Predictability of plant-soil feedback

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Chapter 1

General introduction

Ecology's greatest challenges

It is widely acknowledged that human alterations of the biosphere (e.g., land use change, increased greenhouse gas emissions and consequent global warming, nitrogen deposition, introduction of alien biota into natural systems) are responsible for rapid global declines of biodiversity ('the sixth mass extinction on earth') and world-wide impairment of ecosystem functioning (Vitousek et al. 1997). An equally important and rapidly growing insight is that biodiversity, ecosystem functioning and the provisioning of ecosystem services are all intimately linked (Chapin et al. 2000, Hooper et al. 2005, Cardinale et al. 2012). However, the role of soil organisms in driving these relationships is relatively unknown (Bardgett and van der Putten 2014).

The beginning of the 1990's witnessed a rapid increase in the awareness of the importance of biological diversity for the functioning of earth's ecosystems, both on a scientific level (Schulze et al. 1993) and regarding international agreements and legislations concerning the global conservation of biodiversity (Convention on Biological diversity). The Millennium Ecosystem Assessment (2005) includes a special report on biodiversity, in which scientific insights about the value of biodiversity for humanity (i.e. ecosystem services) are explicitly linked to possible mitigation scenarios by us, citizens of the world.

Since two decades, an increasing number of biodiversity experiments have been set up with the aim of understanding the role of diversity at different levels of biological organization (Loreau 2010) for the functioning of ecosystems. In these studies, diversity (most often number of species or number of pre-defined functional groups) is manipulated in one or more trophic groups (primarily plants), and effects of this manipulation on ecosystem functioning are subsequently studied (e.g., Cardinale et al. 2006). Today, the most important conclusions that can be drawn from past and currently running biodiversity experiments are that biodiversity loss reduces the efficiency by which ecological communities convert biologically essential resources into biomass and recycle nutrients, and that it reduces temporal stability of ecosystem functions (Cardinale et al. 2012).

Cardinale et al. (2012) provided a critical evaluation of the evidence for each of the possible links between biodiversity, ecosystem functioning, and ecosystem services. One of the statements of the authors was that, by now, there is broad consensus for the idea that trophic interactions are key mediators of ecosystem functioning (Duffy et al. 2007), and are thus pivotal to be considered when studying impacts of biodiversity loss on ecosystem functioning. This observation was already made 15 years ago (Chapin et al. 2000), and since

then, the field has expanded rapidly towards the underground, where biological processes reach their highest complexity in terms of both species and functional diversity (van der Heijden et al. 2008, Bargett and Wardle 2010, Bardgett and van der Putten 2014).

At the time of the extensive review on the subject by Hooper et al. (2005), aboveground-belowground interactions still represented an understudied component of biodiversity-ecosystem functioning (BEF) research. Today, it is widely acknowledged that soil organisms are a major driver of plant community dynamics (Bardgett and Wardle 2010), and recent experimental work underlines their important role in driving the often observed positive diversity-productivity relationship in biodiversity experiments (Maron et al. 2011, Schnitzer et al. 2011). Results of the latter two studies strongly suggest that increasing plant community productivity at higher plant species diversity occurs, at least in part, because of decreasing impacts of soil microbial plant antagonists at high vs. low plant species diversity. However, not all plant species show the same growth difference in soil conditioned by plant communities composed of several plant species vs. in soil conditioned by their monoculture (Kulmatiski et al. 2012, Hendriks et al. 2013). This observation prompts for further investigation of the relation between plant species-specific overyielding and alterations of interactions between individual plants and soil microbes in plant communities with different plant community diversity (Kulmatiski et al. 2012). After all, a full understanding of complementarity and selection effects of plant biodiversity on e.g. productivity, to which soil organisms may heavily contribute, can only be reached by considering relative yields of individual plant species in communities with a low degree of diversity as compared to those with high degree of diversity (Loreau and Hector 2001).

The larger part of biodiversity experiments, and consequently also most of the theory about biodiversity-ecosystem functioning (BEF) relationships, comes from model grassland ecosystems in which plant community diversity was manipulated (Loreau et al. 2001, Cardinale et al. 2012). This should not come as a surprise, because plants provide the main carbon source for all organisms in terrestrial ecosystems (Wardle 2002) and are at the center of multi-trophic interactions between the above- and belowground components of ecosystems (van der Putten et al. 2001). Moreover, effects of global change phenomena on the functioning of terrestrial ecosystems are expected to be largely indirect, being driven by changes in plant community structure and cascading effects on higher trophic levels above- and belowground (e.g., Bardgett and Wardle 2010, Isbell et al. 2013).

Soil organisms exert strong control on plant productivity through direct interactions with plant roots as plant growth-depressing or -promoting symbionts and by shaping the physical habitat of soil (van der Heijden et al. 2008, Wardle et al. 2004, Bardgett and van der Putten 2014). To date very little is known about the mechanisms that link plant diversity to belowground diversity and functioning (Bardgett and Wardle 2010). This lack of knowledge on mechanisms precludes an informed interpretation and predictability of plant community diversity effects belowground. The general observation that the study of the mechanistic basis of BEF relationships has remained challenging up to date can be partly explained by the fact that these mechanisms are subject to many of the ecological phenomena and processes that are still heavily debated (Sutherland et al. 2013). Understanding them requires integrating concepts of community ecology and ecosystem ecology, two areas that have largely developed independently from each other (Bardgett and Wardle 2010).

One of the fundamental questions central to ecology is why so many species coexist in nature (Chesson 2002). Often, ecosystem-level properties are used to explain patterns of biodiversity (e.g., Harpole and Tilman 2007). BEF research turns this question around by asking how biological diversity in an ecosystem affects its functioning, which includes biomass productivity (Marquard et al. 2009), stability of functions (Weigelt et al. 2008, Allan et al. 2011) and diversity of functions (Hector and Bagchi 2007, Hillebrand and Mathiessen 2009, Isbell et al. 2011). Turning the question around does not change the theoretical and experimental challenges. This is why the community of present day ecologists needs to employ its full capacity to understand and communicate the importance of biodiversity for ecosystem services; it's ecology's greatest challenge today.

A diversity of diversities[1] *and the study of aboveground-belowground linkages*

Is diversity of soil organisms dependent on plant community diversity and, the other way around, is belowground biological diversity functionally important for supporting plant productivity? These are two broad questions that governed past and present research on the role of soil biota for BEF relationships. The structuring effects of plant community composition on soil biotic communities may operate via changes in the actual identities of the species that make up the plant community, influencing quantity and quality of the live plant biomass, plant litter, root exudates and other organic compounds that may enter the soil food web (e.g., Korthals et al. 2001, Wardle 2002, Porazinska et al. 2003, Hedlund et al. 2003,

[1] *The term 'a diversity of diversities' was coined by Loreau 2010 (see references)*

Wardle et al. 2003, Zak et al. 2003,Viketoft et al. 2009, De Deyn et al. 2011). 'Diversity *per se'* has been an often-used expression in studies of linkages between plant community diversity and soil biotic community structure and functioning. In many cases no effects of plant species diversity *per se* on belowground community structure and functioning were found; variation of soil biotic structure and functioning could be largely explained by the identity of the plant species present, and not by plant species richness (e.g., Viketoft et al. 2009). A first distinction that can be made when considering linkages between plant community and soil biotic community structure is between effects of plant resource quantity (e.g., plant litter biomass) and quality (i.e. plant species diversity or identity). On this point, studies give variable support for either plant resource quality or quantity to be more important in driving the relationship between above- and belowground biodiversity (Wardle 2002).

