat. The function of soil structure possibly mediating the interaction of soil biota and plants as plant-soil feedback (PSF) has to our knowledge never been examined.

Conspecific or direct PSF is defined as the fitness effect a plant achieves from soil being modified in biotic and abiotic character by a plant of the same species (Bever *et al.*, 1997; van der Putten *et al.*, 2013).Interactions between plants and soil biota can drive negative conspecific feedback (Bever *et al.*, 1997; Kulmatiski *et al.*, 2008) as in Janzen-Connell effects (Janzen, 1970; Connell, 1971), which can be strong enough to maintain plant coexistence (Bever, 2003; Mangan *et al.*, 2010; van der Putten *et al.*, 2013; Mack & Bever, 2014; Bauer *et al.*, 2015; Heinze *et al.*, 2015). In a meta-analysis it was found that feedbacks were generally stronger in artificial soil compared with field soil (Kulmatiski *et al.*, 2008), which suggests the soil environmental context may affect feedbacks. Soil structure may be an important candidate: although a direct role of soil structure on PSF has been considered unlikely because effects of structure on plant growth will not be species specific (Ehrenfeld *et al.*, 2005), it may strongly influence the soil biota responsible for the feedback effects. This can happen in multiple ways, and will depend on whether there is a relationship between plant species specificity of soil biota on the one hand and their relative performance under different soil aggregation levels on the other. For fungal pathogens we know that host specificity and virulence are negatively related to typical-soil related lifestyles such as saprotrophic activities (Aguilar-Trigueros *et al.*, 2014), and thus the latter are expected to perform relatively better on more complex, aggregated soils as occurring under natural conditions. In cases of nutritionally-based mutualists like arbuscular mycorrhizal fungi (AMF) we expect the opposite: because nutrients are sequestered inside soil aggregates reducing plant-availability (Angers & Caron, 1998), interactions may become more positive under high soil aggregation levels. Both of these mechanisms would predict a less negative/more positive conspecific feedback under aggregated soils.

Apart from these soil biotic responses to soil structure and their projected effects on plants, plants may differ in their direct response to soil aggregation, which may in turn affect feedbacks. It has been argued that plant functional traits may have profound influences on PSF which has not been studied in detail yet (van der Putten *et al.*, 2013; Baxendale *et al.*, 2014). Different plant functional types (PFT) such as grasses and forbs have been found to differ in feedbacks (Kulmatiski *et al.*, 2008; Heinze *et al.*, 2015), and it has been proposed that high specific root length (SRL) in grasses may increase susceptibility to pathogens (Bever, 1994; Newsham *et al.*, 1995b), while many forbs have higher reliance on mycorrhizal fungi for nutrient foraging (Reinhart *et al.*, 2012). Because PSFs are conceptually linked to a plant species-specific modulation of soil (-biota) by plant tissue, replacing high-SRL roots by AM fungi as absorptive structures can be expected to elicit a reduction in negative feedback strength. Furthermore, it is known that the net effect of AMF on plant performance is highly dependent on environmental factors as well as the plants ability to acquire nutrients from soil (Johnson *et al.*, 1997). These different nutrient acquisition strategies may therefore determine direct plant responses to soil aggregation (e.g. allocation towards AMF versus fine roots).

To investigate the effect of soil structure on feedback, we carried out a greenhouse feedback experiment including species specific root length as an explanatory variable. We tried to disentangle the physical and chemical effects of soil aggregation from the accompanying biological ones by first training the microbial community on different soil structure (Figure 4.1) and then examining the effects of the resulting soil communities in a feedback phase on homogeneous soil (Figure 4.2).

Figure 4.1 Disintegrated (left, MWD=51µm, 29% WSA) and aggregated soil (right, MWD=109µm, 44% WSA) used in the training phase.

Materials & Methods

We conducted a classical feedback experiment where the training phase is meant to accumulate species specific soil biota which - in the feedback phase - affect different plant species.

Seed material

The seeds used for this experiment had been collected in the National Park Hainich (Central Germany) on different experimental grassland plots of the Biodiversity Exploratories (Fischer *et al.*, 2010) in 2011. Grasses were: *Anthoxantum odoratum* L.*, Briza media* L.*, Holcus lanatus* L. and *Dactylis glomerata* L., and forbs were: *Plantago major* L.*, Plantago lanceolata* L.*, Centaurea jacea* L.*, Daucus carota* L.*, Leucanthemum vulgare* LAM. and *Taraxacum officinale* F.H. WIGG. All plants were perennials except for *D. carota*, which is a biennial (Kühn *et al.*, 2004). Seeds were surface sterilized in 7% sodium hypochlorite solution for 3 min (Bartelt-Ryser *et al.*, 2005) to avoid the input of species specific microbes.

Soil

We used fresh field soil from a loamy sandy mineral soil (Albic Luvisol; N = 0.12%,C = 1.87%, C/N ratio 15.58) from a meadow in Dahlem (Berlin, Germany), which has previously been used in experiments in our lab (Rillig *et al.*, 2010; Salem *et al.*, 2013). All species we used in the experiment (except *Briza media*) are common in the greater area. The soil was collected from approximately 10-30 cm depth, air dried, and then mixed and sieved (1 cm mesh). Then, half of it was disintegrated in a cement mill containing boulders (10 min per 15 l batch) to obtain the low-aggregation-status. The disintegrated soil had the same pH and only slightly elevated plant available phosphorus and nitrogen contents compared to the aggregated soil (see Table S4.1 in Supporting Information). We measured water stable aggregates (WSA) before (3 replicates per soil structure level) and after (all replicates) the experiment by wet sieving using the method of Kemper & Rosenau (1986): 4.0 g of soil was weighed into sieves, rewetted through capillary action with de-ionized water, and sieved on a wet-sieving apparatus (Eijkelkamp, Giesbeek, the Netherlands) for 3 min. The material left on the sieve was separated from coarse material (stones, organic debris) and both were weighed after drying at 60 °C. MWD was measured to further characterize the structure of the soil prior to training according to Lehmann & Rillig (2013) by dry sieving of about 200 g soil (3 replicates per soil structure level) through a stack of sieves (2 mm, 1 mm, 250 µm, 53 µm) and calculating the sum of the proportions of the weight and mean diameter of aggregates of the resulting five size classes.

Specific root length

To obtain the SRL as a species specific trait for our plant species not confounded by our soil treatment, we measured it on plants of an independent, earlier experiment (Heinze *et al.*, 2015) that used the same seed material. They were grown in cones (0.41 L; Stueve & Sons; USA) on an autoclaved soil-sand mixture (40:60) with field soil from one less-intensively managed pasture in the National Park Hainich from which most seeds originated. Plants were grown for 18 weeks in 2012 under greenhouse conditions and were then harvested and air-dried for storage. In 2013, roots of three replicates per species were gently washed by hand, dried at 40°C for at least three days, and weighed to obtain dry weight. SRL was calculated using the WinRhizo scanner-based system (Regents Instruments, Inc., Canada) (see mean values in Table S4.5).

Training phase

In a first experiment, set up from end of May until mid-August 2013 for a duration of 10 weeks, we grew 10 plant species on aggregated (H: 44% WSA, MWD=109µm) versus disintegrated (L: 29% WSA, MWD=51µm) soil: the “training phase”. Seeds germinated on Petri dishes on filter paper soaked in de-ionised water. Within a week after germination seedlings were transplanted into rose pots containing 2.5 kg soil and set up in a randomized block design in a greenhouse (light: 16 hrs, dark: 8 hrs), watered daily for the first four weeks (50 ml tap water per day) using an automatic watering system after which they were watered manually additionally every second-third day according to plant needs. The experimental setup consisted of 2 treatments (aggregated / disintegrated) * 10 species * 8 replicates resulting in 160 pots total. The pots planted with *P. major* and *L. vulgare* contained two plant individuals instead of one because seedlings were frail at time of transplant but grew too vigorously to be thinned afterwards. After harvest, we measured aboveground and belowground dry biomass as well as soil aggregation. Roots were taken out of the soil and gently washed by hand. To confirm that microbiota had not been destroyed during the disintegrating process we measured arbuscular mycorrhizal (AM fungal) colonization in the roots by using 6 replicates per species and treatment. We used ink and vinegar staining (Vierheilig & Coughlan, 1998) and counted 100 intersects per replicate under a light microscope to determine colonization rate by AM hyphae as well as arbuscules and vesicles (according to McGonigle *et al.* 1990).

Feedback phase

The trained soil was air dried in closed paper bags and stored dry for < 2 months at room temperature. This soil was used to produce the inoculum for the second phase of the experiment: the “feedback phase”. The inoculum was extracted by mixing 2.5 l soil (pooled from 8 replicates for each species and soil structure level), with 5 l deionised water, stirring vigorously for 2 min, leaving it to settle for 1.5 min and decanting through a 212 µm sieve. Inoculum was stored at 4°C and used within 48 h. Nine replicates of each species received an inoculum trained by the same species (home or conspecific) and another nine received an inoculum each trained by one of the 9 other species (away or heterospecific), which were all either from aggregated or disintegrated soil. *T. officinale* was used as donor plant for inoculum but not as receiver plant in the feedback phase in order to have the same number of replicates for home and away treatments. We used this well established experimental approach (Klironomos, 2002) to create a balanced design but are aware of the fact that each away species may have a different effect on the microbial community which can cause a higher variation in the away treatment (van de Voorde *et al.*, 2011).

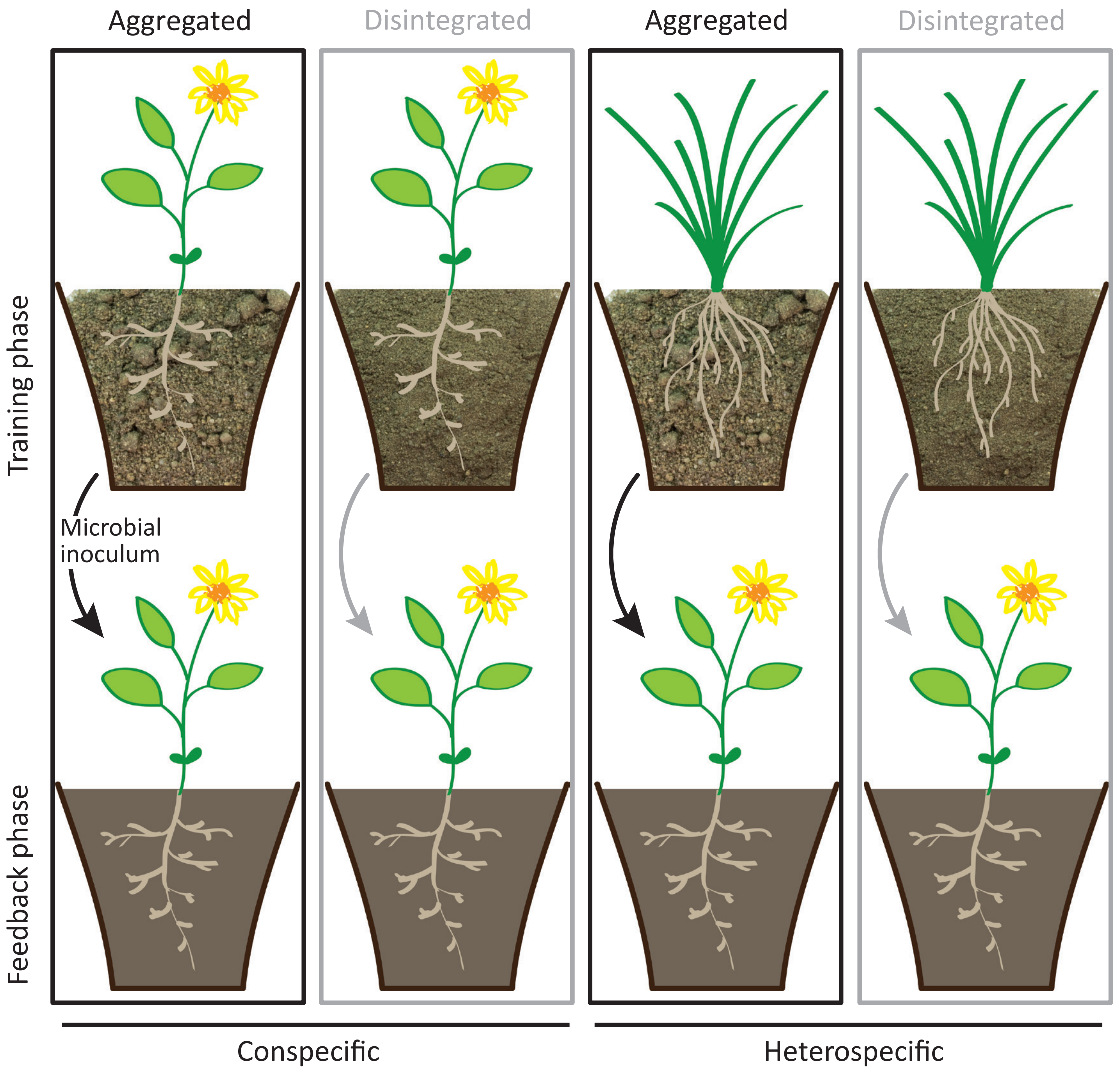

Figure 4.2 Experimental design. In the training phase 10 plant species were grown on aggregated and disintegrated soil with 8 replicates each. The microbial community of that trained soil was added to a common soil for the feedback phase. 9 species received conspecific inocula as well as 9 different heterospecific inocula from both soil structure levels. This resulted in 9 species * 2 soil histories (home/ away) * 2 former soil structure (aggregated/disintegrated) * 9 replicates = 324 experimental units. Black and gray boxes represent the two different soil treatments „aggregated“ and „disintegrated“.

This resulted in 9 species (four grasses and five forbs) * 2 soil histories (home and away) * 2 soil structure levels (aggregated and disintegrated) * 9 replicates = 324 experimental units in a fully randomized design (for the experimental design see Figure 4.2).

During the experiment, set up in October 2013, we used an autoclaved soil (aggregated field soil)-sand mixture (50:50) and added the inoculum from the training phase. We did so to disentangle the biotic effects caused by the soil structure of the training phase from physical and chemical effects, which would have occurred by using the whole trained soil. Seeds were surface sterilized (see above) and immediately sown on the soil-sand mixture covered with a thin layer of sterile sand. They were watered daily and transplanted into cones in the cotyledon-stage while adding 10 ml of the inoculum which saturated the whole root area of the seedling. Plants were grown for 6 weeks to minimize the further effect on physical soil structure and to prevent the roots from getting pot bound and then harvested to measure aboveground and belowground dry biomass.

Statistical analysis

All statistical analyses were conducted using R version 3.0.3 (R Core Team, 2014). In the training phase we tested for effects of soil structure and SRL on (i) plant biomass (total biomass as well as aboveground biomass and belowground biomass separately), (ii) biomass allocation (log(aboveground biomass/belowground biomass)) and (iii) the percentage water stable aggregates (WSA) after the training phase. Therefore, we fitted a general linear mixed effects model with effect coded variables using the package lmerTest (Kuznetsova *et al.*, 2014)and performed a type 3 ANOVA. The model contained the fixed effects “soil structure” (aggregated/disintegrated) and “SRL” (numerical) as well as the random effect “plant species”. For the analysis of the hyphal colonization we fitted a general linear mixed effects model with the fixed effects “soil structure” and “SRL” and the random effects “plant species” and “person” (two persons were counting) and performed a type 3 ANOVA. Pearson’s correlation coefficient was calculated to determine the relationship between the formation of WSA and the SRL.

In the feedback phase we wanted to test for the effects of soil biota originating from different soil structure and trained by different plant species with varying SRL on (i) plant biomass (total biomass as well as aboveground biomass and belowground biomass separately) and (ii) biomass allocation (log(aboveground biomass/belowground biomass)). To determine the feedback effect of “home vs. away” we fitted a general linear mixed effects model with the fixed effects “history (home/away)” and “former soil structure” (aggregated/disintegrated) and the random effect “species” (in the feedback phase) and performing a type 3 ANOVA (adapted from Brinkman *et al.* 2010). The “former soil structure” describes the initial soil conditions under which the microbial community was trained. To have a closer look into the effect of soil structure under different soil histories we then split our dataset by soil history (home/away) and fitted a general linear mixed effects model performing a type 3 ANOVA, with the fixed effects “former soil structure” (aggregated/disintegrated) and “SRL” (numerical) and the random effect “plant species”. Pearson’s correlation coefficient was calculated to determine the relationship between SRL and dry biomass productionas well as biomass allocation.

As the trait “SRL” was collinear with the factor “PFT” (grasses/forbs) we could not analyze them simultaneously. The PFT is nevertheless an important predictor for plant performance. We therefore fitted all models containing the fixed effect (SRL) additionally with the factor “PFT” replacing the former one.

Results

Training phase

In the training phase when using SRL as the predictive trait we found no effect of soil structure on plant biomass production (Table 4.1), but there was a difference in biomass allocation.