Overall, resource quality, indicated by plant species-specific effects on soil biological diversity and functioning, seems to be more important than resource quantity for affecting belowground communities (Bardgett and Wardle 2010). However, an important further distinction to be made in the concept of resource 'quality' is between additive and synergetic effects of plant resource diversity on soil organisms (e.g., Kuyper and Giller 2013). Here, the question is whether resources (for instance plant litter) of a mixed plant community support a more efficient community of soil organisms in comparison with monocultures due to non-additive (synergetic) effects of resource diversity *per se*. There is some evidence that this might occur (Gartner and Cardon 2004, Hättenschwiler et al. 2005, Eisenhauer et al. 2010), but the issue remains debated up to date (Bardgett and Wardle 2010). Importantly, however, the discussion has mainly developed around biotic components of the *indirect pathway* in the soil food web, based on litter and root exudates and the recycling of nutrients (Wardle et al. 2004). There are, however, good reasons to expect that linkages between plant community structure and soil biotic components of the *direct pathway* as governed by root herbivores, pathogens and mutualistic symbionts (Wardle et al. 2004) may be markedly different from those of the *indirect pathway*. For the former, species diversity *per se* is expected to be of particular relevance, because these biota often show a high degree of host plant specificity (e.g., Mills andBever 1998, Grayston et al. 1998, Becklin et al. 2012), and consequently, host plant frequency-dependent effects on their host plants. This phenomenon is well-established for the impacts of pathogens on plant communities (Mordecai 2011), but poorly known for symbiotic mutualists.

Nothing in AG-BG biology makes sense except in the light of plant-soil feedbacks[2]

The soil accumulates legacy effects of organisms that may remain after their death or disappearance. For example, soil organic matter, porosity of the soil, nutrient levels, pathogen abundances, mycorrhizal (spore) abundance, all influence 'soil functioning' at a particular point in time; consequently, soil functioning cannot be understood without considering legacies of past soil biological activity. Moreover, because plant community structure is a main driver of belowground communities, these communities themselves represent a legacy of previous plant growth (e.g., van der Wal et al. 2006).

Interactions between plants and soil biota are reciprocal and dynamic, which means that species composition of the plant community influences soil biotic community structure, as plants influence soil organisms in a species-specific way (e.g., Grayston et al. 1998, Bezemer et al. 2010), and that the community structure of soil organisms in turn impacts on plant community structure in a plant species-specific manner (e.g., van der Putten 1993, Bever et al. 1997, De Deyn et al. 2003). The general name of this phenomenon is 'plant-soil feedback' (PSF); it involves both biotic and abiotic alterations of the soil environment (Ehrenfeld et al. 2005). Biotic and abiotic soil conditions are intimately linked, as soil organisms drive decomposition and influence the physical habitat of soil (Wardle 2002), whereas abiotic soil conditions influence community composition of the soil biota by acting as an environmental filter (e.g., Ettema and Wardle 2002, Ramirez et al. 2014). In recent years, the term PSF has been mostly used to denote short-term feedback loops between plants and soil organisms, which mainly work via the direct pathway in the soil food web (Wardle et al. 2004). As plant species identity can influence the community composition of rhizosphere microbes on a time scale of only a few weeks, and by this, alter the effects that soil biota have on neighboring and successive plants, it is expected that especially these direct interactions (Wardle et al. 2004) will be affected by the precise (history of) species composition of the plant community.

[2] *Derived from T. Dobzhansky's (1964) 'Nothing makes sense in biology except in the light of evolution', American Zoologist 4: p. 449.*

Plant-soil feedback and plant productivity

Short-term PSFs are strongly embedded in theoretical frameworks (Bever et al. 1997, Bever 2003), in which the effects of PSFs on plant community dynamics have been discussed primarily with regard to plant species coexistence. In this framework, coexistence of two plant species is promoted by direct feedback (i.e. intraspecific feedback) being more negative (or less positive) than indirect feedbacks (i.e. interspecific feedback). If this effect is strong enough, PSFs can reverse plant competitive hierarchies when superior competitors become highly abundant in the plant community and build up species-specific antagonists (Mordecai 2010, Petermann et al. 2008, Pendergast et al. 2013).

An important effect of increasing the species richness of a plant community on PSFs is that direct (intraspecific) PSFs decrease in frequency and/or strength for all plant species that comprise the community: they are increasingly replaced by indirect (interspecific) feedbacks. As most plant species produce more biomass when they grow on soil with a legacy of heterospecific plants, plants generally experience less negative indirect PSF than direct PSF (Kulmatiski et al. 2008). Given this notion, all else being equal, increasing species-richness of plant communities should then lead to higher plant community productivity (Kulmatiski and Kardol 2008).

This observation, combined with the insight that PSF effect sizes are widely variable among plant species, leads me to conclude that understanding the contribution of PSF to the positive relationship between plant species diversity and productivity requires an approach that scales up from interactions between individual plants and soil microbes to community level PSFs (e.g., van de Voorde et al. 2011).

Plant traits and phylogeny as predictors of PSF

Hillebrand and Matthiesen (2009) observed that biodiversity effects only hold in a world of ecological niches: different species need to have differential effects on ecosystem functioning for mechanisms like niche complementarity and facilitation to take place. The authors suggested that the use of plant functional traits in predicting species-specific effects on, and responses to, ecosystem processes (Violle et al. 2007, Garnier and Lavorel 2002) can greatly benefit our understanding of biodiversity effects on ecosystem functioning. A key challenge is to identify key attributes of species that influence their interactions with the environment

(e.g., Orwin et al. 2010), and use these traits to explain ecosystem functioning (e.g., Griffin et al. 2009, Roscher et al. 2012).

For the decomposer subsystem (Wardle et al. 2004), the relevance of plant traits for predicting decomposition rates among species (e.g., Cornwell et al. 2008, Orwin et al. 2010) and the relative importance of different biota in driving decomposition (Orwin et al. 2010, De Vries et al. 2012) is well-appreciated. However, for plant-soil interactions belonging to the *direct pathway* (Wardle et al. 2004), the use of plant traits is currently poorly developed.

Phylogenic relationships among co-occurring plant species potentially play an important role in influencing biotic interactions in plant communities (Webb et al. 2002, Cavender-Bares et al. 2009). Similarly to plant traits, the use of plant phylogenetic relatedness to understand PSFs has only recently been gaining attention (e.g., Webb et al. 2006, Liu et al. 2012). Recently, Anacker et al. (2014) found that plants that are more closely related develop PSFs of similar size and direction. However, plant performance can be markedly different between soils, depending on the plant species that grew previously in that particular soil, thereby conditioning the soil biota (e.g., Hendriks et al. 2013). A promising, but unexplored possibility is that plant relatedness influences indirect PSF between plant species, as there is increasing evidence that plant symbionts show a phylogenetically restricted plant host-range (e.g., Liu et al. 2012).

A large part of this thesis (chapters 2, 3 and 4) focusses on the short-term feedback cycle, and here, we expect strong predictive power of plant traits (Ke et al. 2015) and phylogenetic relationships between plants (Liu et al. 2012) to predict PSFs. Predictability of interactions between plants and symbiotic rhizosphere biota of individual plant species with particular traits may be strong, as root pathogens and mutualists need direct access to live plant resources (as opposed to organisms in the detrital food web). Although the accessibility and quality of plants to intimate symbionts is expected to strongly relate to plant traits (Bardgett and Wardle 2010), remarkably little is known about what traits to focus on.

The Jena Experiment

The research outlined in this thesis took place in the framework of the Jena Experiment, currently one of the largest and longest running grassland biodiversity experiments, located on the floodplain of the river Saale at the northern edge of Jena, Germany (Roscher et al. 2004). This experiment offers many possibilities to address the above-discussed gaps in our understanding of biodiversity effects on belowground functioning and feedbacks to plant productivity.

The Jena Experiment was established in 2002 by sowing plant species mixtures on former agricultural land used for the production of wheat and vegetables (Roscher et al. 2004). The experimental design incorporates important earlier insights on how such an experiment should be designed to allow for statistical testing of biodiversity effects (Schulze et al. 1993), e.g., the need to include monocultures of all species (Loreau and Hector 2001). It was also one of the first experiments that included a near-orthogonal variation of species diversity and diversity of pre-defined functional groups (grasses, small forbs, tall forbs and legumes), of which the results (e.g., Marquard et al. 2009) led to the establishment of a new trait-based experiment on the same experimental site (Ebeling et al. 2014). An important benefit of the Jena Experiment is the fact that a tremendous amount of data has been gathered on various ecosystem processes and biotic community structure (see http://www.the-jena-experiment.de/, 'publications'), which is freely available for use within the research consortium. In due time, the data will also be available for a wider users group. This data availability has been pivotal for extrapolating our findings on plant species-specific differences in PSF effects (chapter 3) to species performances in the field (chapter 5), and for building a structural equation model linking plant community diversity to abundances of nematodes in different trophic levels via various community-scale parameters (chapter 6).

Overview of chapters

In **chapter 2**, I discuss key points that need to be considered when performing pot experiments on interactions between plants and microbial symbionts in order to gain insight in the functional significance of these microbes, translated to field settings. More specifically, studies are discussed that perform inoculation of live soils into a background of sterilized soil in order to examine effects of soil microbes on plant growth. This is a widely used approach

(Bever et al. 2010) that aims to separate the effects of soil microbes from the effects of abiotic drivers on plant growth and soil functions (Ehrenfeld et al. 2005). The issues presented in this chapter are of particular relevance for chapters 3, 4 and 5, in which I also performed such studies. The main conclusion of chapter 2, that also should have an impact when reading chapters 3 to 5, is that continuous cross-validation is needed between highly controlled studies and studies with increasing ecological realism. A study of Verbruggen et al. (2012) was taken as a case study to illustrate the many challenges this type of studies face when trying to extrapolate experimental observations to field situations.

In **chapter 3**, I explored the mechanisms underlying direct PSFs by testing the ability of plant traits (see further) to predict the ranking of 48 plant species going from a negative to a positive response to conspecific conditioned soil biota (i.e. soil with a legacy of conspecific plants), using sterilized soil inoculum ('PSFsterilized') or soil inoculum conditioned by other species ('PSFmixed') as controls for the effects of the conspecific conditioned inoculum. PSFsterilized reflected net effects of all soil biota, whereas PSFmixed captured the effects of relatively specialized soil organisms.

I did not aim to demonstrate direct mechanisms underlying the interaction between individual (groups of) soil microbes and plants; instead, I attempted to predict the ranking of a wide range of plant species according to their PSFsterilized and PSFmixed values, based on interspecific variation of four plant traits (specific leaf area, SLA; specific root length, SRL; relative growth rate, RGR; and the level of mycorrhizal colonization of plants grown in conspecific conditioned soil). My overarching hypothesis was that plants with high trait values for SLA, SRL and RGR have most negative PSFsterilized. I based this hypothesis on the well-developed theory of trade-offs between plant growth and defense against aboveground antagonists (e.g., Herms and Mattson 1992, Fine et al. 2006, Lind et al. 2013), which may also occur for interactions between plants and soil biota (Bauerle et al. 2007). Conversely, plants may grow slower and divert energy away from growth to maintain mutualistic root symbionts like arbuscular mycorrhizal fungi (AMF).

There is increasing awareness that plant traits related to resource acquisition are often inter-correlated, giving rise to plants either being 'slow' (resource conservative, well defended) or 'fast' (resource acquisitive, poorly defended) (Reich 2014). Variation of functional traits can however also be decoupled (i.e. poorly inter-correlated), so RGR, SRL and SLA are not necessarily interchangeable. Moreover, especially belowground plant traits have been found to influence soil microbial community structure (Legay et al. 2014). Consequently, SRL may be considered a plant trait that links to PSFsterilized via proximate

mechanisms, whereas a whole-organism trait like RGR may indicate overall carbon allocation to growth vs. defense (e.g., Fine et al. 2006). Additional to these resource-acquisition related traits, I tested whether the AMF colonization, a typical component of positive PSF, was positively correlated with PSFsterilized.

Because I used two different measures of PSF, my study offers the unique opportunity to test, within the same experiment, whether species rank similar for PSFsterilized and PSFmixed. With PSFsterilized, I determined total effects of conspecific conditioned soil biota on plant performance; with PSFmixed, effects of specialized vs. generalized soil communities were determined. Presuming a certain degree of host plant-specificity of both beneficial and antagonistic root symbionts, I hypothesized that plant species that build up strong negative interactions in their soil will benefit from growing in mixed soil, whereas plant species that build up strong positive interactions in their soil will suffer from growing in mixed soil.

While in Chapter 3, I focus on interspecific differences in the tendency of plants to associate with soil microbes in a way that is either beneficial or detrimental to plant performance, in **chapter 4,** I focus on intraspecific variation of PSFs.

Many studies that investigated relationships between species-specific PSFs and plant ecological dynamics treated PSF as a fixed species attribute (e.g., Klironomos 2002, Petermann et al. 2008). In the latter two studies for instance, species-specific PSF was calculated as the average value of several (Klironomos 2002) or two (Petermann et al. 2008) direct vs. indirect PSF values. Klironomos (2002) used this average PSF to show that species with (on average) more negative PSF were subordinate to species with less negative PSF. Petermann et al. (2008) used the species-specific averaged PSF to model the effect of negative species-specific feedbacks on coexistence, showing that average species-specific feedback effects were sufficiently large to stabilize fitness differences between co-occurring plant species.

Clearly, the average value of species-specific PSFs is relevant to a plant's ecological dynamics; however, because plants root in soil patches that had been previously conditioned by specific plant species, the PSF a focal plant experiences in a plant community will depend on the specific combination of the focal species and the species that conditioned the soil (Hendriks et al. 2014). I tested whether the phylogenetic relatedness between focal plants and soil-conditioning plants may predict the performance of the focal species in these soils.

For the design of the experiment described in chapter 4, my presumption was that host plant-specificity of soil microbes is key to understanding indirect feedback effects. Based on the results of chapter 3, I selected 11 focal species representing three 'feedback groups':

plants that have a (I) negative, (II) neutral, or (III) positive response to conspecific conditioned biota (named 'PSFsterilized' in chapter 3).

My hypothesized outcomes of the relationship between the performance of focal plants and their phylogenetic distance to the soil-conditioning plant are depicted in Fig. 1. I assume that both beneficial and antagonistic rhizosphere microbes impacting on plant performance are to an important degree conserved in the phylogenetic tree of plants. I predict that the response of a focal plant to conspecific conditioned soil biota (PSFsterilized, see chapter 3) will determine the relationship between plant performance of a focal plant and the phylogenetic distance (PD) between the focal plant and soil-conditioning plant. Species with strong positive PSFsterilized are expected to lose host plant-specific mutualists, and hence exhibit worse performance, with increasing PD between focal plant and soil-conditioning plant (b); species with neutral PSFsterilized are expected to lose both host plant-specific antagonistic and mutualistic rhizosphere microbes and consequently show a weak or no relationship with PD between focal plant and soil-conditioning plant (c); species with a strong negative PSFsterilized are expected to experience less suppression by antagonistic soil microbes, and hence exhibit increased biomass production, with increasing PD between focal plant and soil-conditioning plant (d).