Plants allocated significantly more biomass aboveground with increasing SRL on disintegrated soil than on aggregated soil (Figure 4.3). When using PFT as the predictor the soil structure also as a single factor significantly affected the belowground biomass production and thereby the biomass allocation (Table S4.4) with grasses allocating more biomass aboveground overall. The formation of WSA was significantly affected by the PFT (Table S4.4) with forbs producing a higher percentage of WSA than grasses (Figure 4.4). We found a negative correlation between SRL and WSA formation on aggregated soil that significantly differed from disintegrated soil (Figure 4.4). The initial soil structure of the disintegrated soil with 29% WSA did not change overall but included some plants (like *D. glomerata*) which actually decreased soil aggregation down to less than 20% WSA, while others (like *L. vulgare*) increased it to 33% WSA. The aggregated soil with initially 44% WSA increased in aggregation to an overall mean of 48%. We could rarely detect decreases in WSA in the aggregated soil (the strongest was 2 % by *B. media*) while plants with low SRL as *D. carota* increased aggregation up to 55% WSA (Figure 4.4). The AM-colonization as well as the number of arbuscules differed significantly between the soil structure level and was higher on the disintegrated soil (mean AM-colonization in aggregated soil: 37% < 48% in disintegrated soil, Table S4.2 & S4.3, Figure S4.1) indicating that the disintegrating process did not negatively influence mycorrhizal fungal root colonization. Total colonization as well as number of arbuscules and vesicles were significantly negative correlated with SRL (Table S4.2) and forbs were highly significantly more colonized than grasses (Table S4.3, Figure S4.1).

Table 4.1. Summary of the linear mixed effects models for the training (A) and feedback (B) phases. Main effect of history (home vs. away) as well as main and interactive effects of soil structure (aggregated vs. disintegrated) and specific root length (SRL) on dry biomass (b, total biomass; ab, aboveground biomass; bb, belowground biomass; ln(ab/bb), biomass allocation) and water stable aggregates (WSA) are estimated. SRL was fitted as a continuous variable that is constant per plant species. Degrees of freedom (df), *F* values and *p* values from ANOVA are presented. Significant values (p < 0.05) are presented in bold.

A

		b		ab		bb		allocation		WSA	
effect	d.f.	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
soil	1	0.016	0.900	0.168	0.682	0.322	0.571	0.104	0.747	102.871	**<0.001**
SRL	1	0.044	0.839	0.121	0.735	0.472	0.508	1.422	0.261	2.331	0.158
soil*SRL	1	0.749	0.388	1.273	0.261	5.267	**0.023**	8.508	**0.004**	4.423	**0.037**

B

		b		ab		bb		ln(ab/bb)	
effect	d.f.	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
history	1	8.602	**0.004**	14.388	**<0.001**	0.850	0.357	0.504	0.478
soil	1	0.470	0.493	0.005	0.944	2.176	0.142	0.105	0.746
history*soil	1	0.018	0.894	0.026	0.872	0.231	0.631	2.225	0.137
				home					
soil	1	4.262	**0.041**	0.792	0.375	9.746	**0.002**	11.205	**0.001**
SRL	1	0.311	0.591	2.899	0.123	1.502	0.215	19.316	**0.002**
soil*SRL	1	4.453	**0.036**	1.384	0.241	8.064	**0.005**	8.596	**0.004**
				away					
soil	1	0.103	0.748	0.002	0.968	0.453	0.502	0.029	0.864
SRL	1	0.194	0.670	2.489	0.149	2.332	0.161	19.950	**0.002**
soil*SRL	1	0.011	0.918	0.009	0.926	0.103	0.749	0.457	0.450

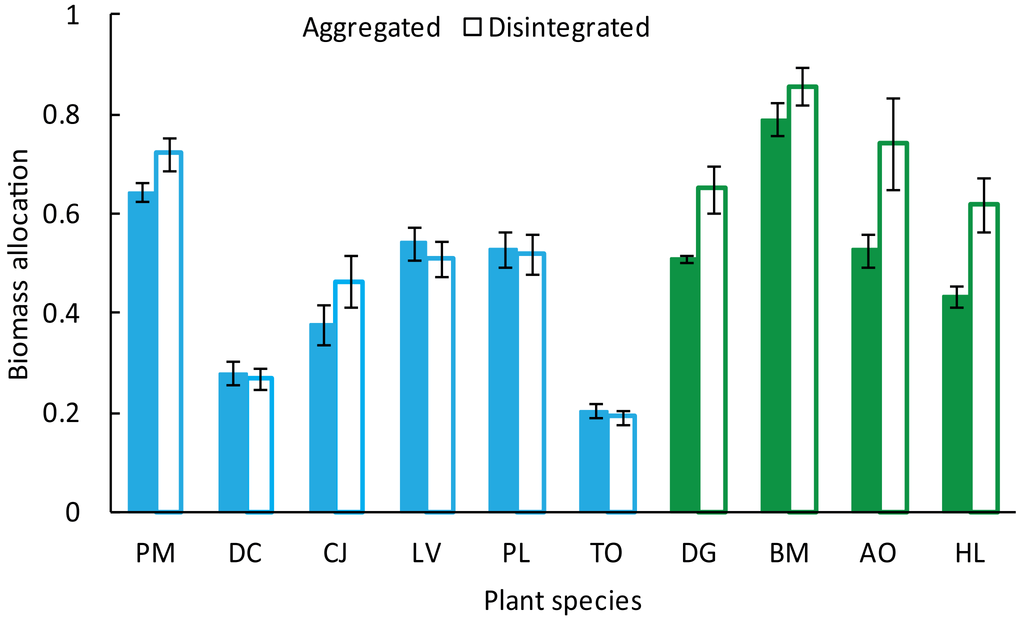

Figure 4.3 Training phase. Biomass allocation [log(aboveground biomass/belowground biomass)] on aggregated versus disintegrated soil. Species are sorted by specific root length (SRL) from low to high. Blue colouring indicates forbs, green colouring indicates grasses. Data represent mean ± SE. (PM) *Plantago major*, (DC) *Daucus carota*, (CJ) *Centaurea jacea*, (LV) *Leucanthemum vulgare*, (PL) *Plantago lanceolata*, (TO) *Taraxacum officinale,* (DG) *Dactylis glomerata*, (BM) *Briza media*, (AO) *Anthoxantum odoratum*, (HL) *Holcus lanatus*. The interaction of the effects „soil structure“ and „SRL“ significantly affects the biomass allocation with *p*=0.004 in a linear mixed effects model with the random effect „plant species“ (see Table 4.1).

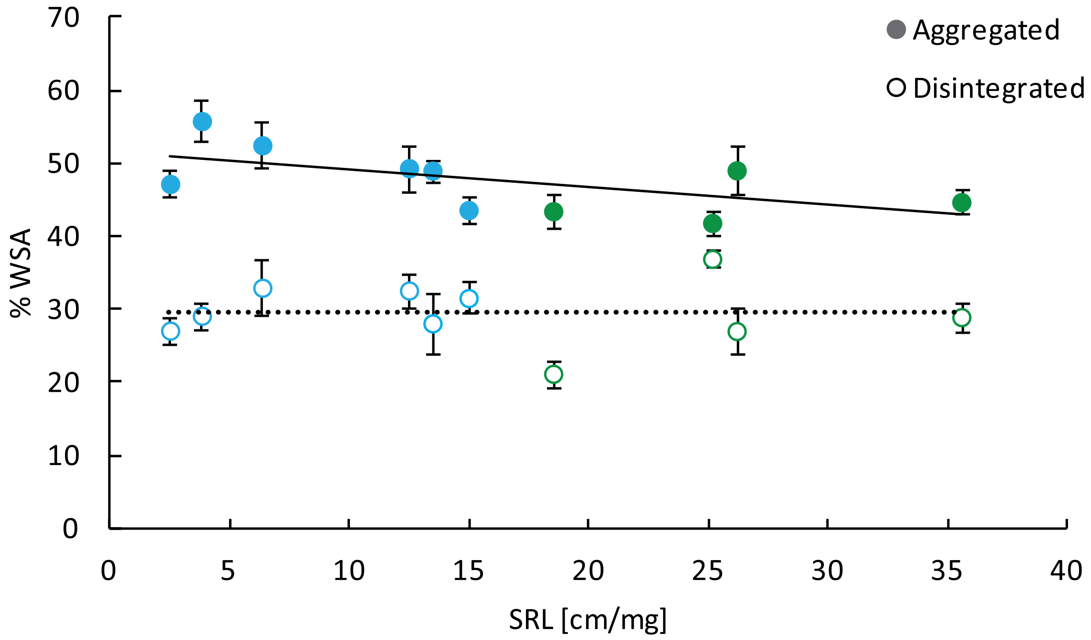

Figure 4.4 Training phase. Formation of water stable aggregates (WSA) on the two soil structure levels in correlation to the specific root length (SRL) of the species. Blue colouring indicates forbs, green colouring indicates grasses. Data represent mean ± SE. Initial WSA were 29% in disintegrated and 44% in aggregated soil. The relationship of SRL and %WSA is significant in aggregated soil (solid line, r^2=0.360, p=0.003) but not in disintegrated soil (dashed line, r^2=0.000, p=0.962). The interaction of the effects „soil structure“ and „SRL“ significantly affects the formation of WSA (p=0.037) in a linear mixed effects model with the random effect „plant species“ (see Table 4.1).

Feedback phase

In the feedback phase we found a significant effect of soil history (home vs. away) on total plant biomass as well as on aboveground biomass (Table 4.1) with plants experiencing a negative conspecific feedback overall. Belowground biomass was not significantly affected by the soil history, so all changes in total biomass took primarily place in the aboveground biomass. The effects of heterospecific-trained soil microbes on biomass production did not differ between disintegrated and aggregated soil (Table 4.1, Table S4.4). The only effect pronounced within the heterospecific treatment was a correlation of biomass allocation and SRL (Table 4.1), as well as a significant effect of PFT on biomass allocation (Table S4.4) which also occurred in conspecific trained soil. Biomass allocation in the heterospecific treatment was positively correlated with SRL (r=0.588, p<0.001) indicating that plants with higher SRL allocated more biomass aboveground irrespective of training soil structure being aggregated or disintegrated.

The biomass effects of conspecific-trained soil microbes - being negative overall - were significantly affected by the soil structure of the training phase in the model with SRL as the predictive trait (Table 4.1). With the predictive factor PFT the single effect of soil was not significant

(Table S4.4). Under the influence of conspecific microbes from disintegrated soil, plants produced less biomass than with those from aggregated soil (i.e. negative conspecific feedback was stronger; Figure 4.5). The results of the linear mixed effects models illustrated a significant interaction between the former soil structure and the SRL (Table 4.1) and the PFT respectively (Table S4.4) in affecting biomass. With conspecific microbes from disintegrated soil, biomass production was mediated by SRL (r=0.230, *p*=0.039). This effect was mainly caused by differences in root biomass production, which therefore also resulted in significant effects on biomass allocation (Table 4.1, Table S4.4). In disintegrated soil plants with higher SRL produced relatively more root biomass (Figure 4.6) compared to all other treatments resulting also in a less negative feedback. Plants with a SRL up to app. 20 cm/mg experienced negative effects on root biomass while species with a SRL higher than that benefitted from conspecific microbes from disintegrated soil in comparison to all other treatments. This effect was most prominent in belowground biomass (Figure 4.6) but was also reflected in total biomass production. The SRL clearly separated forbs (low SRL) and grasses (high SRL) (Table S4.5). The mediating effect of SRL could only be observed with conspecific microbes from disintegrated soil. Biomass production under the influence of conspecific microbes from aggregated soil was not correlated with SRL.

Summarizing these observations, the aboveground biomass showed feedback effects irrespective of soil structure while within the conspecific feedback the soil structure affected belowground and thereby total biomass production mediated by SRL which discriminates between forbs and grasses.

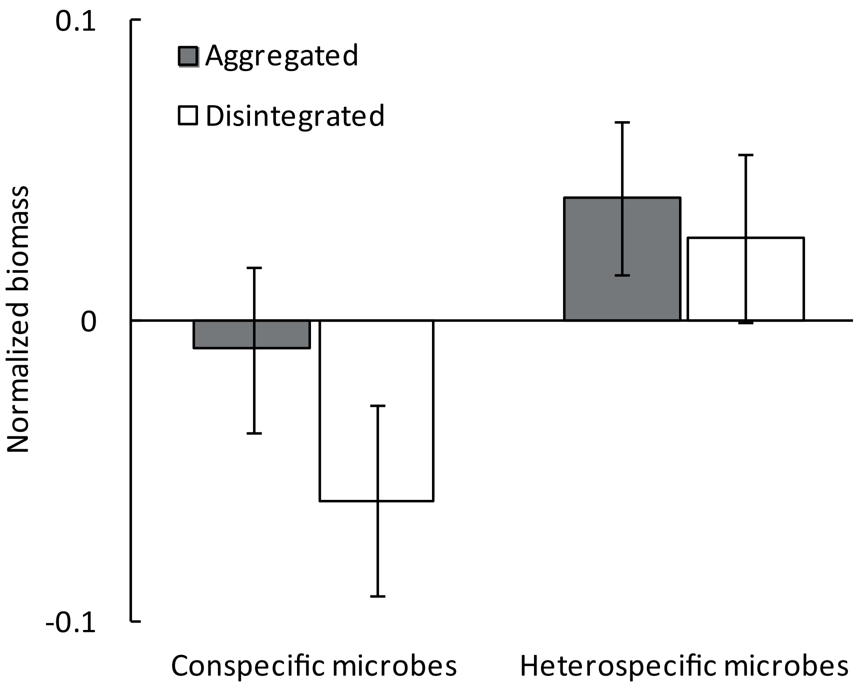

Figure 4.5 Feedback phase. Effect of different training soil structure on plant biomass after inoculation with conspecific or heterospecific microbes respectively. For a better visualization biomass data are normalized per plant by (x-species mean)/ species mean over the entire experiment to account for species specific differences. Data represent mean ± SE. For conspecific microbes the soil structure significantly affects plant biomass production (*p*=0.041) in a linear mixed effects model with the additional fixed effect „SRL“ and the random effect „plant species“ (see Table 4.1).

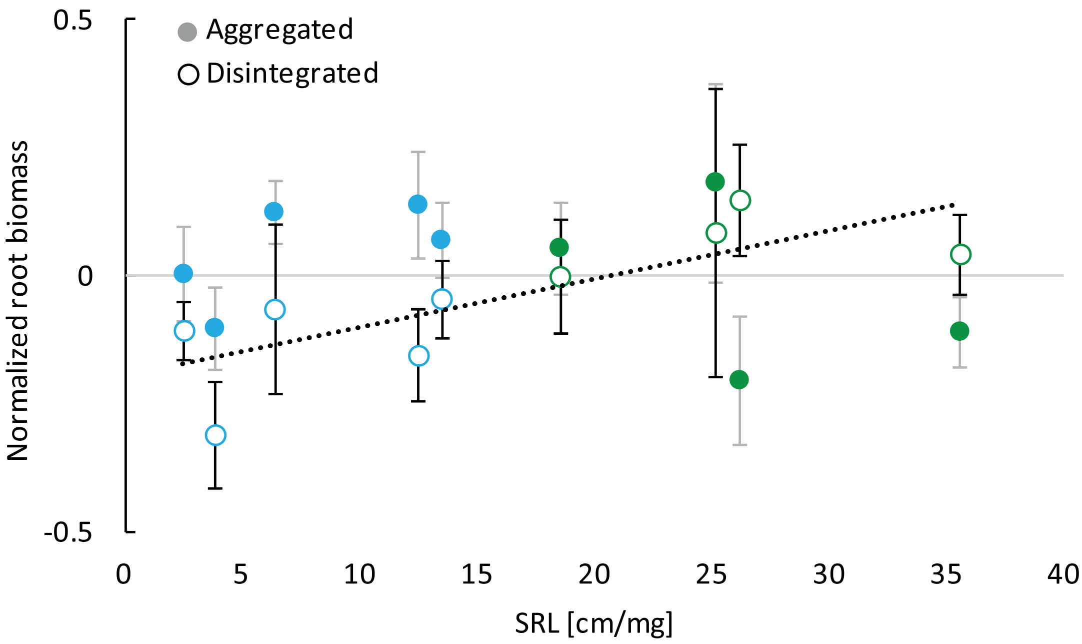

Figure 4.6 Feedback phase. Effect of conspecific soil microbes. Displayed is the specific root length (SRL) as a mediating factor of root biomass production. For a better visualization biomass data are normalized per plant by (x-species mean)/ species mean over the entire experiment to account for species specific differences. Blue colouring indicates forbs, green colouring indicates grasses. Data represent mean ± SE. The relationship of SRL and normalized dry root biomass is significant in disintegrated soil (dashed line, r^2=0.603, p=0.025) but not in aggregated soil (r^2=0.068, p=0.239). The interaction of the effects „soil structure“ and „SRL“ significantly affects the root biomass production (p=0.005) in a linear mixed effects model with the random effect „plant species“ (see Table 4.1).