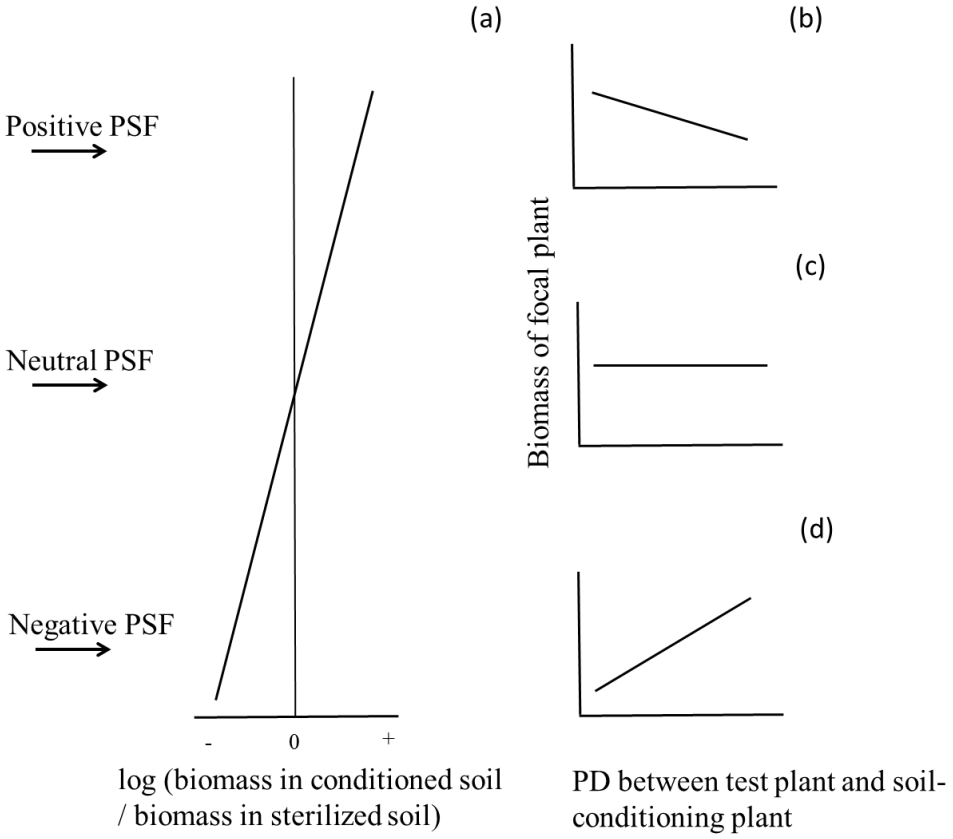

Figure 1 Hypothesized relationship between the plant-soil feedback (PSF) value of a focal plant (a) and the effect of phylogenetic distance (PD) between focal and soil-conditioning plant on focal plant biomass, separately shown for plants that have a negative PSF (b), a neutral PSF (c) and a positive PSF (d). See main text for explanation.

In **chapter 5**, I examined the relationship between species-specific direct vs. indirect PSFs and the contribution of the species to biomass overyielding in species-rich plant communities vs. monocultures in the Jena Experiment. Overyielding of plant communities occurs when the productivity of a mixed plant community composed of a specific set of species is greater than the average productivity of those species grown in monocultures. This can be the result of both selection and complementarity effects (Loreau and Hector 2001). The selection effect is calculated as the co-variation between monoculture yield and species relative yields in mixtures (i.e. the observed yield in mixture divided by its expected yield, the latter being its productivity in monoculture corrected for (i.e. divided by) the number of species in mixture). Positive selection effects indicate that species that are highly productive in their monoculture become relatively dominant in mixture. This causes the observed yield to be higher than the expected yield, which assumes equal density of the plant species that were sown together. However we can expect the effects of PSF on plant productivity in species-rich vs. species-poor plant communities (see above) to be mainly reflected in a positive 'complementarity' effect, which occurs when the average relative yield is above one.

In a recent greenhouse experiment, Kulmatiski et al. (2012) showed that negative species-specific PSF (determined in the absence of competition) related to high overyielding in highly simplified three-species communities. The authors reasoned that PSF alterations may provide an additional explanation for community overyielding with increasing plant species diversity. Species-specific negative soil feedbacks are expected to diminish in plant communities with an increasing number of species, which effectively decreases the amount of conspecific conditioned soil in the vegetation. Importantly, the results of Kulmatiski et al. (2012) suggest that plant species with the most negative PSF will overyield the most.

A few studies to date have shown that PSF values derived from pot experiments can be predictive for the population dynamics of a plant species in the field, for instance for the relative abundance of a species (e.g., Klironomos 2002, Mangan et al. 2010). In these two studies, short-term feedback effects on plant performance were predictive for the species performance in the field. A recent study, however, found no such relationship (Reinhart et al. 2012). Controlled experiments and field studies have shown that PSF effects can interact with the competitive environment (Caspar and Castelli 2008, Peterman et al. 2008, Shannon et al. 2012). Therefore, facilitative effects of plant neighbors may need to be considered in concert with competitive interactions.

In **chapter 6** I take a broader perspective on the linkages between plant community diversity and functioning of belowground biota by examining the effects of species and

functional group richness of plant communities on nematode community structure across feeding groups (plant feeders, microbial feeders and predators) on a plant-community level. Similar studies to this one have been published over the last decade (e.g., Wardle et al. 2003, De Deyn et al. 2004a, Viketoft et al. 2009, Sohlenius et al. 2011), but none of these has considered specific aspects of resource quality, next to resource quantity. In those studies, 'resource quality' referred to either plant species or functional group richness or identity effects on nematode community structure. As such, they represent an indirect measure of potential top-down and bottom-up controls of plant community structure on nematode abundances. In order to understand the mechanisms underlying effects of plant community structure on nematode community structure, relevant aspects of resource quality and quantity have to be identified, which include both biotic and abiotic parameters of soil (Yeates 1993). I do this by building a structural equation model (SEM), incorporating various plant and soil variables that were measured on a plot scale. These included organic matter content in soil, microbial biomass and C/N ratio of plant material.

Finally, in **chapter 7**, I integrate the results of my thesis and provide directions for future research by critically evaluating my contributions to the research questions I addressed in my thesis.

Predictability of plant-soil feedback

The research of my thesis is concerned with the predictability of PSFs. It could be argued that it is hard to set out PSF research without aiming for improved predictability, since the general awareness that PSFs are widespread and strongly affect plant community dynamics has already been established more than a decade ago (Bever et al. 1997). Consequently, virtually all current PSF research aims to improve PSF predictability. However, my research focused on both the predictability of the size as well as the direction of a plant's PSF, both direct and indirect PSF (van der Putten et al. 2013), and predictability of the influence of a plant's PSF on the plant community (more specifically: biomass overyielding). By using a large set of plant species, I was able to test rigorously for different factors that may predict PSF variation at the inter- and intraspecific level. In the last chapter, where I looked at nematode communities in the Jena Experiment, predictability of aboveground-belowground linkages could be tested by using various plant and soil parameters in Structural Equation Model (SEM) analyses.

Chapter 2

The curse of the black box

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Abstract

Background: Soil is a foremost provider of (agro-)ecosystem services, making plant-soil interactions pivotal in agriculture research. The functioning of soils entails complex interactions between soil biota and the abiotic soil environment and is therefore often considered as a 'black box'. The study of Verbruggen et al. (this volume) tries to crack the black box open by examining the role of soil microbial communities from conventional and organic farming fields for the growth of *Zea mays* and phosphorus retention in the soil.

Scope: In this commentary on the paper of Verbruggen et al. (2012) we use the study to illustrate that investigating soils, and specifically the role of soil biota in ecosystem functioning, is not straightforward, given the overwhelming soil biodiversity and the complexity of soil as a habitat. We discuss the key elements that need to be considered in order to translate results of highly controlled experiments with inoculated soil biota to their functioning in the field.

Conclusions: Verbruggen et al. contribute to our understanding of the functional role of AMF in agro-ecosystems. Yet the results only allow us to merely speculate about the realized functional role of AMF communities in the field, a very interesting avenue for future research.

In a world where organic agricultural practice is put forward as a sustainable alternative to conventional farming, understanding the implications of these two contrasting management types for the provision of essential soil functions is more than welcome (Gomiero et al. 2011). From both natural and human dominated ecosystems, it is well known that soil biota play an integral role in soil processes that are essential for sustaining plant productivity and other soil based ecosystem functions (Brussaard et al. 1997). Soil biota are the main drivers of mineral nutrient cycling (Coleman et al. 2004) and also play an important role in causing and suppressing pathogenesis in plants (Garbeva et al. 2004), in the formation of soil structure and in the sequestration of soil carbon (Bronick and Lal 2005, De Deyn et al. 2008). The activity of micro-organisms, meso- and macrofauna in the soil thus affects both abiotic and biological soil properties which are important for plant growth.

Particularly interesting and relevant for plant performance are the ubiquitous micro-organisms present in the rhizosphere, i.e. the soil environment in the immediate surroundings of the roots. A complex interplay of antagonistic and mutualistic interactions between plants and rhizosphere organisms can give rise to large variations in plant performance resulting from the combined effect of these plant-soil biological interactions (Garbeva et al. 2004, Buée et al. 2009). Antagonistic organisms in the rhizosphere include parasitic nematodes, pathogenic fungi and bacteria (Jackson and Taylor 1996). Among the mutualistic micro-organisms associated with plant roots, arbuscular mycorrhizal fungi (AMF) are the best known. These fungi live partly in plant roots and partly in the soil matrix and provide their host plant with nutrients, mainly phosphorus, in exchange for carbon compounds of the host plant. The effects of AM fungi on plant performance are however not fixed and can be dependent on the specific combination of AMF and plant species (Klironomos 2003), on interactions between AMF and the surrounding soil dwelling organisms (e.g. Azcón-Aguilar and Barea 1997, Vásquez et al. 2000) and – importantly – on the abiotic soil context in which the plant-AMF interactions take place (Hoeksema et al. 2010). For AMF but also for other groups of soil organisms, especially those which directly interact with plant roots, it has been shown that their effects on plant performance interact with the abiotic properties of the soil, with often stronger impacts of soil biota in soils with lower availability of mineral nutrients (De Deyn et al. 2004b, Hoeksema et al. 2010).

Sustainable farming relies on internal biological processes which maintain soil fertility and crop protection under low external input of fertilizers and chemical pest management. A main challenge is thus to identify and protect the functional components of soil biodiversity that provide these ecosystem services (Altieri 1999, Kuyper and Giller 2011). A full understanding of biological soil functioning and the associated ecosystem services under different agricultural management types can, however, only be obtained by taking an integrated perspective, considering short-term as

well as long-term interactions between soil biota, abiotic soil properties and plant performance (Barrios 2007).

Studying soils and specifically the role of soil biota in ecosystem functioning is not straightforward, given the overwhelming diversity of soil biota and the opaqueness of soil as a habitat. Indeed, it may not be a surprise that soils are often considered as a 'black box'. Studies on the relation between soil biota and ecosystem functioning are facing the difficulty of choosing between a holistic or reductionist approach. In the holistic approach, a high relevance to natural systems is attained by using natural, complex, soil communities and testing their combined impact on ecosystem response variables, but without knowing the exact underlying mechanisms in play, so that the soil system remains a 'black box'. The reductionist approach on the other hand provides mechanistic insights in soil functioning by focusing on the mechanism by which a specific species or specific group of soil biota affects ecosystem processes in isolation. In this commentary, we discuss the advantages and the pitfalls hidden in both the holistic and reductionist approach using the paper by Verbruggen et al. (2012) (this volume) as a case study, where elements of both approaches have been combined.

The study of Verbruggen et al. (2012) aimed to explore potential differences in the functioning of soil microbial communities between agricultural fields with a history of either organic or conventional farming, and to separate the effects of soil microbial activity from the effects of other soil properties that could affect the processes under study. In two greenhouse experiments, the functional role of field-specific microbial communities was investigated for two ecosystem processes: phosphorus leaching (after artificial rainfall) and plant biomass production (using *Zea mays* as test plant). The soils were collected from agricultural fields on sandy soils which were managed in a conventional or organic way, pairwise co-occurring for each management type and distributed over five regions in The Netherlands. Living soil from the different fields was added to a larger fraction of sterilized soil (composed from a mixture of soil from an organically managed field and nutrient poor sand), the latter being uniform over all treatments. A similar approach of soil inoculations into a background of homogenized sterilized soil is often used in plant-soil feedback studies which aim to separate impacts of soil biota on plant growth from potential differences in abiotic soil properties (Kulmatiski and Kardol 2008, Brinkman et al. 2010).

Intrinsically, by applying an inoculation approach as in Verbruggen et al. (2012), it is not possible to really *isolate* the functioning of the soil microbial community and its effects on plant performance from the abiotic soil properties, as they function within the abiotic setting provided. In the field however, soil biota modify their abiotic environment, both in physical and chemical sense, at various scales in space and time (Wright and Upadhyaya 1998, Barrios 2007). Levels of plant available soil nutrients and soil structure for instance are genuine components of soil microbial

effects, which are however - intentionally - not studied in inoculation experiments (i.e. the aim is to limit abiotic carry-over effects from the inoculum soil to the soil mixture used in the experiment). Moreover, the functional significance of the rhizosphere community, including AM fungi, can be dependent on abiotic factors in the soil, and can therefore – in principle – only be properly assessed within this abiotic context. Apart from these limitations, inoculation experiments are certainly a good way to *separate* effects of soil microbes from the abiotic context they are operating in, potentially giving valuable insights in the role these microbes play in their original soil environment. Within this original soil environment, coupling soil processes and functions to the activity and abundance of specific belowground organisms or taxa is difficult, one reason being the complex belowground multitrophic network all soil organism are embedded in (Wardle 2006)). Indeed, many biologically driven soil processes are the result of the combined activity of functionally different organisms across the different trophic levels (Wurst et al. 2012). To what extent the functional significance of one organism group in the soil-plant system can be understood by the study of individual effects, without considering multitrophic interactions, is likely dependent on the effect size of the organisms of interest and the degree of interaction with other groups of belowground biota (Wurst et al 2012, Ladygina et al. 2010). This complexity of the belowground ecosystem also implies that observational field studies involving soil microbes and their effect on soil functioning and plant growth cannot provide solid mechanistic insight in the processes at play. Consequently, controlled experiments are a requisite in elucidating the mechanisms behind soil processes in the field. Nevertheless, a combination of highly controlled (e.g. an inoculation experiment with only one microbial group in a sterile background soil) and more natural experiments (e.g. manipulating AMF abundance in the field using selective fungicide treatments) should allow for a more complete understanding of the functioning of a specific group of organisms. Experimentally gained information on individual interactions (for example an AMF-plant interaction in sterile soil) can be integrated with information, obtained from field experiments and observational studies, on possible biological and abiotic factors influencing the individual interaction (for instance the presence of interacting biota and nutrient levels in the soil), and thus the functional significance of the interaction of interest in the original soil environment. This crosstalk of results from field and controlled experiments also guides the design of more complex controlled experiments, where additional factors (e.g. nutrient status and/or increasing complexity of soil communities) can be included.