Discussion

As far as we know soil structure has never been taken explicitly into account when investigating PSF. The major conclusion we can draw from our experiment is that soil structure can have a profound influence on the outcome of PSF. We initially hypothesized a stronger negative feedback on disintegrated soil due to a higher host specificity and virulence of pathogens under these conditions as well as to lower dependency on mutualists. Our results support this hypothesis. The SRL was not measured within the experiment because reciprocal effects would have made it impossible to distinguish cause and effect. It can be criticised, that with such an approach the SRL is not directly linked to the effects observed as it only represents a potential and not an actual morphological character of the experimental plants. This is a general issue with trait based approaches using data from databases (Cordlandwehr *et al.*, 2013) that we tried to diminish to some extent by using data of an independent experiment with the same seed material representing a species specific trait not effected by any of our treatments. The correlation of SRL, as a suggested explanatory variable for the observed effects, turned out to be a significant mediator of the training process in disintegrated soil. The SRL separates the two PFTs (forbs and grasses) which on their own explain most of the effects observed. It is a challenge to identify the mechanisms underlying a process that coincides with clear PFTs. Furthermore, if a mechanistic explanation has been found it is most likely not the only factor that drives the process. It is still obvious, that a significant correlation of SRL with various effects during training and feedback phase indicates a direct mechanistic relation and explains an important component of the different effects observed for grasses and forbs. To discuss the impact of SRL on PSF in different soil structure the two phases of the experiment need to be examined in detail.

Training phase

The results of the training phase illustrate the physical effects of soil structure on plant performance. The soil and its microbial community was initially the same except for the impact of the disintegrating process. Therefore, the effects observed were caused by physical structure of the soil (either directly on plants or indirectly via different microbial performance during the training phase due to soil structure) or by different nutrient availability due to aggregation status. Plant available phosphorus as well as nitrogen were slightly elevated in the disintegrated soil but as plant biomass was not affected by soil structure, this seems to not have influenced plant performance directly. Therefore, we assume that the effects observed are not primarily caused by nutrient availability but by physical structure of the soil. The high colonization by AM fungi in both treatments showed that the process of disintegrating did not destroy the large mycorrhizal fungal propagules and so most likely did also no harm to propagules of other microbiota of comparable or smaller size. Plants with higher SRL (grasses) showed less AM colonization and all plants were more colonized in disintegrated soil with even more arbuscules. Rillig and Steinberg (2002) found that AM-colonization did not differ when extraradical hyphae colonized substrate of different aggregate sizes (simulated by glass beads) with roots growing in vitro without access to the hyphal compartment. If differences in AM colonization were to arise from differences in soil nutrients we would expect a stronger colonization in the aggregated soil with less available nutrients, which was not the case. Furthermore, it is known that a simultaneous increase in P and N buffers the effects a single P increase would induce for arbuscular mycorrhizal colonization of plants (Johnson, 2010; Camenzind *et al.*, 2014). We therefore suggest that the difference in colonization in our experiment is an indirect effect of the soil structure due to root performance: as roots can grow more easily in soil with larger aggregates and resulting larger pores this could lead to a lower dependency on mycorrhizal partners especially for water uptake. The gradient of SRL clearly separated forbs and grasses in our experiment with the latter having the higher SRL, which is also known from the literature (Craine *et al.*, 2001). Grasses, at least members of the Poideae subfamily (C3), are known to have a low mycorrhizal responsiveness (Reinhart *et al.*, 2012). This supports our result of lower colonization rates in plants with higher SRL irrespective of the soil.

In the training phase total plant biomass was not affected by soil structure. But on disintegrated soil plants with a higher SRL and accordingly grasses allocated proportionally more biomass aboveground. The general linear mixed effects model containing the factor “PFT” reveals a single effect of soil structure with a general biomass allocation towards aboveground plant parts on disintegrated soil. Actually Figure 3 clearly shows that the effect of soil structure on biomass allocation cannot be observed in most forbs with low SRL and is therefore mainly driven by the interaction with SRL or PFT respectively. Following the concept of a ‘functional equilibrium’ of biomass allocation (Brouwer, 1963; Poorter *et al.*, 2012) this could be translated to differences in belowground limitation along a SRL gradient on disintegrated soil. We cannot exclude whether that limitation is by soil nutrients or not, but the fact that AM-colonization was higher in disintegrated soil, which contained slightly more plant available phosphorus does not point in this direction. The fact that especially grasses (with higher SRL and less AM colonization) produced less root biomass on disintegrated soil rather raises the question if there could be different phylogenetically conserved strategies in coping with soil structure or if there are other mechanisms causing this effect. Studies on the effect of soil structure on root performance are scarce, but results obtained using maize seedlings suggest that plants can alter allocation patterns towards roots in response to larger soil aggregates, which was unrelated to nutrient status of the plants but appears to be a direct response (Alexander & Miller, 1991). We propose that the aggregated soil is easily penetrable by roots irrespective of the SRL (in the range of our experimental plants, see Table S4.5) with nutrients to some degree being sequestered inside aggregates forcing the plants to allocate biomass belowground for soil exploration and nutrient acquisition. In the disintegrated soil the SRL (mainly caused by root fineness) determines the ability of exploring the soil structure with smaller pores where nutrients are more equally distributed and less sequestered inside large aggregates (Linquist *et al.*, 1997). That causes an advantage for plants with higher SRL to obtain soil nutrients and reduces the need for an additional investment in root biomass.

We know that apart from abiotic factors such as soil texture (Wick *et al.*, 2009), plant roots and microbiota such as mycorrhizal fungi can have a profound influence on levels of soil aggregation (Six *et al.*, 2004; Rillig & Mummey, 2006). In experimental plant communities of the Jena experiment grasses (with high SRL) had the strongest positive contribution to soil aggregation, as compared to forbs and legumes (Pérès *et al.*, 2013). We found the opposite when testing the effect of SRL on the formation of WSA. In the aggregated soil, the formation of WSA was negatively correlated with SRL. This could be either due to root penetration resulting in destruction of macroaggregates (Materechera *et al.*, 1994; Angers & Caron, 1998; Six *et al.*, 2004) or to reduced length of AM fungal hyphae. As AM hyphae can increase soil aggregation in various ways (Tisdall & Oades, 1982; Rillig & Mummey, 2006) the lower colonization rate of plants with higher SRL is very likely to negatively affect the formation of WSA. In the disintegrated soil the effects of the training phase varied from slight elevation to clear decrease in aggregation. The process of disintegration led to the destruction of macroaggregates (250 µm - 4 mm) falling apart into stable microaggregates (<250 µm). Angers *et al.* (1997) showed that the formation of highly water stable microaggregates takes place within macroaggregates. Six *et al.* (1998, 2004) concluded that disturbance (like tillage or other processes unfolding disintegrating forces) reduce the amount of macroaggregates resulting in a reduced formation of new microaggregates. That could be a possible explanation for the lacking net formation of new aggregates in our disintegrated soil. We cannot identify the mechanism behind the further disintegration caused by some plants but we suppose that the partially destroyed aggregates where damaged to a point where they were predestined to further fall apart to some extent. This effect was not correlated to SRL so there seem to be other plant- or soil microbial community specific traits driving the process of further disintegration.

Feedback phase

At the beginning of the feedback phase the soil aggregation was equal in all treatments. Therefore, the results observed were caused by the biological components of the trained soil primarily reflecting soil microbial differences due to initial soil structure level of the training phase.

Plants growing with heterospecific microbes showed no response to the microbes originating from different soil structure levels of the training phase. The SRL was positively correlated with the biomass allocation towards aboveground parts of the plant. This effect is comparable to the one we found in the training phase on disintegrated soil where microbes from field soil represent a predominantly heterospecific-trained inoculum. The autoclaving of the uniform soil of the feedback phase as well as the mixing with sand is likely to have disintegrated the soil structure favouring grasses with their high SRL and allowing them to allocate more biomass aboveground like we argued above to account for this effect in the training phase.

Plants growing with conspecific microbes produced less biomass than the ones in the away treatment. The conspecific feedback was therefore negative, leading to the conclusion that host specific antagonists likely accumulated during the training phase in both soil structure levels. Following the basic concept of PSF this translates to an expected stabilization of plant diversity by coexistence (Bever, 2003) in a community irrespective of soil structure. However, the negative conspecific feedback was most pronounced with microbes from disintegrated soil. The fact that this effect was only significant in the general linear mixed effects model with SRL strengthen the argument that the SRL was a significant mediator of this effect with plants with a low SRL experiencing the most negative feedback. Following our initial hypothesis this could be either due to more negative effects by pathogens or less positive effects by mutualists. As plants with lower SRL had a high percentage of colonized roots by AM fungi on disintegrated soil in the training phase a reduced positive influence of mutualists in the respective soils of the feedback phase is not likely. It seems more plausible that host specific pathogens were the drivers of the reduced biomass production observed in the feedback phase. As PSF is a complex reciprocal process it is hard to distinguish whether the fungal or the plant partner (or any other biological participant) is responsible for an observed effect. Taking into account that during training on disintegrated soil, belowground biomass production and biomass allocation were correlated with SRL, it seems most likely that the effects we observed were caused by different plant performance during training along a SRL gradient. We argue that the physical structure of disintegrated soil (with smaller pores and more equally distributed nutrients) represented a disadvantage for plants with low SRL - namely forbs - in terms of effective soil exploration. That may have caused the need to allocate relatively more biomass belowground and increase exposure to soil pathogens. The consequence is a conspecific pathogen enrichment causing negative biomass effects in the feedback phase. Our initially hypothesized concept that specialist pathogens might perform better in disintegrated soil seems to be part of a much more complex interaction.

Aboveground biomass in the feedback phase was not affected by the former soil structure nor by the SRL. All changes related to soil structure took place belowground reflected by significant effects on biomass allocation in the conspecific inoculated plants. The stronger negative conspecific feedback effect with microbes from disintegrated soil (Figure 4.5) therefore mainly arose from a reduction of belowground biomass especially in plants with low SRL. A plausible explanation for this would be that plants have lost roots to species-specific pathogens and that this was a higher burden on plants with relatively expensive, low-SRL roots: these plants may have invested more in AM symbiosis than in root biomass (Veresoglou *et al.*, 2012), which may make these roots even more expensive to replace. On the contrary, plants with higher SRL may compensate root loss to pathogens more easily by producing new fine roots. Mommer *et al.* (2011) suggested that fine roots are relatively cheap in terms of biomass investment to explain the observed advantage for plants with high SRL (experiment on *A. odoratum*) in nutrient foraging. Our results appear to support de Kroon *et al.* (2012) who suggested that the responses of roots to soil microbes are underestimated regarding their impact on plant community dynamics. More specifically, we show that feedbacks to soil structure are primarily reflected in belowground parts of plants, while feedbacks to conspecific versus heterospecific training are mainly reflected in aboveground plant parts.

Conclusion

This study has been the first to examine the effects of soil structure on PSF. We find the complex interaction of plant roots and microbial colonizers - being pathogenic or mutualistic - that creates PSF is to some extent dependent on soil structure, leading to stronger negative feedback in disintegrated soil. Furthermore, our results indicate that this effect is strongly mediated by a plant specific root morphological trait, i.e. SRL that coincides with the distinction between grasses and forbs. It has previously been argued that plant traits could explain PSF effects in grassland communities (Baxendale *et al.*, 2014). However, our finding of more negative feedback for low SRL plants in disintegrated soil goes against the expectation that finer roots are more susceptible to fungal pathogens (Bever, 1994; Newsham *et al.*, 1995b). A potential explanation is that higher nutrient scavenging capabilities and replaceability of high SRL roots in the small pored disintegrated soil more than counteracts this expected stronger negative feedback. Clearly, more research is needed on the relationship between SRL and PSF, as well as on the degree to which this mechanistic trait contributes to the differences between grasses and forbs and soil structure is a primary candidate of potential moderator variables to take into account. It has been shown that soil structure in terms of WSA and size distribution is positively correlated with succession (Duchicela *et al.*, 2013; Cheng *et al.*, 2015; Erktan *et al.*, 2015). In that context our findings raise the question if plants with lower SRL may be more limited by PSF in early successional stages. This question has not been addressed explicitly so far, but Erktan *et al.* (2015) found SRL to be negatively correlated with aggregate stability along a successional gradient. Zangaro *et al.* (2008, 2012, 2014) found high SRL and low root diameter to be associated with early successional stages in different ecosystems in Brazil. Regarding our results it would be an exciting research avenue to study how root traits affect soil structure, PSF, and the potential interaction between these factors in a vegetational succession context.

Acknowledgements

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Chapter 5

The influence of host traits and environment on the phylogenetic structure of intraradical communities of arbuscular mycorrhizal fungi

Running title: Community phylogenetics of Glomeromycotina

with Kriszta Vályi, Masahiro Ryo, Daniel Prati and Stefan Hempel

Abstract

The arbuscular mycorrhiza (AM) is a multispecies symbiosis between plant roots and Glomeromycotina, in which AM fungi completely rely on the plant partner for carbon, and forage in the soil for other nutrients. We used a community phylogenetics and host trait based framework to explore how host phylogeny, root and leaf traits, ecological preferences, and land use influence the phylogenetic structure of intraradical AM fungal communities. We carried out this analysis in 150 grassland plots using pyrosequencing. AM fungal communities in the root were consistently phylogenetically clustered, a possible sign of more “filtering” in the root niche than competitive exclusion among the AM fungal co-colonizers. The ability of plants to selectively reward their symbionts was previously shown to vary not only with plant identity and phylogeny, but with environmental conditions as well. Less host filtering and the resulting increased competition could explain the observed decrease in phylogenetic clustering under certain environmental conditions or host traits. Taking these results together, we propose a heterarchically structured system of influence between the symbiont community, the host and the environment to determine AM fungal community structure.

Introduction

The simple theoretical framework of community phylogenetics (Webb *et al.* 2002) states that the relative importance of competitive exclusion (and sometimes other species interactions: Vamosi *et al.* 2009; Mayfield & Levine 2010) and "environmental" filtering causes communities to exhibit nonrandom phylogenetic structure. Phylogenetic patterns in communities of macroorganisms have been studied at least since Darwin (1859), who stated that species of the same genus are expected to be more similar in traits and therefore compete more severely.

In symbiotic microbial communities, in addition to abiotic habitat filtering and local competitive exclusion, the host becomes another significant determinant of endosymbiont communities (Vályi *et al.*, 2016). Just as the abiotic environment, the host may directly or indirectly select symbionts with a certain trait complex from the local species pool, i.e. relevant host traits can be interpreted as additional niche axes for the symbionts. Current molecular techniques allow researchers to collect DNA-based data even from endosymbiotic communities and investigate their phylogenetic patterns. Due to the lack of species level trait data and sometimes even taxonomic description, phylogeny is often used as a proxy of ecologically relevant functional traits in these "cryptic" microbial groups (Martiny *et al.*, 2015), for instance in bacteria (Horner-Devine & Bohannan, 2006), or in AM fungi (Horn *et al.*, 2014, 2017; Montesinos-Navarro *et al.*, 2016), the latter a prominent group of obligate endosymbionts associated with the vast majority of plant species.

The framework of community phylogenetics is well suited to community analyses of AM fungi (Glomeromycotina (Spatafora *et al.*, 2016), formerly Glomeromycota) , because their traits related to competition for space in soil and the host roots are conserved (Maherali & Klironomos, 2007) and thus the connection between phylogenetic relatedness and functional trait similarity has been established.

Recent studies established the relation between *aboveground* plant traits and plant habitat preferences and mycorrhizal status (Hempel *et al.*, 2013; Menzel *et al.*, 2016). But studies that take host traits, especially belowground traits into account as potential defining factors for AM fungal *community structure* are missing, even though plant roots are hypothesized to have evolved as a habitat for mycorrhizal fungi (Brundrett, 2002; Comas *et al.*, 2014) and root trait variation among plant species is immense (Bardgett *et al.*, 2014). Therefore, among host characteristics, root traits have the potential to strongly define AM fungal communities, providing an additional information compared to using host species identity as an explanatory variable alone (Violle *et al.*, 2007).

In this study, we combined for the first time host and symbiont phylogeny, intrinsic and extrinsic host traits and environmental characteristics to explain root AM fungal phylogenetic structure. Host related niche axes were described by leaf traits and morphological root traits (intrinsic traits) and ecological preference (extrinsic traits). A combined index of fertilization, grazing and mowing was used to describe the environment in a series of grasslands representing a land use intensity gradient. We formulated the following hypotheses:

1. Phylogenetically related plants have phylogenetically more similar AM fungal communities.

2. AM fungi within roots exhibit nonrandom phylogenetic structure.

3. Host plant traits (a. root traits b. leaf morphological and ecological traits) have an effect on the phylogenetic structure of root AMF communities.

4. Phylogenetic structure of root AM fungal communities also changes with sampling region and increasing land use intensity.

Methods

Sampling sites

Soil sampling was carried out in the 150 grassland plots (50 × 50 m of size) of the three sampling regions (S: Schorfheide-Chorin, H: Hainich-Dün and A: Schwäbische Alb) of the German Biodiversity Exploratories, each representing a land use gradient of typical grassland use in Central Europe (Table S5.1, Fischer *et al.* (2010)), including grasslands with low input and also fertilized, grazed and mown meadows and pastures.