For the specific goal of investigating which biological agents cause variation in soil functioning and ecosystem services between agricultural fields of different management, it is, as explained above, not possible to rule out the effects of long term legacies of the microbial community on abiotic and biological characteristics of the soil, limiting the possibility to draw causal linkages in a biological

manipulation experiment. For instance, a fungicide treatment applied in the field will not exclude the long term effects of fungi on for example soil structure. In this particular case, the problem is that indirect fungal effects on plant performance we may want to exclude (i.e. those that operate on long timescales and are not the effect of direct plant-fungus interactions) are still present in the manipulated system. On the other hand, the causal linkages between microbial community composition, soil functioning and plant performance which can be investigated properly in controlled inoculation experiments only comprise the short term effects of the microbial community, because the long-term effects (e.g. soil structure, organic matter content, water retention capability) are wiped away during the experimental set up of the inoculation experiment. Specifically for AM fungi, the experimental procedures of an inoculation study – notably the sieving and mixing of soils - can also fundamentally change their functioning by destructing mycelial networks and promoting fast over slow growing AMF species (Evans and Miller 1990, Helgason et al. 1998). Put short, it becomes clear that essential trade-offs exist when choosing between different approaches to study the functioning of microbes (or other soil organisms) in an ecosystem context.

An essential point we want to make is that by combining different experimental methods and observational studies, a mechanistic explanation of the effects in a simplified experimental design can be tested for consistency in field situations, where many factors and interactions occur simultaneously. Experimentally clear-cut results might be hard to translate to patterns observed in the field (e.g. Vandegehuchte et al. 2010). If such an inconsistency of experimental and field data is found, hypotheses can be formulated on why this might be so – which factors are we missing out, and which biological interactions might be in play in the field, not included in the experimental treatments?

Hopefully, the above made clear that inoculation experiments face many limitations in assessing microbial community effects on the functioning of soils. However, they can considerably add to our understanding of soil biology and associated ecosystem services. To gain insight in the mechanistic basis of soil microbial effects on ecosystem functioning using inoculation approaches, and to assess the possible discrepancies between controlled experiments and field conditions, we advocate that the following questions need to be taken in consideration.

1. Which members of the microbial community are likely to be the causing agents for the observed effects, and how can we validate this causality?

2. To what extent does the microbial community established under experimental conditions reflect the original field community of interest? This is an essential point when extrapolating experimental results to field conditions.

3. Appreciating the complex nature of plant-soil biological interactions and their soil abiotic context dependency, can we expect the functional significance one ascribes to (members of) the microbial community to be the same in the original field where the experimental inoculum originated from?

4. Given the limited (short term) timescale of most inoculation experiments, which long term effects of the microbial community on plant performance are possibly missed out? Dealing with this problem is a matter of trying to integrate short term effects with long term effects, which will need to be assessed in separate studies, for example a mesocosm inoculation study and a long-term inoculation experiment in the field.

We explore the results presented in Verbruggen et al. (2012) with the above three questions in mind. In their first experiment, Verbruggen et al. (2012) found that maize plants reach significantly lower biomass in soil inoculated with live soil from the fields under study compared to a sterile control soil. However, no significant difference in plant biomass was found between organic versus conventional inoculum origin, while plant biomass was clearly more variable in the conventional inoculum treatment compared to the organic inoculum treatment. These results suggest a net negative impact of soil biota on plant growth irrespective of field management type, but more predictable 'black box' outcomes with soil biota originating from organic fields.

It is more rule than exception that plants grow better in sterile soil than on living soil (Kulmatiski et al. 2008). In other inoculation experiments, this phenomenon has been attributed to antagonistic organisms in the root environment, in many cases overruling positive plant-soil biological interactions. So who is causing the trouble for the maize in this experiment? Two observations support the interesting conclusion that AM fungi might be an important factor in this experiment, negatively affecting plant growth. First, in a soil treatment where AMF spores of *Glomus intraradices* were added to the sterile background soil, maize growth was significantly reduced, suggesting more costs than benefits of this plant-symbiont association under the experimental conditions (Johnson et al. 1997). Second, the extent of AMF colonization in the roots

was negatively correlated with plant biomass across all the treatments – including a sterile control, an AMF addition treatment and the live inocula originating from the different fields. This negative correlation between AMF colonization and plant biomass was retained when the sterilized treatment was removed from the analysis. Clearly, these observations are no proof for a causal relationship between AMF colonization extent and growth reduction of the maize plant. As the authors point out, other soil organisms could have been responsible for a direct negative effect. The strong negative correlation between intra-radical AMF abundance and plant biomass, together with the observation that a common AMF species reduced maize growth, make it however likely that indeed at least a part of the variation in plant growth in the experiment was attributable to plant-AMF interactions. It has to be noted that maize growers are not necessarily interested in aboveground biomass but rather in grain yield and its quality. Clearly, measuring such responses will require longer-term experiments.

In their second experiment, the authors further explored effects of the AMF communities on plant growth and phosphorus retention in the soil. Before harvesting the first experiment, watering was ceased for four weeks to promote sporulation of AM fungi. A selection of six soils from the first experiment, representing the full spectrum of AMF colonization variation, was used as an inoculum source for the second experiment. Treatments included 0% inoculum (sterile control), 4% and 12% inoculum (percentages are dry weight fraction). In order to standardize the microbial community composition across all the AMF treatments, a microbial wash composed of an AMF-free mixed filtrate from all inocula was added to all treatments.

In this second inoculation experiment, Verbruggen et al. (2012) found significantly lower intra- and extra-radical AMF colonization levels in the treatments with inoculum originating from conventional fields compared to those from organic fields. This lower AMF abundance in both the roots and in the soil was associated with higher plant biomass production, but traded-off with higher soil P-leaching. Although amounts of P leaching were not consistently related to the origin of the inocula, P leaching was significantly negatively correlated to AMF hyphal density in soil. Interestingly, molecular analysis revealed AMF species-specificity for both effects on plant growth and phosphorus retention. However, in response to question 2 (how well do the soil biota from controlled experiments represent the natural field communities?), the authors found that compositional divergence from the original fields had clearly occurred. AMF species richness was reduced to roughly half of the observed AMF richness in the fields. In how far other AMF properties such as total AMF abundance and community composition was affected by the experimental treatments was not further explored. Yet maize roots collected from the field did not show significant differences in % AMF colonization between conventional and organically managed fields (Table 1 in Verbruggen et al. 2012), while in the pot experiments using soil as

inoculum, colonization levels did differ between both management types (marginally non-significant higher colonization in the organic soils in experiment 1, significantly higher colonization in the organic soils in experiment 2). This discrepancy could potentially be due to the overruling impact of disturbance on AM fungi hyphal growth in the field and greater possibility for diverging AMF growth in a pre-culturing step. Under field conditions, hyphal networks of AMF will develop, but especially physical disturbance such as soil ploughing are very destructive to these mycorrhizal structures (Helgason et al. 1998). In the study system of Verbruggen et al. (2012), the organic and conventionally managed sites differed in the application of mineral fertilisers and pesticides and in crop rotation, but tilling practices were not different (they were not mentioned as being so) and the effect of tilling may overrule effects of pesticide and fertiliser use. Comparisons between fields with different levels of physical disturbance may thus yield greater differences in colonisation levels. When using soil inoculum, AM fungi need to establish a network from germinating spores and viable AMF remains in root fragments. The use of soil inoculum after a pre-culturing step in the greenhouse rather than directly from the field can have promoted larger AMF densities after the pre-culturing step with soil inoculum from organic fields, given their larger diversity and assuming niche complementarity between these AMF species (van der Heijden et al. 1998).

Ultimately, the authors aimed to improve our understanding of microbial soil functioning under contrasting management types. Soil processes and the associated ecosystem services are the result of a complex interplay of abiotic soil factors and biological activity in the rhizosphere and bulk soil. Communities of AMF might have different functional roles in soils of different management type due to differences in their abundance and composition (Oehl et al. 2003, Verbruggen et al. 2010) and/or due to the different abiotic and biotic context in which they need to function. From an agro-ecological perspective, this means that the net effect of AMF on the studied processes might be quite different in conventional versus organically managed systems. In conventional farming, AMF might only imply costs for the plant, because mineral phosphorus and other nutrients are generally added to the fields. In an organic farming context, AMF might be essential to prevent P limitation in the long run. This means that the functional significance of AMF can indeed be dependent on management type. One could further speculate about important interactions between AMF and other soil organisms, changing AMF community composition during the experimental treatments, and so on - all of this possibly dragging the obtained results out of a proper field context.

The considerations discussed above do however not preclude the quality and importance of the paper of Verbruggen et al. (2012). The study provides very interesting perspectives on the multi-functional role of AM fungi in agricultural systems and potential trade-offs between several functions provided by AMF. However, we believe that firm conclusions on the actual functional

outcomes of AMF communities from agricultural systems with either conventional or organic management are hard to make on the basis of the results presented in Verbruggen et al. (2012). The potential trade-off between nutrient retention and plant productivity in the AMF-maize interaction might hold true on a mechanistic basis, meaning that these individual effects of AMF (carbon cost for the plant and positive effect on P retention) potentially exist in field situations. The ultimate question for farming policy, however, is if this trade-off holds true in the full context of field conditions and in a wide range of soil types and levels of soil nutrients. Organic farming relies on internal, biologically driven nutrient cycling by retaining nutrients in the soil system in the form of organic matter and mineralization of internal and externally provided organic matter by soil biota (Altieri 1999). AMF communities play an important role in these processes – as the study of Verbruggen et al. (2012) confirms (for P rentention). Loss of AMF diversity or activity, especially of those adapted to the local abiotic and biotic environment in which they have to function, might thus negatively affect both P retention and crop production in the long run. Moreover, AMF are known to provide other ecosystem functions beyond P retention, such as suppression of soil pathogens and improving soil structure, which need to be taken into account in order to make up the balance between costs and benefits of AMF mediated impact of agricultural practices (Hart and Trevors 2005).

We conclude that Verbruggen et al. (2012) make an interesting contribution to our understanding of the functional role of AMF communities for the studied processes, but that the results of this paper only allow us to merely speculate about the realized functional role of the management-specific AMF communities in the complex web of below ground biological interactions and the complexity of the abiotic soil environment. We recommend that highly controlled experiments, such as the one discussed in this commentary, be complemented by long-term field studies where short as well as longer-term impacts of (manipulated) soil biota communities can be investigated, ideally across soils with different abiotic properties. As an intermediate between field and highly controlled mesocosm studies with inoculation of single (trophic) groups of soil biota, mesocosm studies with increasing biotic complexity and in different abiotic settings (e.g. nutrient levels) could provide an additional stepping stone in increasing our mechanistic understanding of the functioning of specific soil biota in their natural complex biotic and abiotic environment.

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Chapter 3

Plant traits predicting soil feedback

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Abstract

Plant trait and plant-soil feedback (PSF) concepts both advanced our understanding of plant community dynamics, but how they are interlinked is hitherto unknown.

We compared biomass production of 48 grassland species grown in soil conditioned by conspecifics with that of plants grown in sterilized soil (PSFsterilized) or in a mixture of the 48 different conditioned soils (PSFmixed). PSFsterilized reflects net effects of all soil biota, whereas PSFmixed captures the effects of relatively specialized soil organisms. We correlated these PSFs to relative growth rate (RGR), specific leaf area (SLA), specific root length (SRL) and percent arbuscular mycorrhizal fungi (AMF) colonization. Furthermore, we compared interspecific variability and species-rankings of PSFsterilized and PSFmixed.

Plant species with high SRL and low AMF colonization had most negative PSFsterilized, while species with opposite trait values experienced neutral to positive PSFsterilized. PSFmixed resulted in similar species ranking and showed similar relations to the plant traits as PSFsterilized, albeit less strong.

We conclude that SRL and AMF colonization, but not SLA and RGR, are good predictors for plant-soil feedback effects. Plant species with thin roots and poor mycorrhizal colonization are more likely to suffer from growth reducing soil biota than species with thick roots and high AMF colonization.

Introduction

Interactions between plants and soil organisms are known to be important determinants of plant performance, affecting plant population and community dynamics (Wardle et al. 2004, Bever et al. 2010). At the same time, plant trait approaches have gained attention in predicting effects of plants on ecosystem processes (Violle et al. 2007, De Deyn et al. 2008, Lavorel and Grigulis 2012, Baxendale et al. 2014), plant performance along environmental gradients (Lavorel and Garnier 2002), and plant ecological strategies (e.g., Grime 1977, Díaz et al. 2004, Wright et al. 2004, Reich 2014). Thus far, plant trait frameworks have been mainly based on principles of resource capture, abiotic stress (in)tolerance, competitive ability, and dispersal strategies (e.g., Grime 1977, Tilman 1988, Craine 2009). Traits are often found to be inter-correlated, presumably because of the existence of trade-offs among these traits (Westoby et al. 2002, Reich et al. 2003). Based on this, so-called trait syndromes have been established (e.g., Wright et al. 2004). Although there is increasing attention for plant traits that are related to plant interactions with belowground biota (Bardgett et al. 2014) little is known about plant traits that may predict how plants affect and respond to soil through plant-soil feedback (PSF) effects (Bever et al. 1997, van der Putten et al. 2013). Here we examine how plant traits may be indicative of PSF effects in terms of sign (positive or negative) and magnitude.

PSF can be studied in a variety of ways, both regarding the experimental methods used and the type of statistical analysis performed (Brinkman et al. 2010). In the present study, we tested growth of 48 grassland plant species in three soil treatments: (I) soil conditioned by conspecifics (conspecific soil), (II) a mixture of all 48 species-specific conditioned soils (mixed soil) or (III) sterilized soil. In all treatments we used sterilized soil inoculated with living (treatments I and II) or sterilized soil inoculum, as this approach enables equalizing abiotic properties between the soil treatments (Troelstra et al. 2001). We compared plant growth in conspecific conditioned versus sterilized soil (named PSFsterilized) and plant growth in conspecific conditioned vs. mixed conditioned soils (named PSFmixed). These PSF values have been used interchangeably in some previous meta-analyses (Kulmatiski et al. 2008, Meisner et al. 2014), but their interpretation might differ: PSFsterilized is expected to reflect net effects of all soil biota on plant performance, whereas PSFmixed will reveal effects of relatively specialized soil biota on plant performance.