Sample preparation

As part of a larger sampling campaign of several projects within the German Biodiversity Exploratories, in each of the 150 grassland plots 14 soil cores along two orthogonal transects were taken and the top 10 cm of all cores per site were pooled; sampling was done within two weeks in May of 2011. Five randomly selected healthy looking root fragments with a length of 4 cm and diameter less than 1 mm per plot were subsampled from these pooled soil samples, resulting in 750 root samples which were used for AM fungal and plant molecular analyses. This random sampling strategy was chosen in order to obtain root fragments in a way that was not biased by the aboveground plant status (dormant, vegetative growth, flowering etc.) within a predefined land use background. Roots were washed thoroughly with distilled water, freeze dried and pulverized using metallic beads. Afterwards, total DNA from the powder was extracted with the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, USA). DNA was amplified by a nested PCR approach, first with GlomWT0/Glomer1536, then with NS31/AM1a+b primer pairs (Morris *et al.*, 2013), using 25 different Multiplex Identifier Adaptors (Roche Diagnostics GmbH, Germany) in order to label sequences belonging to different samples. AM1a and AM1b are modified AM1 primers designed to capture AMF families not captured by the original AM1, which excludes some taxa (Daniell *et al.*, 2001). The primers target the small subunit (SSU) of the nuclear encoded ribosomal DNA (rDNA). A detailed protocol of the PCR conditions is given in the Supporting Information (Methods S5.1).

Equal amounts of DNA were mixed into pools of 25 samples based on DNA content quantification by the image-analysis software GelQuant.NET (v. 1.8.2, BiochemLabSolutions.com). Pools were purified via agarose gel extraction with NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany) and were 454 pyrosequenced on a Roche GS FLX+ system with Titanium chemistry. Raw sequencing data from one of the three sampling sites (Hainich) was previously used in Vályi *et al.*, (2015) for testing different hypotheses, but was bioinformatically re-analyzed.

Bioinformatic analysis

Processing of flowgram data from pyrosequencing was done by Mothur (Schloss *et al.*, 2009), using the sff.multiple command, discarding reads with less than 300 flows and reads shorter than 200 bp. Sequences were unpacked, screened, denoised and trimmed. Reads with more than 1 base difference in the barcode or 2 bases in the forward primer were discarded. Afterwards, the 750 samples had an average of 4218 reads per sample (SD: 3193).

As we were interested in the structure of the AM fungal communities rather than their identity, we used an open reference OTU (Operational Taxonomic Unit) picking approach (sensu Bik *et al.* 2012), to define sequence clusters using the CROP clustering tool (Hao *et al.*, 2011), which performs an unsupervised Bayesian clustering. This method works with a Gaussian mixture model with a flexible OTU cutoff threshold.

The processed sequences were compared with sequences deposited in the Nucleotide collection (NCBI Resource Coordinators, 2016) of the National Center for Biotechnology Information with BLAST (Basic Local Alignment Search Tool). Based on the BLAST results, Glomeromycotina sequences were extracted with MEGAN (Huson *et al.*, 2011). Average Glomeromycotina reads per sample were 995 reads (SD=1451).

Multi-tag pyrosequencing might introduce a bias to community composition due to the preferential amplification of certain barcoded primers during PCR (Berry *et al.*, 2011). Since such unequal representation of samples will lead to a differential sampling intensity, we resampled our dataset to an equal number of reads per sample. As the amplicons in our study were short and about the same length, we believe that read numbers in the resampled dataset could be used as a proxy for relative abundance of the OTUs (Ihrmark *et al.*, 2012).

The OTU table was randomly resampled to 500 Glomeromycotina reads per sample with replacement by bootstrapping, repeated 100 times, and then averaged. To avoid excessive upsampling, samples consisting of less than 400 sequences were removed prior to resampling, as were singleton OTUs. Downstream data analysis was performed on a data table consisting of 495 root communities and 144 Glomeromycotina OTUs. In this resampled dataset the average OTU richness per sample was 10.94 (SD=7.17).

AM fungal phylogenetic structure analysis

We calculated pairwise genetic distances between the center sequences of each Glomeromycotina OTU with ESPRIT (Sun *et al.*, 2009), that uses the Needleman–Wunsch algorithm (Needleman & Wunsch, 1970). Phylogenetic beta diversity, which is the phylogenetic dissimilarity of AM fungal communities of different plant root samples (hypothesis 1), was addressed by calculating inter-community mean pairwise distance (IC-MPD) by the comdist function in picante. Both ses.mpd and comdist are adapted to R from Phylocom (Webb *et al.*, 2008).

To test hypothesis 2, we addressed the phylogenetic structure of AMF root communities of single root samples by calculating the standardized effect size of mean pairwise distances (SES-MPD), an equivalent to the additive inverse of the nearest relative index (Webb *et al.*, 2002). SES-MPD was calculated by the ses.mpd function in picante package (version 1.6-2, (Kembel *et al.*, 2010)) of R, using the "taxa.label" algorithm with 999 randomized null communities, which uses null model randomization of distance matrix labels across all taxa to calculate effect sizes of deviations from mean phylogenetic distance between samples (i.e. AMF community of each individual root sample). Negative SES-MPD values are correlated with clustering, positive values with overdispersion.

Host plant identity

For testing the effect of plant traits and phylogeny, detailed information on host plant identity and phylogeny was required. The vegetation of the plots was assessed by cover estimates of a 4x4 m core area by visual estimation (Schmitt *et al.*, 2011). Plant identity of the root samples were assigned based on the sequence of the trnL-intron (see Vályi et al. 2015), in combination with coverage data from vegetation surveys. Plant DNA from the root extract was amplified with trnL c/d primers (Taberlet *et al.*, 1991), cleaned and Sanger-sequenced. Data was BLASTed against NCBI nucleotide collection and best hits based on maximum scores were matched with the vegetation of the plot, which allowed us to identify 52 different host plants (Table S5.2).

A phylogenetic tree of the detected plant species was created by pruning the Daphne phylogeny (Durka & Michalski, 2012) to include only our plant taxa. Phylogenetic distance was calculated by the cophenetic.phylo function from the ape package of R (Paradis *et al.*, 2004).

Root trait measurements

The most common plant species found in our sampling sites were grown in 2012 in a greenhouse in Muri, Switzerland under uniform conditions (for details see Methods S5.2). Roots were separated from the aboveground plant parts, washed, dried and weighed by a precision balance. Root length, root surface area and root volume were determined using the WinRhizo scanner based system (version 2007d, Regent Instruments Inc., Québec, Canada). Fine roots, the absorptive part of the root system, were defined as roots thinner than 0.2 mm in diameter, and constituted on average 79 % of scanned root length. We measured root length and surface area separately for all roots and fine roots. Subsequently, specific root variables were calculated by dividing the total values by root dry weight. Out of the 52 plant species identified by root DNA, 43 plant species were included in the root trait analysis (Table S5.2).

Leaf traits

Leaf anatomy traits were acquired from the BIOLFLOR database (Klotz *et al.*, 2002; Kühn *et al.*, 2004). Plant species are characterized as having either scleromorphic, mesomorphic, hygromorphic or helomorphic leaves. Plants with hygromorphic leaves are characterized as delicate plants of shade and semi-shade and relatively high humidity. Their root system is not very extensive and ensures no quick resupply of water to the plant.

Plant ecological traits

Ecological strategy types (following the system of Grime 2001) were acquired from the BIOLFLOR database. Plant species are characterized either as competitors, stress-tolerators, ruderals or one of the intermediate combinations. Ellenberg indicator values (Ellenberg *et al.*, 1992) for light and moisture preference reflect the realized environmental optima of plant species of Central Europe and are expressed as ordinal numbers. Light preference is characterized as low (1–3), medium (4–6), high (7–9) or indifferent. Ellenberg values were acquired from FLORKART (hosted as floraweb by the German Federal Agency for Nature, available under http://www.floraweb.de).

Land use

In order to test hypothesis 3, we included land use information in our analyses. Land use intensity was measured by the LUI index (Blüthgen *et al.*, 2012), which is an additive index summarizing the standardized intensity of fertilization (organic or inorganic N-fertilizer applied by farmers measured in kg nitrogen per hectare), frequency of mowing, and the intensity of livestock grazing (reflected by density of livestock) on the grassland sites of the Biodiversity Exploratories project. It was calculated for the years between 2006 and 2010 and then averaged (Klaus & Blüthgen, 2013).

Statistical analyses

All statistical analyses were performed using the statistical software R version 3.2.1 (R Core Team, 2016). To test the relationship between host plant relatedness and the distance between the phylogeny of their AM fungal phylogenetic communities (Hypothesis 1), we fitted a linear model using host cophenetic distance and inter-community mean pairwise phylogenetic distance of AM fungi in the roots.

To analyze the effects of sampling area, land use intensity and host plant traits jointly in relation to the magnitude of AM fungal clustering (hypotheses 3 and 4), and to assess their relative importance, we carried out a random forest analysis (Breiman, 2001) with the "party" package of R (v.1-2) (Strobl *et al.*, 2009). This modeling approach is nonparametric and quantifies the relative importance of variables, while being able to function with nonlinearity, variable interactions, and missing values. Relative importance score is calculated for each explanatory variable based on the relative contribution on explaining the variability of a response variable (Breiman, 1996). Using the algorithm in Hapfelmeier & Ulm (2013), we also evaluated statistical significance of the importance of each explanatory variable ($\alpha = 0.05$). As the number of explanatory variables does not restrict random forest models, we included all variables including host traits, land use intensity and sampling region into one model. The parameters of the random forests, the numbers of trees and random subset of explanatory variables, were set to 1000 and 5, respectively. To confirm nonlinear relations of statistically significant variables with the magnitude of AM fungal clustering (SES-MPD), we generated partial dependence plots with the mlr package (version 2.9 (Bishl *et al.*, 2016))of R. Partial dependence plots visualize the modeled effects of selected explanatory variables on the response variable, while taking the effects of the other explanatory variables into account beforehand. For the detailed list of variables included in the random forest analyses, see Methods S5.3.

To explore the potentially different effects of different root traits on phylogenetic clustering (SES-MPD) of the AM fungal communities in one root piece (Hypothesis 3a) we fitted separate linear models. We checked correlation between root traits using Pearson's correlation.

To test whether the magnitude of AM fungal clustering linearly changes with increasing land use intensity (Hypothesis 4), we fitted a linear mixed effects model using the lme command in the package nlme (Pinheiro *et al.*, 2016) with sampling region and LUI and their interaction as an explanatory and SES-MPD as a response variable, including the sampling plot as a random term. Linear model assumptions here and in further models were checked visually using plot diagnostics in the package stats.

Results

More related plants have phylogenetically more similar AM fungal communities

We calculated mean pairwise phylogenetic distances between AM fungal communities of all root pieces, and pairwise genetic distances between the host plants we identified from the same root samples. Host plant relatedness had a small, but significantly positive effect (p=0.0022, Multiple R-squared: 6.813e-05, Adjusted R-squared: 6.082e-05) on inter-community mean pairwise phylogenetic distance of AM fungi, i.e. more related plant species did have phylogenetically more similar AM fungal communities.

The distribution of AM fungi among root communities is nonrandom with respect to phylogeny

SES-MPD values were negative in almost all (99.4%) root communities (on average: -2.29 ±0.87), indicating that the communities were more phylogenetically clustered than expected by chance. Three communities consisted of only one taxon, in which therefore SES-MPD could not be calculated.

Host plant traits contribute to the phylogenetic structure of root AMF communities

a. root traits

In the random forest analysis, specific root volume was the most important root trait variable (Figure 5.1), that showed marginal significance (p = 0.06, p > 0.1 for the others) with a negative effect on the magnitude of clustering (Figure 5.2a). As root trait variables were not available for all entries of the entire data table used in the random forest analysis (ca. 30% of missing values,

Table S5.2), we conducted separate linear model and correlation analyses for the root traits to complement a potential reduction in the power of the tests in the random forest algorithm.

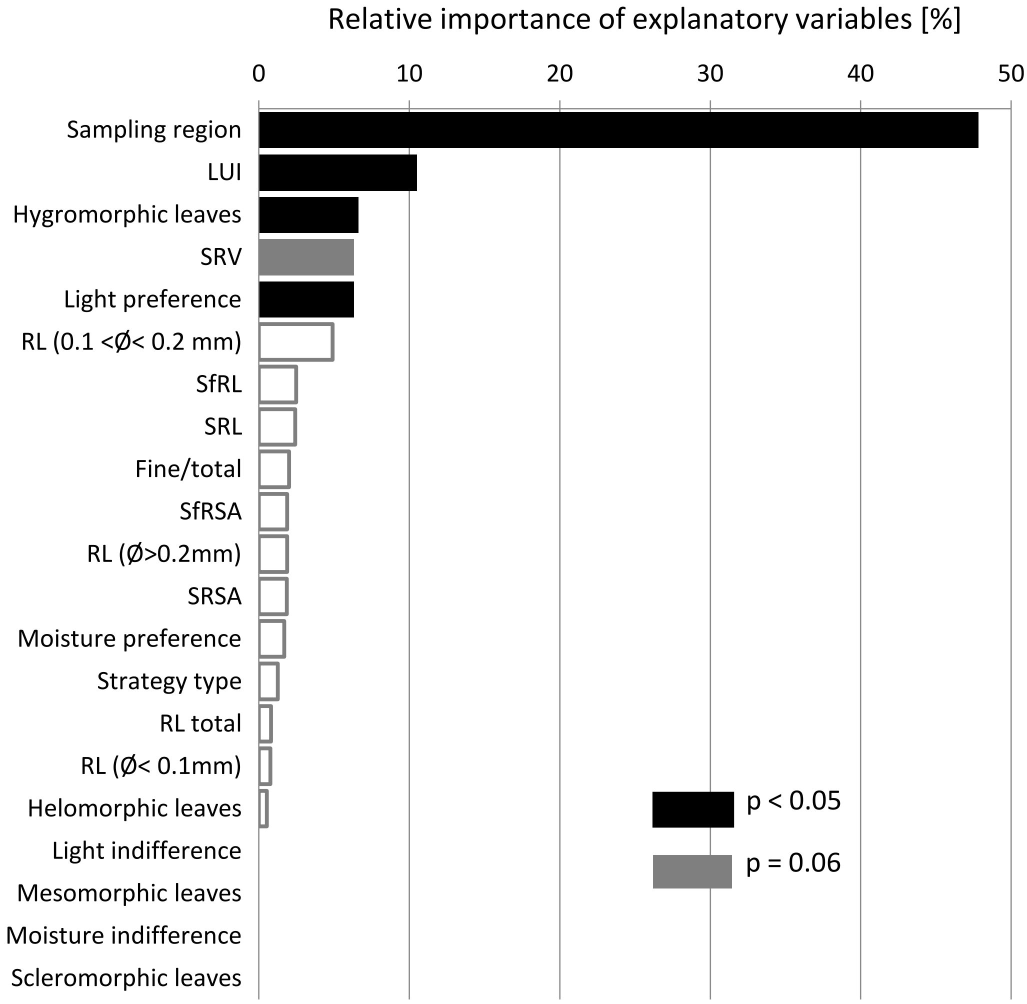

Figure 5.1 Relative importance of root traits, leaf traits, ecological plant traits, and land use intensity on the phylogenetic clustering of AM fungal communities in root samples in the random forest model (i.e. relative contribution to model accuracy; $R^2 = 0.21$). LUI: Land use intensity index, SRV: Specific root volume, RL: absolute root length, Ø: root diameter, S(f)RL: Specific (fine) root length, Fine/total: Ratio of the absolute length of fine roots S(f)RSA: Specific (fine) root surface area. Note that SRV (p=0.06) contained ca. 30% of missing values, which causes a reduction in the power of the test.

Root traits were strongly correlated with each other (Pearson’s r between 0.92 and 0.99), except for specific root volume, which correlated weakly with the rest of the root variables (Pearson’s r between 0.38 and 0.67). All specific root variables had a small, significantly negative effect on the magnitude of clustering (positive on the value SES-MPD). In the linear models, the higher the specific length, area or volume of the root, the less phylogenetically clustered were the AM fungal communities in it (Table 5.1). In accordance with the results of the random forest analysis for specific root volume, its predictive power was one order of magnitude higher compared to the other root trait variables, emphasizing its importance.

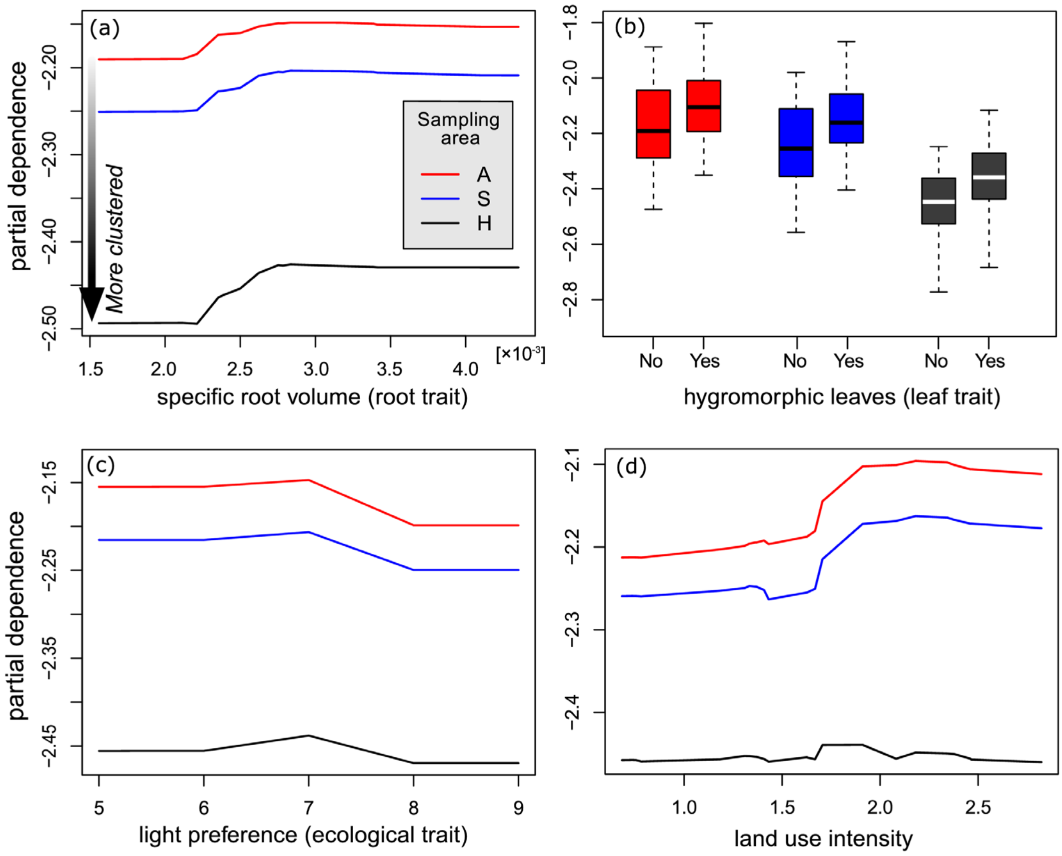

Figure 5.2 Partial dependence plots for the modeled relationships between the magnitude of phylogenetic clustering of AM fungal communities in roots (SES-MPD) and the statistically significant explanatory variables in the random forest model. Different lines show interactions with sampling area (A: Schwäbische Alb, S: Schorfheide-Chorin, H: Hainich-Dün).

Table 5.1 Linear model parameters of the effect of root traits on the magnitude of phylogenetic clustering (SES-MPD) of AM fungal communities in roots. Bold letters signify significant effect ($p < 0.05$).

	P	Multiple R^2
Specific root length	**0.0155**	0.0154
Specific fine root length	**0.0211**	0.0140
Specific root surface area	**0.0275**	0.0128
Specific fine root surface area	**0.0347**	0.0117
Specific root volume	**0.0229**	0.1125

b. Leaf morphological and ecological traits

The presence of hygromorphic leaves and light preference also revealed statistical significance ($p = 0.01$ and 0.04, Figure 5.1) using random forest analysis. AM fungal communities were more clustered in plants that had hygromorphic leaves (Figure 5.2b). Plants with higher light preference scores had more clustered AM fungal communities as well (Figure 5.2c).

Root AM fungal communities become slightly less clustered with increasing land use intensity, depending on sampling region.

Although neither land use intensity nor its interaction with sampling area had a significant linear effect on the magnitude of clustering in a linear mixed effect model ($p = 0.259$ and 0.3736, respectively), random forest analysis revealed their effects as nonlinear patterns with thresholds ($p = 0.01$; Figure 5.1). Moreover, the patterns differed between the sampling areas ($p < 0.01$) (Figure 5.2d). AM fungal phylogenetic clusters start to dissolve at medium land use intensity in two of the sampling regions (increase in partial dependence around LUI=1.5; Figure 5.2d), while the strongest clustered pattern found in Hainich was not influenced by land use intensity. These significant differences between the sampling areas can be explained by several factors, including geology and climate (Table S5.1).

Discussion

In our analyses of a yet unprecedented dataset of almost 500 AM fungal root communities from 150 sampling sites, we found clear effects of all hypothesized drivers on the phylogenetic structure of AM fungal communities.

Our results show that more closely related plants had phylogenetically more similar AM fungal communities, but the phylogeny of the plants explained only a small part of the variation. It was previously demonstrated that even though different plant species associate with different AM fungal OTUs from the same soil (Gosling *et al.*, 2013), plant relatedness does not result in more similar AM fungal communities in terms of composition on the level of OTUs (Veresoglou & Rillig, 2014). Taking this together with our results, we can conclude that the traits of the relevant to partner association are not coded on OTU level, but instead closely related plant species tend to associate with AM fungi from certain higher level clades. This is congruent with the conservation of functional traits and host benefits of AM fungi in greenhouse experiments (Powell *et al.*, 2009), which showed that much of the variation of these traits was associated with nodes representing early divergences within the Glomermycotina.

Root AM fungal communities are phylogenetically clustered

We also showed that AM fungal phylogenetic structure was significantly different from random, specifically, it was consistently phylogenetically clustered. Given conserved traits (which was shown for spatial niche use of AM fungi by Maherali & Klironomos (2007)), phylogenetic clustering is understood as a sign of habitat filtering influencing community assembly more than local competitive exclusion (Webb *et al.*, 2002). As we examined communities from within the root, this "habitat" filtering effect can be attributed to both the host plant and its environment.

We explore these effects further in the following paragraphs.

Root traits are important determinants of AM fungal community structure

Out of all host traits that potentially contribute to the interaction between plant and symbionts, root traits influence mycorrhizae most directly. Morphological root traits appeared to be multidimensional. While specific root length and surface area were strongly correlated for both the whole root system and fine roots, specific root volume was less correlated with them. Specific root volume explained the most variation in the phylogenetic structure of the fungi: the higher the specific volume of the root, the less phylogenetically clustered were the AM communities. Specific root volume is expressed as the ratio of volume and dry mass, and it is also considered to be an important predictor of plant strategies in terms of investment and longevity (Roumet *et al.*, 2006).

High specific root volume can be attributed to less dense tissue, e.g. because of higher volume of air channels in the cortex, the habitat of AM fungi in the plant (Brundrett, 2002). Growth rate of AM fungi within roots is reported to be faster in plant species with air channels in the cortex than in species where hyphae spread by intracellular growth (Brundrett & Kendrick, 1990). Faster AM fungal growth could have led to less clustering through increased competition. In contrast, plants with lower specific root volume are on the resource conservative end of the spectrum, and can be characterized by a root system with few fine roots (Roumet *et al.*, 2006). These plant species might have to recruit specific AM fungal communities to ensure sufficient water and nutrient input (Kong *et al.*, 2014). The more clustered structure of the AM fungal communities we have shown in these plants might signal this.

Effect of land use in dissolving phylogenetic clusters is not drastic on root communities and is site dependent

Land use intensity was the most important variable after sampling region in defining phylogenetic structure of AM fungal communities. More intensive land use (mowing, grazing and fertilization) was correlated with less clustering in accordance with hypothesis 2 in two of the three sampling regions. This is in line with previous research that has shown that elements of land use intensity, for example high level fertilizer treatments can shift AM fungal phylogenetic structure even to overdispersion in soil (Liu *et al.*, 2015). But even though the phylogenetic structure of AM fungal communities in the root did exhibit a modest shift in the same direction, it just became less clustered, not overdispersed. In AM fungi, Horn *et al.*, (2014) and Saks *et al.*, (2014) found phylogenetic clustering in roots when studying natural habitats, but clustered or random structure in soils.

This difference can be explained by host effects which interact with land use intensity (Vályi *et al.*, 2015) in shaping AM fungal communities.

Intrinsic host traits and preferences towards abiotic environmental conditions can influence symbiont communities through direct filtering or influencing competition

The host filter and the habitat filter are not independent: for example, the plant's resource exchange with their symbionts varies not only according to plant identity but with environmental conditions as well (Bever, 2015; Walder & van der Heijden, 2015), suggesting a complex system of interactions with the symbiont community. Plants with different intrinsic and extrinsic (ecological) traits respond differently to environmental conditions. This is a possible explanation for why plants with different light preferences had different AM fungal phylogenetic structures (Figure 5.2c). Plants with hygromorphic leaves and plants associated with shade habitats had less clustered AM communities. It was experimentally shown that shade decreased the ability of plants to selectively reward beneficial AM fungi due to decreased carbon allocation to roots (Zheng *et al.*, 2015). Plants of shade that have hygromorhic leaves and prefer more humid soil, have less extensive root system (Klotz *et al.*, 2002). The lack of possibility of spatial separation for symbionts in root systems with a simple spatial structure also decreases preferential allocation of carbon (Bever *et al.*, 2009). Decreased preferential allocation, the resulting less host filtering and stronger competition between co-occurring AM fungal species. This scenario can explain the decrease in clustering we have shown in our study.

As another example of the interaction of the host and environmental filter, increasing soil fertility was shown to result in less nutrient allocation to roots and thus to AM fungi (Liu *et al.*, 2012). Less clustering (in this study) or outright overdispersion (Liu *et al.*, 2014) with increasing land use intensity could signify not only a direct environmental effect, but also an interaction between host and environment: plants not investing in filtering AM fungal species due to sufficient nutrient input.

Taking these results together, it emerges that if the nutrient allocation from host to AM fungi decreases, because of outside environmental conditions, it leads to increased competition and less clustering. In soil communities outside the plant this can even lead to overdispersion (Figure 5.3).

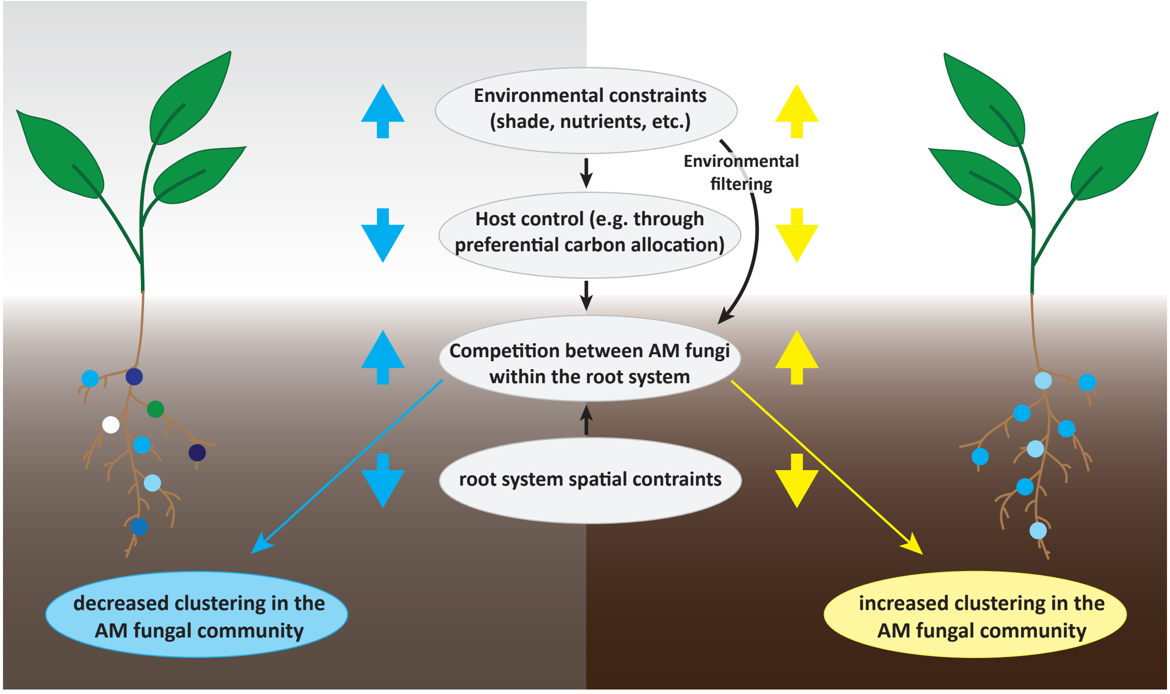

Figure 5.3 Proposed framework of factors influencing AM fungal phylogenetic community structure in roots. The host, its symbiotic community and their abiotic environment constitute a heterarchically structured system of control, in which each element might influence other elements. This figure shows two possible hypothetical scenarios. In the left side of the figure, the control of the host plant on its symbionts via preferential carbon allocation is hampered by suboptimal environmental conditions (in the figure we used shade as an example) relative to its ecological preferences. Diminished host control results in higher competition among AM fungi in the root system. Because of AM fungal traits relevant to spatial niche use are conserved, higher competition results in less phylogenetic clustering. Spatial constrains in the root system also influence competition among co-colonizing AM fungi. For example, growth rate within roots with more air channels (less constrained, left side of the figure) is reported to be faster, potentially leading to increased competition, and thus less clustering. In contrast, environmental constraints relevant to the symbionts themselves (such as low nutrient concentration in the soil) decrease competition and result in more clustering (right side of the figure).

Research on other obligately symbiotic organism groups that form multispecies communities frequently explains symbiont community structure by host phylogeny and traits that have either direct effects or influence community structure through influencing competition between co-colonizing symbionts. For example, host phylogeny explained the structure of microbial communities of marine sponges: although the identity of specific microbial OTUs varied substantially among the hosts, more closely related sponge species tended to harbor microbial communities with more similar relative abundance and dominance structure (Easson & Thacker, 2014). In a variety of parasites, clustering was explained by host mediated effects: facilitation mediated by immunosuppression (protozoa, helminths, bacteria, viruses: Cox 2001; fleas: Krasnov *et al.* 2006). It was also shown that developmental and life history differences between different symbiont organism groups might cause subtle differences in the relative importance of the host trait-symbiont interaction. Mites, as opposed to fleas had a tighter association between host traits and parasite diversity, probably because of the dependence of both imago and preimaginal stages on the host body (Korallo *et al.*, 2007). Spatial structure and historical events might be more important in defining the phylogenetic structure of symbionts of mobile hosts than those of sessile ones (Krasnov *et al.*, 2013), or when examining patterns at a global scale (Kivlin *et al.*, 2011).

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Chapter 6

Root traits are more than analogues of leaf traits: the case for diaspore mass

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Chapter 7: General discussion

The aim of the present thesis was to better integrate root morphological traits - as being strongly underrepresented in trait based ecology today (Bardgett *et al.*, 2014; Laliberté, 2016) - into ecological research with a special focus on abiotic and biotic plant soil interactions. Chapter 2 discussed root traits as possible drivers of biotic plant-soil interactions under different land-use intensity. Chapter 3 displayed the importance of root traits for soil aggregation while chapter 4 evaluated the mediating power of root fineness for abiotic and biotic plant-soil interactions. Chapter 5 assessed the impact of root traits on arbuscular mycorrhizal community structure. Finally, chapter 6 displayed the general predictive power of root traits for trait based ecology and highlighted the multidimensionality of the root economics spectrum. The following discussion will evaluate the results of all chapters in the light of the general aim of this thesis and combine them to draw additional conclusions on top of the single studies.

The effect of land-use intensity on plant-soil interactions mediated by root traits

Land-use in semi-natural grasslands mainly comprises fertilization, mowing and grazing (Blüthgen *et al.*, 2012). The interplay of these land-use factors is known to affect both abiotic and biotic soil conditions with a continuative effect on plant biodiversity (Klimek *et al.*, 2007; Socher *et al.*, 2012, 2013). Yet, knowledge about underlying mechanisms is still lacking in many cases. Chapter 2 aimed to identify quantitative patterns in plant community composition under low and high land-use by studying plant-soil interactions of rare and abundant species. We could confirm our hypothesis that potentially strong competitors were held in check by negative plant-soil interactions restricting them from outcompeting smaller species. Furthermore, vegetation records revealed that increasing land-use intensity had a strong negative effect on forb species richness overall. The explanation we found by our experimental approach was that forbs experienced their strongest negative soil feedbacks on soil from high land use plots, while grasses experienced less negative or even positive effects on these soils. Searching for possible mechanisms underlying the observed patterns, post-analysis revealed a significant lower SRL for forbs compared to grasses ($p<0.001$) in the species set of the experiment. It is known from previous studies that soil aggregation increases with successional stage (Erktan *et al.*, 2015) but also decreases with increasing land use intensity (Barto *et al.*, 2010) – a fact that we could validate in the soils of our experiment in chapter 2. A core finding of chapter 4 was the fact that on low aggregated soil, PSF is positively correlated with SRL. Thus, the varying effects of land-use intensity on the plant-soil interactions of the two functional groups in chapter 2 could to some extent be mediated by differences in root fineness. Following this argumentation, grasses experienced more positive, and forbs more negative soil effects from the low aggregated soil of high land-use, because of their differences in root fineness.

Hence, a common ecological finding of chapters 2 and 4 is that plants with a low SRL (including most forbs) are most limited under high land-use intensity or on field sites of early successional stages because of the lower soil aggregation status. Little is known about differences in plant community mean root traits along land-use intensity gradients. Prieto *et al.* (2015) found a shift from resource conservative to resource acquisitive root traits with increasing land-use. They discussed the results in relation to nutrient differences in the soil ignoring soil structure or soil biota as possible mediators of the observed pattern. Our results are therefore the first hint towards a close relationship of root fineness and soil structure leading to varying plant-soil interactions as a function of land-use. In chapter 6, we collected morphological root traits including different proxies of root fineness. Bringing together those data and the vegetation records from the Biodiversity Exploratories could offer several future research opportunities. Based on our findings from chapter 2, 3 and 4, it would be a promising future research question whether community weighted means of root traits related to root fineness change with soil aggregation mediated by land-use. Furthermore, to close the loop - it would be interesting whether soil aggregation changes with those community weighted means or either with the complementarity of traits in the community. Answering these questions could help to design seed mixes for optimal enhancement of soil aggregation in future restoration programs after intense land-use or to choose cover crops for agricultural fields to reduce erosion risks.

The reciprocal effects of soil structure and root traits

As chapter 3 strongly emphasizes, root traits - as well as fungal traits - are affected by soil aggregation and do affect formation and stabilization of aggregates at the same time. All interactions between biota and soil structure therefore must be regarded as a feedback loop when trying to implement mechanistic findings into the field ecological context.

As described above, chapter 2 raised the question if varying plant-soil interactions under different land-use intensity could be caused by changes in soil aggregation status. To further study this hypothesis, we carried out the first experimental study to integrate the reciprocal effects of soil structure and root traits into the PSF concept (chapter 4). We aimed to disentangle the direct abiotic soil effects from the indirect soil biotic effects caused by training on soil of different structure. SRL was thereby studied as the mediator of plant specific differences in the formation of soil aggregates (as proposed in chapter 3), the training of the soil microbial community as well as the experience of soil feedback. We were able to show that plant species differences in SRL mediated the accumulation of soil microbiota as a function of soil structure affecting the outcome of PSF. The most important concept underlying the interpretation of the study was that surface maximization for nutrient and water uptake can be achieved by investment either in fine roots or in mycorrhizal symbiosis (Peat & Fitter, 1993; Wilson & Hartnett, 1998; Comas *et al.*, 2014; Koziol *et al.*, 2015).

These strategies vary between grasses and forbs, but also along a continuum of SRL in the study. Therefore, all observed effects can be argued to be caused either by root morphology or plant functional type (grasses vs. forbs). Nevertheless, the stepwise design of the study disentangling single factors of the complex feedback loop gave mechanistic insights, leading to the conclusion that SRL was the driver of the observed effects. Cortois *et al.* (2016) also found SRL to contribute significantly to explaining PSF in addition to the plant functional type. Koziol *et al.* (2015) found mycorrhizal responsiveness to be negatively correlated with the number of root tips as a proxy for fine root branching intensity. In our study, plants with lower SRL and higher AMF colonization had a positive effect on soil aggregation, congruent with our suggestions in chapter 3 and the ability of AMF to form and stabilize soil aggregates (Rillig & Mummey, 2006). On disintegrated soil, plants with lower SRL had to invest more biomass to the belowground parts to be able to penetrate the small pored soil and to offer roots to get colonized by AMF. At the same time, they also exposed more roots to fungal pathogens leading to a higher accumulation of antagonists and hence a more negative feedback outcome. This core finding led to the conclusion that on disintegrated soils of high land-use intensity or early successional stages, species with lower SRL could be particularly strongly limited by PSF supporting the results of Koziol *et al.* (2015). As argued above, this forms a coherent concept with the results of chapter 2, where forbs experienced the most negative feedback on high land-use soils. In the light of global change, future restoration of abandoned areas or regeneration of high land-use regions could profit from considering these findings. Plants with high SRL would have a better chance to establish over several generations on disintegrated soils. Although the new aggregation process in the soil would be comparably slow because of low amount of AM fungal hyphae, this approach could help to establish self regenerating systems that follow natural succession.

The effect of root traits on AMF community structure

Soil microbial taxa involved in plant-soil interactions are numerous. AMF are probably the best studied group because of their known fundamental effects on plant fitness (Smith & Read, 2008) and also trait expression (Friesen *et al.*, 2011). The arbuscular mycorrhizal symbiosis involves more than two partners as AMF form multispecies communities within plant roots. As the roots are both their symbiotic partner and their habitat, it is very likely that root traits might not just be mediated by AM colonization but reciprocally mediate AMF community structure in addition to soil environmental factors. In chapter 5, we found the phylogenetic clustering of AMF communities to be influenced by root traits, especially the specific root volume (SRV). AMF communities in roots with high SRV and hence a low root tissue density were less clustered. We conceptualize that a root with higher SRV and more air channels would resemble an open habitat with more competition between the AMF species, leading to a decrease in phylogenetic clustering within the community. Among the root traits, SRV was the only trait directly depicting tissue density as it is its mathematical inverse. Theoretically, fineness and tissue density both influence SRL and SRSA. In our study the weak correlation of those with SRV - in accordance with the meta analysis of Weemstra *et al.* (2016) - revealed tissue density to not have had the major impact on variation in SRL and SRSA. Therefore, those traits can be regarded mainly as proxies of root fineness. Coherently, root fineness did not affect AMF community structure in chapter 5, but root tissue density - depicted as SRV - did. Apart from answering questions about root trait effects on AMF community structure, chapter 5 therefore represents a good example of the multidimensionality of root traits (Kramer-Walter *et al.*, 2016). Like proposed by Weemstra *et al.* (2016), it reveals that certain root trait patterns are related to the root system's interaction with AMF independent of the one-dimensional plant economics spectrum. This strengthens the argumentation that root traits are more than just analogues of leaf traits, and in fact they hold additional information, especially in the soil ecological context, as pointed out throughout this thesis.

The unique predictive power of root traits

Chapters 2-5 all underline the fact that root traits are underrepresented despite being informative for trait based ecology. Additionally, Figure 1 shows that especially morphological root traits are poorly covered. To address this need of data and to evaluate the overall importance of root traits in explaining whole plant ecological patterns, we collected data of 141 grassland species and carried out analyses with a machine learning approach including aboveground, belowground and whole plant as well as ecological and life history traits as presented in chapter 6. We found a notable portion of our root traits not to be explained by the wide set of aboveground traits including those assumed to reflect a whole plant economics spectrum. This strongly suggests that roots hold unique information not predictable by aboveground traits. Belowground traits depicting root fineness were explained by a different set of predictors than those depicting root tissue density. In line with the results of chapter 5 and Weemstra *et al.* (2016) this strongly supports the multidimensional nature of root traits. Root tissue density was mainly predicted by leaf dry matter content as expected under the assumption of a fast-slow economics spectrum (Reich, 2014). Against all expectations, we found the basic assumptions of a whole plant economics spectrum (Freschet *et al.*, 2010; Mommer & Weemstra, 2012) in terms of the close relationship between above and belowground surface maximization not to be among the most important predictors of variation in root fineness. Instead of that, diaspore weight was the most important predictor of root fineness, leading to the concept illustrated in figure 6.4: Seedlings of plants with heavier diaspores, that fall down close to the mother plant come into contact with maternally trained soil biota. This favors the development of coarser roots that are less susceptible to pathogens (Newsham *et al.*, 1995a) and profit more from AMF (Comas *et al.*, 2014). This concept is a strong example of the close interaction between soil microbes and roots leading to a pattern in root trait variability that holds information about soil ecological and even whole plant ecological relationships that are not depicted in leaf traits. This is strong evidence for the necessity of root traits to be measured rather than just assumed by their proposed correlation to aboveground traits. Furthermore, the main finding of chapter 6 confirms the statement of Laliberté (2017) that mycorrhiza should get integrated when working with root traits. This is because they are strongly related to and have a known mediatory power for root trait expression (Friesen *et al.*, 2011). This integration could be done by including data on mycorrhizal colonization or dependency, but also by integrating mycorrhizal fungal traits like proposed in chapter 3. This would be an important step towards the aim to make trait based approaches more mechanistic.

The underestimated complexity of continuous root fineness

One of the most frequently considered characteristics of root systems in general trait based ecology, as well as in this thesis, is their fineness, mainly depicted by specific root length, average root diameter or branching intensity. A general assumption underlying most discussions about root fineness is that grasses have finer roots than forbs. This was also true for the small species sets of chapter 2 and 4 of this thesis. Searching the literature, it turns out that this assumption is generally based on studies with very restricted species sets (Siebenkäs *et al.*, 2015) if any. When scientists use this general assumption to explain specific patterns in their experiments without own measurements, most authors cite a study from Craine *et al.* (2001) where root and leaf traits of 76 grassland species in Minnesota USA were measured. Taking a closer look into this study, it first appears that “grasses” were divided into C3 and C4 grasses, and that differences between the functional groups were not analyzed for single traits, but PCA axes. C3 grasses and forbs significantly differed along one PCA axis that mostly accounted for differences in SRL and root diameter, with forbs having coarser roots overall. C4 grasses did not differ significantly from forbs, and the overlap of all groups was strong. The authors concluded that plant functional groups should be used carefully as most traits actually represent a continuum. Interestingly, this study was cited both as a proof (e.g. Siebenkäs *et al.*, 2015) and as a disproof (e.g. Tjoelker *et al.*, 2005) for differences in root fineness between grasses and forbs, revealing a strong uncertainty of the trait ecological community in that context.

To address this uncertainty, I can test the assumption that grasses and forbs differ in their root fineness by using the large root trait dataset from chapter 6. It reveals a slightly higher median SRL and lower average diameter for grasses, but no significant difference between the two plant functional groups (Figure 7a/c). Although this is the largest existing root trait dataset measured under common conditions so far, it seems not large enough to detect a significant difference in proxies of root fineness between grasses and forbs. Consulting the newly launched fine-root

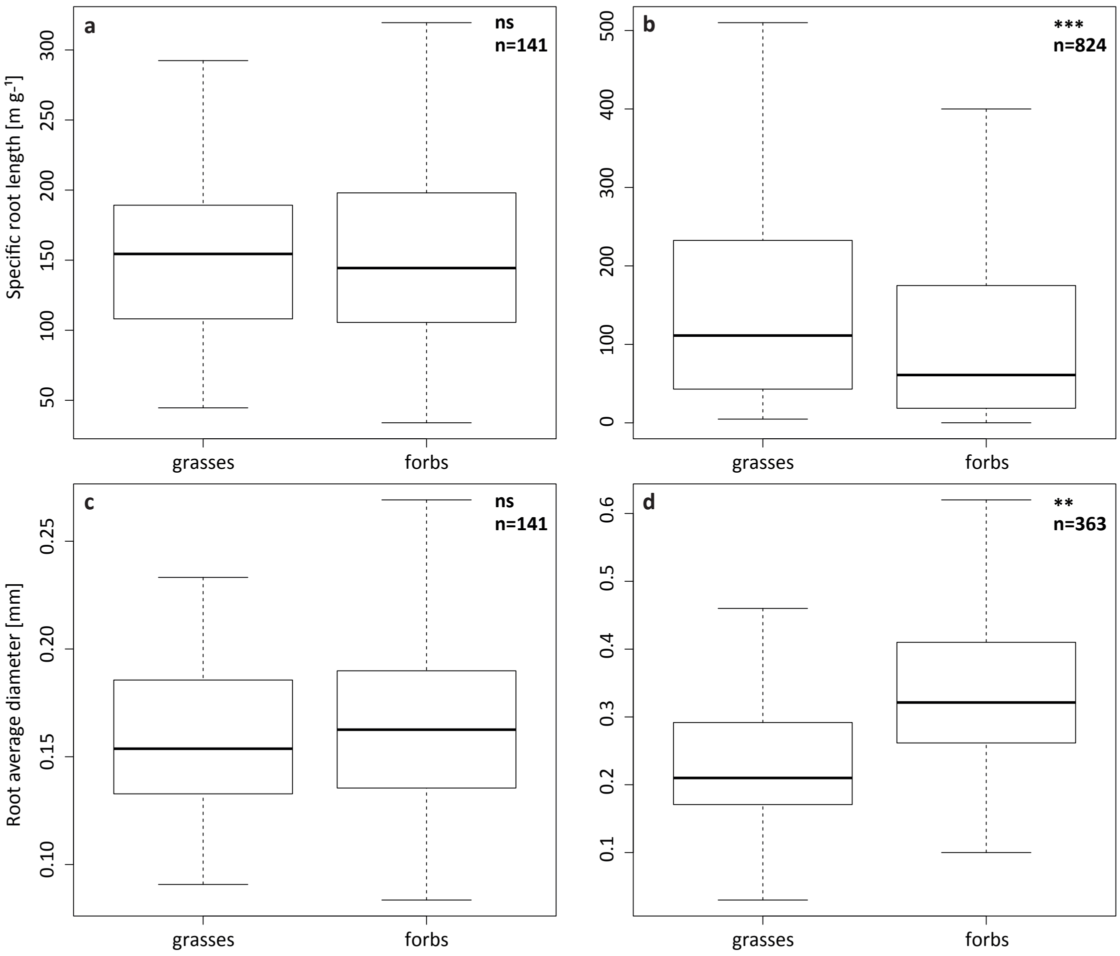

Figure 7 Comparison of different proxies of root fineness between the two functional groups "grasses" and "forbs" on the basis of a/c) the root trait dataset from chapter 6 and b/d) combined data of different datasets extracted from the fine-root ecological database (FRED). FRED 1.0 was searched for entries of "specific root length" or "root average diameter" respectively for the two plant growth forms "herb" and "graminoid" including data from field as well as pot experiments. Displayed are the median, quartiles and minimum and maximum values excluding outliers. ns, not significant; **, $P < 0.01$; ***, $P < 0.001$.

ecological database (FRED) (Iversen *et al.*, 2017) which combines more than 800 datasets on root traits, offers the opportunity to test for a difference in root fineness between grasses and forbs on a much larger dataset. A Student`s T-test reveals significant differences between the two functional groups in FRED with grasses having a higher SRL or lower root diameter and hence a finer root system (Figure 7b/d). Interestingly, the two functional groups have strongly overlapping ranges of values in both datasets. It is therefore striking that a large sample size is needed to detect significant differences. On the one hand, this finding does support the assumption that grasses have finer roots than forbs in general. On the other hand, it shows that there is no distinct difference between the two functional groups. It remains to be tested, if within a local community the need for niche differentiation causes more distinct differences in root fineness between the plant functional groups. Nevertheless, the general assumption of grasses having finer roots than forbs should be handled carefully for smaller species sets. When using root fineness as a mechanistic explanation of observed patterns in a study, the actual root traits of the corresponding species should be considered rather than assuming differences based on plant functional groups. Preferably, root traits should be handled as continuous variables like we did in chapter 4, 5 and 6 rather than factorial discriminators between groups as additionally done in chapter 4.

Conclusion

The main focus of this thesis was to evaluate the general importance of roots for trait based ecology by integrating them into the framework of plant soil interactions. I found root traits - especially those predicting root fineness (SRL and RAD) - to explain patterns in plant-soil feedback as well as soil aggregation and root tissue density to mediate AMF community structure. I highlighted several future research areas that could be studied using morphological root traits. Especially land-use intensity and succession seem to strongly influence plant soil-interactions mediated by root traits with an effect on plant community composition. Furthermore, I showed that root fineness is not predictable by SLA as assumed under the hypothesis of a plant economics spectrum (Freschet *et al.*, 2010; Reich, 2014). Instead, I propose a concept explaining patterns of root fineness by diaspore weight by integrating the maternal training of the soil microbial community into the picture (Figure 6.4). These findings all highlight the importance of roots for trait based ecology due to their mediation of plant-soil interactions. Furthermore, this thesis strongly emphasizes that belowground traits are more than analogues of aboveground traits and that general assumptions on their correlations should be handled carefully. It highlights the explanatory power of root traits not only in answering soil ecological questions, but also for detecting whole plant ecological patterns and indicates a strong need for future root trait measurements given the lack of data in databases. My dataset on 141 species - being the largest today - is an important step in that direction. Further data collection on different species as well as different traits and their plasticity including especially those that relate to plant-soil interactions, is strongly needed and will help to further integrate roots as powerful traits for plant ecology.

Summary

Studies examining patterns of biodiversity, community composition and ecosystem functioning are increasingly being carried out on the basis of plant functional traits today. Until recently, the focus has been on aboveground plant parts - mainly leaf traits. Instead, belowground traits are strongly underrepresented both in databases and studies and often assumed to be analogues of leaf traits within a whole plant economics spectrum. However, increasing evidence suggests that root traits might be decoupled from leaf traits under certain aspects and that they are multidimensional. Particularly they are hypothesized to be informative for explaining mechanisms of plant-soil interactions because of the close association of roots with the soil biotic and abiotic conditions. This thesis studies the importance of root traits for trait based ecology by assessing their impact on different plant-soil interactions affecting grassland plant biodiversity.

In a greenhouse experiment on plant-soil interactions we found that forbs, which had a lower specific root length (SRL) suffered most from soil of high land-use intensity resulting in lower field abundance compared to grasses (chapter 2). The soil of high land-use intensity had smaller aggregates, comparable to soil of early successional stages. This abiotic soil characteristic and its interaction with root traits was also studied in a plant-soil feedback (PSF) experiment (chapter 4). In this study we could show that plants with low SRL experienced the strongest negative PSF on soil with smaller aggregates because of the disadvantage in exploring the soil with smaller pores. Conclusively, in early successional stages, plants with low SRL are most limited by soil biota leading to lower abundances in the field. As soil and roots reciprocally interact, the effects of plant root and mycorrhizal fungal traits on the process of soil aggregation are discussed in chapter 3 also highlighting the close association of roots and arbuscular mycorrhizal fungi (AMF). In chapter 5 we further show that the specific root volume mediates AMF community structure while not being correlated with proxies of root fineness. This underlines the hypothesis that root traits are multidimensional because of their close association with the soil environment. Following our own advice, we collected the largest known root trait dataset on 141 grassland species and assessed their general predictability by aboveground morphological, ecological and life history traits with a random forest analysis (chapter 6). We found a notable portion of unexplained variation in root traits supporting the hypothesis that they hold unique information. Root tissue density decoupled from root fineness which was not predicted by the specific leaf area as generally assumed. Instead we propose a new concept explaining root fineness by seed mass via soil microbial effects. Furthermore, I highlight the complexity of interspecific variability in root fineness and the importance of integrating species specific traits rather than categorical discriminators between plant functional groups for mechanistic implementations.

This thesis offers insights into several biotic and abiotic plant-soil interactions showing that

each of them is strongly mediated by root traits. We could show that root traits are multidimensional and more than analogues of leaf traits holding unique information for biodiversity and community ecology due to their effects in plant-soil interactions.

Zusammenfassung

Heutzutage werden Biodiversität, Lebensgemeinschaften und Ökosystemfunktionen zunehmend mithilfe funktioneller Merkmale („traits“) untersucht. Dabei lag der Fokus bislang auf oberirdischen Pflanzenorganen - vor allem den Blättern. Im Gegensatz dazu sind die unterirdischen traits deutlich unterrepräsentiert und werden meist als analog zu den Blatttraits betrachtet, obwohl sie scheinbar multidimensionaler und unter bestimmten Bedingungen von Blatttraits unabhängig sein können. Durch die direkte Interaktion der Wurzel mit den biotischen und abiotischen Bodenfaktoren könnten sie relevant für die Forschung an Pflanze-Boden-Interaktionen sein. Diese Dissertation untersucht die ökologische Relevanz von Wurzeltraits indem sie deren Bedeutung in unterschiedlichen Pflanze-Boden-Interaktionen und die resultierenden Effekte auf die Biodiversität untersucht.

In einem Gewächshausversuch wuchsen im Vergleich zu Gräsern, die dikotylen Krautigen mit ihrer kleineren spezifischen Wurzellänge (SRL) schlechter auf Böden aus hoher Landnutzung mit negativer Auswirkung auf ihre Abundanz (Kapitel 2). Böden aus starker Landnutzung zeigten kleinere Aggregate, ähnlich denen früher Sukzessionstadien. Diese abiotische Bodeneigenschaft wurde in einem „plant-soil-feedback“ (PSF) Experiment in Hinsicht auf Wurzeltraits untersucht (Kapitel 4). Wir konnten zeigen, dass Pflanzen mit kleiner SRL ein stärkeres negatives PSF auf Boden mit kleinen Aggregaten erfahren, da sie die kleineren Bodenporen schwerer durchwurzeln können. In frühen Sukzessionstadien werden Pflanzen mit niedriger SRL stärker durch Bodenbiota limitiert und sind deshalb weniger abundant. Kapitel 3 diskutiert die Effekte von Wurzel- und Mykorrhizatraits auf Bodenaggregation, da es sich hierbei um eine reziproke Interaktion handelt. In Kapitel 5 zeigen wir, dass das spezifische Wurzelvolumen, nicht aber die Wurzelfeinheit, Auswirkungen auf die Struktur der Lebensgemeinschaft der arbuskulären Mykorrhizapilze hat. All dies unterstreicht die Hypothese, dass Wurzeltraits aufgrund ihrer engen Assoziation mit dem Boden multidimensional sind.

In Kapitel 6 präsentieren wir mit 141 Grünlandarten den größten bekannten Datensatz zu Wurzeltraits und untersuchen ihre Vorhersagbarkeit durch oberirdische morphologische, ökologische und lebenszyklische traits. Ein nennenswerter Anteil der Variabilität in den Wurzeltraits blieb unerklärt, was die Hypothese unterstützt, dass hier einzigartige Informationen verborgen liegen. Die Wurzelgewebedichte zeigte sich unabhängig von der Wurzelfeinheit, welche nicht durch die spezifische Blattfläche erklärt wurde. Stattdessen konnten wir die Wurzelfeinheit über bodenbiotische Effekte und das Diasporengewicht erklären. Die Komplexität der interspezifischen Variabilität von Wurzeltraits wird herausgearbeitet, ebenso wie die Notwendigkeit der Verwendung artspezifischer traits für mechanistische Fragestellungen im Gegensatz zur kategoriellen Unterscheidung funktioneller Gruppen.

Diese Dissertationsschrift untersucht diverse biotische und abiotische Pflanze-Boden-Interaktionen und zeigt, dass sie alle durch Wurzeltraits beeinflusst werden. Wir konnten belegen, dass Wurzeltraits multidimensional und mehr als nur Analoge zu Blatttraits sind. Vielmehr beinhalten sie durch ihre Bedeutung in Pflanze-Boden-Interaktionen einzigartige Informationen für die Biodiversitäts- und Lebensgemeinschaftsökologie.

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Contribution to publications

Heinze J, **Bergmann J,** Rillig MC, Joshi J. 2015. Negative biotic soil-effects enhance biodiversity by restricting potentially dominant plant species in grasslands. *Perspectives in Plant Ecology, Evolution and Systematics* 17: 227–235.

> I participated in the harvest of the greenhouse experiment, conducted soil structural analyses (mean weight diameter and water stable aggregates) and took part in writing the manuscript.

Rillig MC, Aguilar-Trigueros CA, **Bergmann J,** Verbruggen E, Veresoglou SD, Lehmann A. 2015. Plant root and mycorrhizal fungal traits for understanding soil aggregation. *New Phytologist* 205: 1385–1388.

> I took part in developing the conceptual framework especially for the root traits, wrote the part of the manuscript about trait variability and plasticity and reviewed the whole manuscript.

Bergmann J, Verbruggen E, Heinze J, Xiang D, Chen B, Joshi J, Rillig CM. 2016. The interplay between soil structure, roots, and microbiota as a determinant of plant–soil feedback. *Ecology and Evolution* 6: 7633–7644.

> I conceived and performed the greenhouse experiment of the feedback phase together with EV and did the laboratory work including measurement of plant biomass, preparation of inoculum, root trait measurement and soil structural analysis. I designed and conducted the statistical analysis and wrote the manuscript.

Vályi K, **Bergmann J,** Ryo M, Prati D, Hempel S. The influence of host traits and environment on the phylogenetic structure of intraradical communities of arbuscular mycorrhizal fungi.

> I measured and calculated the root traits and took part in writing the manuscript.

Bergmann J, Ryo M, Prati D, Hempel S, Rillig MC. Roots traits are more than analogues of leaf traits: the case for diaspore mass. *New Phytologist (accepted)*

> Me and SH conceived the study. I measured the root traits and collected the data from databases. I designed and conducted the analysis together with MR, developed the presented concept and wrote the manuscript.

Curriculum vitae

For reasons of data protection, the curriculum vitae is not included in the online version.

Supporting information

Table S2.1 Geographical locations and information on land use of sites used for soil collection in this experiment. Land-use intensity is represented by LUI (land-use index; high = intensive; low = less intensive). Within each region two paired grassland sites differing in land-use intensity (high vs. low) were chosen. Explicit values for land-use intensity were given in brackets.

Region	Site	Livestock	LUI	Longitude	Latitude
Schwäbische Alb	Mown pasture	Cattle	High (2.51)	48°23'33.69''	9°22'33.69''
	Pasture	Sheep	Low (0.68)	48°25'18.65''	9°29'55.24''
Hainich - Dün	Mown pasture	Cattle	High (2.28)	51°13'11.41''	10°24'39.90''
	Pasture	Sheep	Low (0.51)	51°13'15.75''	10°22'0.82''
Schorfheide - Chorin	Mown pasture	Cattle	High (2.49)	52°58'49.13''	13°50'53.83''
	Pasture	Cattle	Low (0.98)	52°59'14.31''	13°49'39.18''

Table S2.2 Soil chemistry and aggregation values for the soil sites used for the investigation of biotic soil-effects. Data represent means ± SE. Land-use intensity is represented by the LUI (high = intensive; low = less intensive). The table is devided into two parts because of space restrictions.

Region	LUI	pH [a)]	Total Phosphorus [b)] (g P/kg dry soil)	Total Nitrogen [b)] (g N/kg dry soil)	Ammonium [b)] (mg NH_4^+/kg dry soil)
Schwäbische Alb	**high**	7.3	1.20 ± 0.101	7.65 ± 0.074	1.11 ± 0.185
	low	7.9	0.61 ± 0.006	3.66 ± 0.053	0.42 ± 0.047
Hainich-Dün	**high**	7.2	1.21 ± 0.046	5.24 ± 0.049	16.19 ± 0.484
	low	7.7	0.87 ± 0.018	1.94 ± 0.050	3.67 ± 0.144
Schorfheide-Chorin	**high**	6.7	0.57 ± 0.006	2.22 ± 0.091	0.75 ± 0.067
	low	5.3	0.32 ± 0.035	1.54 ± 0.046	7.14 ± 0.386

Region	LUI	Nitrate [b)] (mg NO_3^-/kg dry soil)	Nitrite [b)] (mg NO_2^-/kg dry soil)	WSA [c)] (%)	MWD [c)]
Schwäbische Alb	**high**	151.82 ± 3.303	0.93 ± 0.047	77.34 ± 2.66	1.69
	low	30.89 ± 1.651	0.45 ± 0.019	93.78 ± 2.97	1.78
Hainich-Dün	**high**	47.66 ± 2.162	0.27 ± 0.035	79.69 ± 4.44	1.78
	low	12.36 ± 0.624	0.25 ± 0.012	82.99 ± 5.57	2.24
Schorfheide-Chorin	**high**	71.50 ± 2.860	0.27 ± 0.017	61.42 ± 0.79	1.29
	low	83.85 ± 3.475	0.43 ± 0.033	66.21 ± 0.19	1.41

[a)] Soil pH was measured at a soil/water ratio of 1:5 (WTW 325 pH meter; Germany).

[b)] Concentrations of total phosphorus (P) and nitrogen (N) as well as ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-) available to plants were measured modified from Kneis et al. 2006.

[c)] Soil aggregation values (WSA = water stable aggregates; MWD = mean weight diameter) were measured according to Kemper & Rosenau 1986 (WSA) respectively Barto et al. 2010 (MWD).

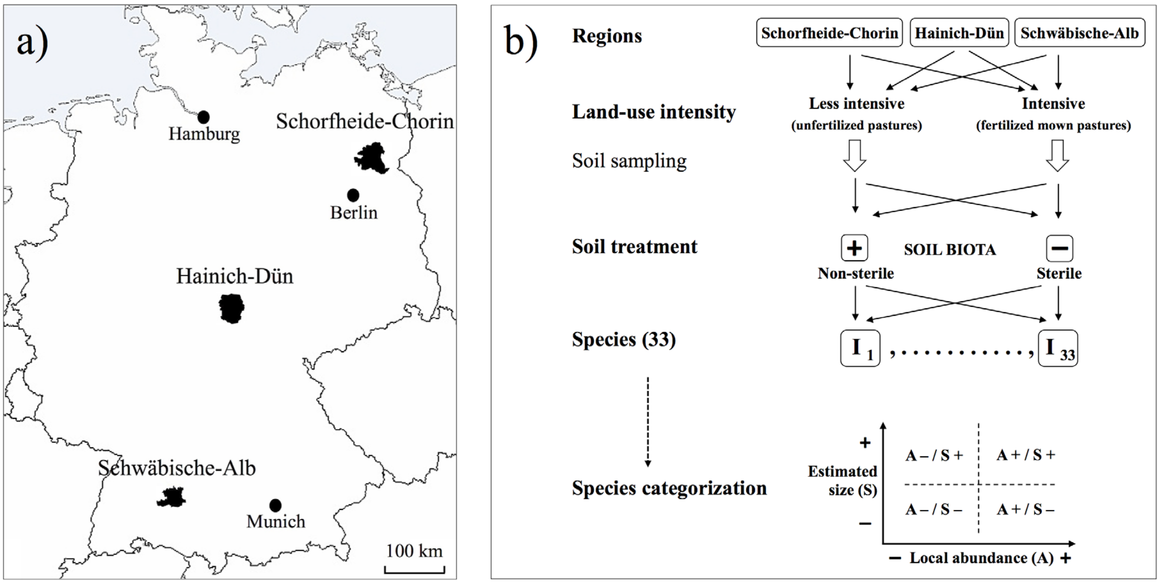

Figure S2.1 Location of the three regions within Germany (a) and conceptual figure of the experimental design (b). Soils were sampled in one intensively and one less intensively managed grassland site per region (six sites in total). In the greenhouse experiments, 33 plants species differing in local abundance and individual size were grown on sterile (- soil biota) and non-sterile (+ soil biota) soils to test the hypothesis that native plants with large individual size that are not locally dominant (despite their size and therefore potentially high competitive ability) are more strongly restricted and therefore held in check by negative biotic soil feed-backs than more abundant and smaller species. Using field soil from sites differing in land-use intensity, we investigated whether biotic soil-effects are modulated by land-use intensity.

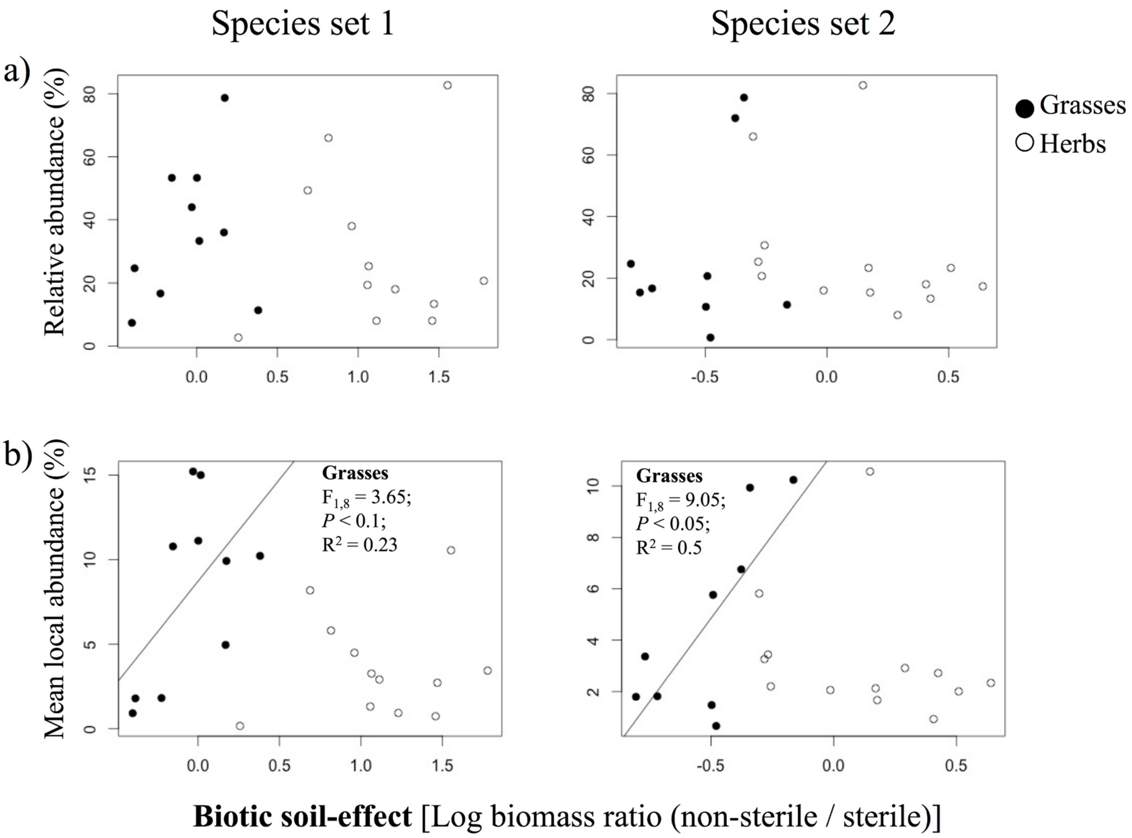

Figure S2.2 Relationship between relative abundance (a), mean local abundance (b) and biotic soil-effects of grasses (filled circles) and herbs (open circles) in central European grasslands in species set 1 (left) and species set 2 (right).

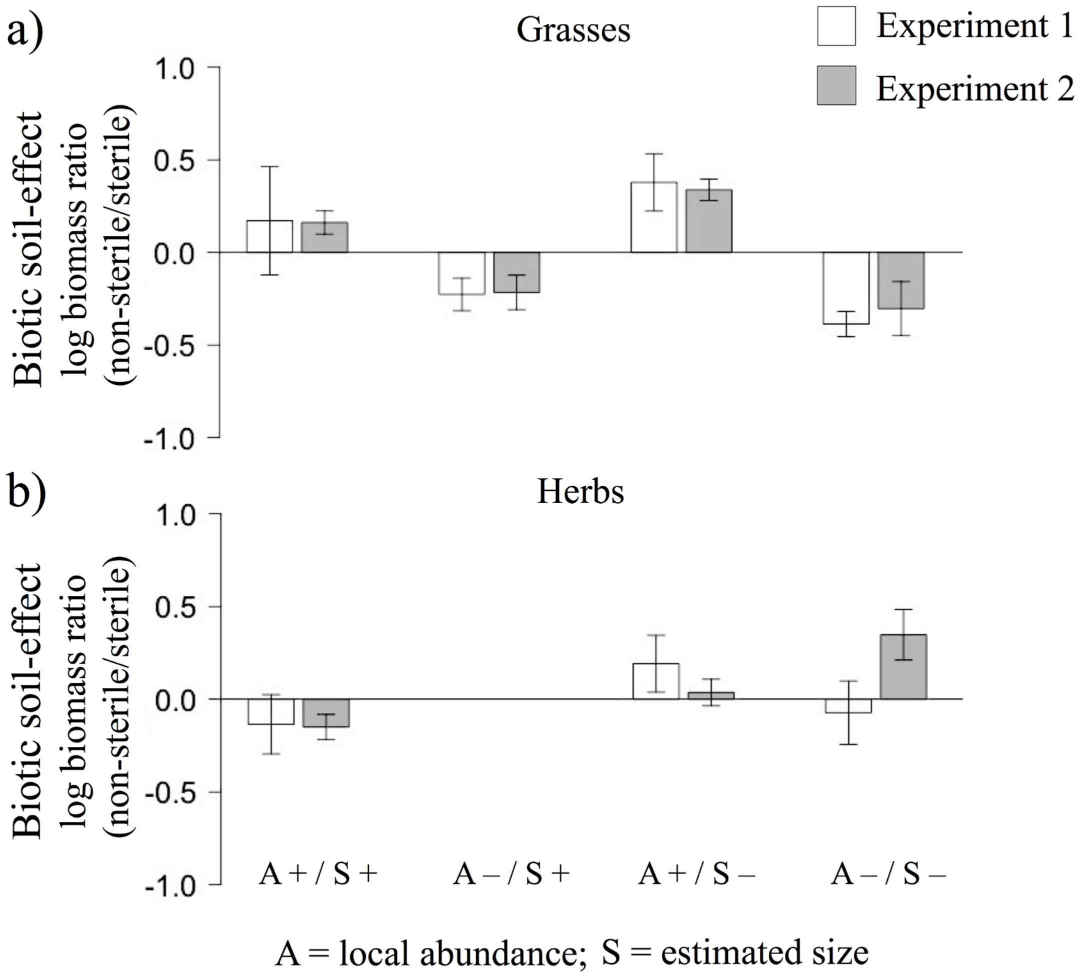

Figure S2.3 Biotic soil-effects for (a) 4 grasses and (b) 7 herbs that were replicates in experiment 1 (white bars) and experiment 2 (grey bars). Species were classified in four different categories (A + / S+; A – / S +; A + / S –; A – / S –): A = local abundance; S = estimated size; + = high / large; – small / low. Within one experiment biotic soil-effect values for the categories were centered on the average of the appropriate experiment. Data represent means of centered biotic soil-effect values for categories in the two experiments ± SE.

Table S4.1 Nutrient analysis of the soil used in the training phase. Data represent mean ± SE.

soil	pH (20°C)	Pdl [mg/100g]	Pcal [mg/100g]	NO3-N [mg/100g]	NH4-N [mg/100g]
aggregated	6.92 ± 0.04	4.68 ± 0.02	2.96 ± 0.08	3.91 ± 0.03	0.01 ± 0.00
disintegrated	6.97 ± 0.03	5.25 ± 0.09	3.50 ± 0.05	5.20 ± 0.06	0.03 ± 0.00

Table S4.2 Summary of the linear mixed effects model (lmer) testing for main and interactive effects of soil structure and specific root length (SRL) on hyphal colonization, arbuscules and vesicles during the training phase. SRL was fitted as a numeric effect. Degrees of freedom (df), *F* values and *p* values from ANOVA are presented. Significant values (p < 0.05) are presented in bold. Downward arrows indicate a negative correlation.

		colonization		arbuscules		vesicles	
effect	d.f.	*F*	*p*	*F*	*p*	*F*	*p*
soil	1	11.243	**0.001**	5.769	**0.018**	3.237	0.075
SRL	1	27.410	**<0.001↓**	30.016	**<0.001↓**	25.868	**<0.001↓**
soil*SRL	1	1.312	0.255	1.108	0.295	1.012	0.317

Table S4.3 Summary of the linear mixed effects model (lmer) testing for main and interactive effects of soil structure and plant functional type (PFT) on hyphal colonization, arbuscules and vesicles during the training phase. Degrees of freedom (df), *F* values and *p* values from ANOVA are presented. Significant values (*p* < 0.05) are presented in bold.

		colonization		arbuscules		vesicles	
effect	d.f.	*F*	*p*	*F*	*p*	*F*	*p*
soil	1	16.833	**<0.001**	7.487	**0.007**	1.780	00.185
PFT	1	61.492	**<0.001**	115.671	**<0.001**	30.684	**<0.001**
soil*PFT	1	0.757	0.386	0.012	0.914	2.881	0.093

Table S4.4 Summary of the linear mixed effects models for the training (A) and feedback (B) phase using the plant functional type („PFT“; grasses vs. forbs) as explanatory factor. Main effect of history (home vs. away) as well as main and interactive effects of soil structure (aggregated vs. disintegrated) and PFT on dry biomass (b, total biomass; ab, aboveground biomass; bb, belowground biomass; ln(a/bb), biomass allocation) and water stable aggregates (WSA) are estimated. Degrees of freedom (df), *F* values and *p* values from ANOVA are presented. Significant values (p < 0.05) are presented in bold.

A

		b		ab		bb		ln(a/bb)		WSA	
effect	d.f.	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
soil	1	2.031	0.156	1.299	0.256	9.724	**0.002**	22.337	**<0.001**	218.702	**<0.001**
PFT	1	0.153	0.704	0.521	0.487	2.085	0.179	4.717	0.055	5.033	**0.049**
soil*PFT	1	2.424	0.122	0.605	0.438	8.734	**0.004**	14.295	**<0.001**	1.480	0.226

B

		b		ab		bb		ln(a/bb)	
effect	d.f.	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
				home					
soil	1	0.099	0.753	0.088	0.767	1.092	0.298	1.691	0.195
PFT	1	0.250	0.629	2.038	0.187	1.007	0.342	12.499	**0.006**
soil*PFT	1	4.563	**0.034**	1.507	0.221	7.978	**0.005**	8.633	**0.004**
				away					
soil	1	0.199	0.657	0.012	0.912	0.512	0.476	0.660	0.418
PFT	1	0.112	0.746	1.526	0.248	1.607	0.237	10.098	**0.011**
soil*PFT	1	0.042	0.838	0.154	0.696	0.014	0.905	0.958	0.329

Table S4.5 Mean specific root length (SRL) of the 10 species used in the experiment. These data originate from a separate experiment with seeds from the same field collection than in the presented feedbackexperiment.

Species	SRL [cm/mg]	
Plantago major	2.48	forbs
Daucus carota	3.81	
Centaurea jacea	6.33	
Leucanthemum vulgare	12.46	
Plantago lanceolata	13.44	
Taraxacum officinale	14.95	
Dactylis glomerata	18.49	grasses
Briza media	25.14	
Anthoxantum odoratum	26.16	
Holcus lanatus	35.55	

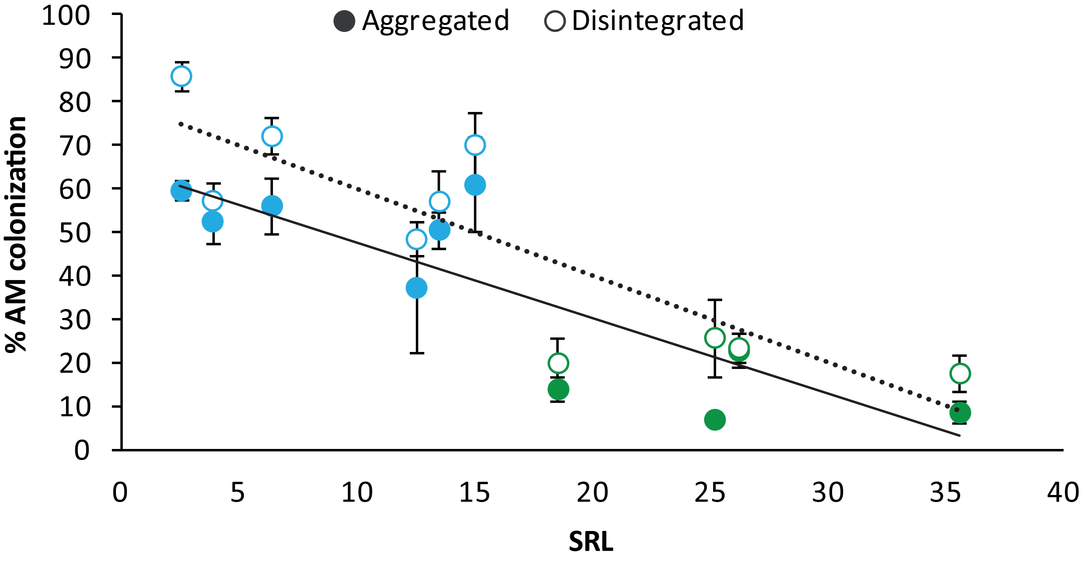

Figure S4.1 Training phase. AM-colonization of plant roots in the two soil structure levels in correlation to the specific root length (SRL) of the species. Blue colouring indicates forbs, green colouring indicates grasses. Data represent mean ± SE. The relationship of SRL and % AM colonization is significant in aggregated soil (solid line, r^2=0.713, p<0.001) as well as in disintegrated soil (dashed line, r^2=0.732, p<0.001).

Table S5.1 Sampling regions of the German Biodiversity Exploratories used in this study, from Fischer et al. 2010

	Schorfheide-Chorin	Hainich-Dün	Schwäbische Alb
Location	NE Germany	Central Germany	SW Germany
Size	~1300 km^2	~1300 km^2	~422 km^2
Geology	Young glacial landscape	Calcareous bedrock	Calcareous bedrock with karst phenomena
Human population density	23 km^{-1}	116 km^{-1}	258 km^{-1}
Altitude a.s.l.	3–140 m	285–550 m	460–860 m
Annual mean temperature	8–8.5°C	6.5–8°C	6–7°C
Annual mean precipitation	500–600 mm	500–800 mm	700–1000 mm

Table S5.2 Plant species and their frequencies, based on BLAST search of their trnL-intron sequences in the NCBI and matched with vegetation survey on the field. Root traits data: A=available, NA=not-available

Plant species name (NCBI)	Number of samples	Root traits data
Agrostis capillaris	8	A
Agrostis stolonifera	16	A
Alopecurus geniculatus	2	A
Alopecurus pratensis	40	A
Anthoxanthum odoratum	3	A
Anthriscus sylvestris	1	NA
Arrhenatherum elatius	73	A
Bellis perennis	1	A
Brachypodium pinnatum	12	A
Briza media	2	A
Bromus erectus	13	A
Bromus hordeaceus	11	NA
Bromus inermis	2	A
Carex flacca	1	A
Carex hirta	1	NA
Cerastium fontanum	1	NA
Cerastium holosteoides	1	NA
Dactylis glomerata	47	A
Deschampsia cespitosa	2	A
Elytrigia repens	50	A
Festuca ovina	3	A
Festuca pratensis	22	A
Festuca rubra	25	A
Helictotrichon pratense	3	A
Helictotrichon pubescens	7	A
Holcus lanatus	7	A
Juncus articulatus	1	A
Koeleria pyramidata	1	A
Lolium perenne	27	A
Luzula campestris	2	A
Phalaris arundinacea	3	A
Phleum pratense	4	A
Picris hieracioides	1	NA
Plantago lanceolata	7	A
Plantago media	1	A
Poa angustifolia	39	A
Poa pratensis	33	A
Poa trivialis	42	A
Prunella vulgaris	1	A
Ranunculus acris	1	A
Ranunculus bulbosus	1	A
Ranunculus repens	5	A
Sesleria albicans	1	NA
Stellaria graminea	2	A
Taraxacum sect. Ruderalia	1	A
Thymus pulegioides	1	NA
Trifolium montanum	2	A

Trifolium pratense	7	A
Trifolium repens	15	A
Trisetum flavescens	36	A
Urtica dioica	1	NA
Veronica chamaedrys	2	A

Methods S5.1 PCR conditions.

For PCR I. and II. a/b KAPA HiFi PCR Kit was used (Kapa Biosystems, Boston, Massachusetts, USA).
PCR I., per well
1 µl DNA extract

Mastermix:
Fidelity Buffer: 5 µl
dNTP: 0.75 µl
GlomerWT0: 0.75 µl of 20 µM primer solution
Glomer1536: 0.75 µl of 20 µM primer solution
H2O 16.25 µl
KapaHifi 0.5 µl
PCR I program (minutes:seconds)
Once:
95°C - 3:30
----
5 times:
98°C - 0:20
60°C* - 0:15
72°C - 0:30
----
25 times:
98°C - 0:20
55°C - 0:15
72°C - 0:30

Once:
72°C - 03:30
4° C - ∞
*=-1° / cycle
PCR IIa/b per well (two reactions in parallel)

PCR I + 25 microliter H2O: 0,75 microliter

NS31_A_MID: 0,75 microliter of20 µM primer solution (A is Adaptor A for Pyrosequencing, MID is the barcode)
Mastermix:
Fidelity Buffer: 5 µl
dNTP: 0.75 µl
AM1a_B or AM1b_B: 0.75 µl of 20 µM primer solution
H2O: 16.75 µl
KapaHifi 0.5 µl

PCR IIa/b program (minutes:seconds)
Once:
95°C - 3:30
----

30 times:
98°C - 0:30
63°C - 0:30
72°C - 1:00

Once:
72°C - 05:00
4° C - ∞

Afterwards PCR II a+b products of the same sample were mixed.

Host plant identification:
Per well/tube
1.5 µl DNA extract

Mastermix:
5 µl FIREPol® 5 x Master Mix "Ready to Load" (Solis BioDyne, Tartu, Estonia)
0.5 µl Trnl-C 20 µM primer solution
0.5 µl Trnl-D 20 µM primer solution
17.5 µl H2O

Program (minutes:seconds)
Once:
98°C - 0:30
35 times:
95°C - 0:30
50°C - 0:30
72°C - 2:00
Once:
72°C – 1:00
4°C - ∞

Methods S5.2 Greenhouse conditions

Seeds from commercial seed suppliers and botanical gardens in Germany were surface sterilized and germinated in Petri dishes on an autoclaved substrate consisting of an 80:20 % mixture of washed sand and commercial soil (Rasentragschicht AarGround, AareKies Brienz AG, Switzerland). The substrate had a pH of 6.5, a carbon concentration of 9.69 mg/g ± 2.04 (mean ± SD, n = 3) and a nitrogen concentration of 0.88 mg/g ± 0.15.

After germination, seeds were transplanted into flower pots containing the same substrate and inoculated with a microbial wash from field soil filtered through a 250 µm sieve to allow colonization by AM fungi. After 4-6 weeks (depending on the species specific growth) plants were harvested before the roots were pot bound.

Methods S5.3: Variables included in the random forest analysis, and the levels of the categorical variables

Root traits (continuous):
absolute fine root length between the given diameters:
- 0 to 0.2 mm
  within this:
  - 0 to 0.1 mm
  - 0.1 to 0.2 mm

total root length
ratio of fine root length and total root length
specific (divided by root biomass) root length
specific fine root length
specific root surface area

specific fine root surface area
specific root volume

Leaf traits (nominal):
scleromorphic leaves
mesomorphic leaves
hygromorphic leaves
helomorphic leaves
Ecological preference:
Moisture preference
indifferent or not
Ellenberg score (ordinal)
Light preference
indifferent or not
Ellenberg score (ordinal)
Strategy type
Competitor (or not)
stress tolerator (or not)
ruderal (or not)

The supporting information for chapter 6 is available at:

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