Predictability of the outcomes of PSF interactions is poorly developed, although predicting soil microbial processes by plant traits is gaining increasing interest (Orwin et al.

2010, Baxendale et al. 2014, Ke et al. 2014). For example, traits of fast versus slow growing plant species differentially influence the quality of litter and the microbial decomposition pathway via nutrient cycling-related mechanisms (Baxendale et al. 2014). Subsequent soil feedback effects will influence the traits of the subsequent plant species that can thrive best under the modified conditions, which may lead to self-enhanced processes in plant community development and ecosystem functioning (Wardle et al. 2004). Other studies have shown that the direction and effect sizes of PSF effects governed by soil microbiota likely depend on plant functional type (Kulmatiski et al. 2008, Meisner et al. 2014): especially grasses and early successional plant species showed predominantly negative soil feedback, likely due to the build-up of host-specific plant pathogens (Kulmatiski et al. 2008, Kardol et al. 2006). However, in spite of some broad scale overview studies, still little is known about how functional plant traits may relate to PSF.

It is well recognized that trade-offs exist between plant resource capture through investment in acquisitive traits and vulnerability to natural enemies (e.g., Herms and Mattson 1992, van der Putten et al. 2003, Rasmann et al. 2011). For example, plant growth rate may relate positively to herbivory rate of leafs (e.g., Coley 1988, Fine et al. 2006), as well as of roots (Bauerle et al. 2007). Plants are known to have sets of traits that are usually inter-correlated, for example high relative growth rate (RGR) often correlates with high specific leaf area (SLA) and high specific root length (SRL) (Reich 2014). From this perspective, plant species could be regarded as being 'slow' (resource conservative, well defended) with low RGR, SLA and SRL or 'fast' (resource acquisitive, poorly defended) with high RGR, SLA and SRL (Grime 1997, Westoby et al. 2002, Reich 2014).

Many plant species associate with arbuscular mycorrhizal fungi (AMF), which can stimulate plant growth both via enhancing nutrient acquisition (Smith and Read 2010) and by plant protection from root pathogens (Newsham et al. 1995). The benefits to plant growth of colonization by AMF remain hard to predict (Johnson et al. 1997, Hoeksema et al. 2010), but it can be expected that plant species with low SRL gain larger benefits, at least when pathogen pressure is relatively low (Newsham et al. 1995, Smith and Read 2010, Blumenthal et al. 2009). Observations that early secondary successional (fast growing) plant species have negative PSF and later secondary successional (slow growing) species have positive PSF (Kardol et al. 2006), generated our overall hypothesis that PSF will become more negative with increasing trait values that are characteristic for acquisitive species (high RGR, SLA, SRL), whereas PSF will become more positive with increasing colonization by AMF. Specifically we tested the hypotheses that (1) species ranking according to their PSF value is

similar for PSFsterilized and PSFmixed, that is, soil sterilization and mixing species-specific soils have a similar effect on a species' plant biomass production, relative to biomass production in conspecific soil; (2) SRL, SLA and RGR are negatively and (3) AMF% colonization is positively related to PSFsterilized and PSFmixed.

Materials and methods

Study system

The plant species used for the plant-soil feedback (PSF) experiments are typical for mesophilic Central-Western European grasslands (Supp. Table. 1). We used 48 of in total 60 study species present in the Jena biodiversity experiment (Roscher et al. 2004). Because of poor germination, 12 plant species were left out. Seeds were provided by commercials suppliers that collect seeds from wild populations in Germany, and the soil (Eutric Fluvisol, developed from loamy sediments) originated from the Jena field site (Jena, Thuringia, Germany, 50° 55' N, 11° 35' E) (Roscher et al. 2004). A two-stage PSF experiment was carried out in pots in a climate-controlled greenhouse. To determine the plant traits relative growth rate (RGR), specific root length (SRL) and specific leaf area (SLA) individuals of all species were grown in additional experiments under controlled conditions in the Botanical garden of Leipzig University and in a greenhouse of NIOO-KNAW at Wageningen, the Netherlands (see below).

Plant-Soil Feedback (PSF)

Plant germination

In December 2010, plant seeds were surface-sterilized by soaking in 5% or 25% household bleach (5% sodium hypochlorite solution) for 30 seconds. 25% household bleach was used when 5% bleach was not a sufficient concentration to prevent fungal infection of seeds. The sterilized seeds were rinsed with demineralized water and placed in a growth cabinet (16h/8h 22°C/16°C light/dark) on water-saturated glass beads in plastic boxes closed with a transparent plastic lid. Based on Roscher et al. (2004), prior to germination, some seeds were scarified or treated with gibberellic acid (Sigma Chemical co., St. Louis, USA). After germination, which took 3-12 days, seedlings were transferred to a climatized room at 4°C

and 16h/8h light/dark conditions, which kept them all in the same post-germination stage until planting.

Soil conditioning phase

End December, 384 pots of 1.5 liter each were filled with 1500 g of soil, consisting of a mixture of 80% sterilized soil and 20% unsterilized soil (based on dry soil weight). Pots were arranged in the greenhouse (16h/8h light/dark) in 8 replicate blocks. Two seedlings per species were planted per pot and pots were spatially randomized within blocks. Soil moisture level was re-set to 25% (w/w) by adding demineralized water until pots were at pre-set weight equivalent to 30% moisture. This was repeated every second or third day. After two months of growth, plants were harvested and the soil was collected from each pot individually in order to be used in the feedback phase. During harvest, aboveground biomass was clipped and dried at 70°C for minimally 72 h. Adhering soil was shaken off roots before rinsing with tap water and drying them at 70°C for minimally 72 h.

Soil from each plant species was stored separately in plastic bags at 4°C. Cross-contamination of soils from different plant species during harvest was avoided by cleansing all used material in 70% ethanol in between working steps. A 50 ml subsample was taken from all plant species-specific conditioned soils and dried at 40°C during 72 h in order to analyze nutrient concentration and moisture content. Plant-available P was determined according to Olsen et al. (1954). Soil mineral N was extracted by shaking 10 g (dry weight) soil with 50 ml 1 M KCl for 2h. NH_4^+-N and NO_3^--N were determined calorimetrically in the KCl extract.

Feedback phase

All 48 soils from the conditioning phase were split into three equal parts. One part was left untreated and kept separate per species (named: conspecific conditioned soil), the second part was left untreated and used to prepare a mixture of all soils based on equal dry weight proportions of the species-specific soils (named: mixed conditioned soil). The third part was kept separate per species and was sterilized by gamma irradiation (25 KGray; named: sterilized soil). All soil treatments thus obtained were mixed individually with a sterilized background soil from the Jena experimental field site as 65% sterilized soil and 35% living inoculum soil (of one species or of the mixture of all species) or sterilized inoculum soil (w/w).

To test the plant responses to the soils, seeds of all plant species were germinated as

done before, and seedlings were planted in pots with either 550 g of the conspecific, mixed conditioned or sterilized soil inoculum treatment. Treatments were carried out in a randomized block design with eight replicates, resulting in an experimental design of 48 plant species x 3 soil treatments x 8 replicates = 1152 pots. All seedlings that died in the first week after planting were replanted and pots were spatially randomized within blocks. Plants were watered every 3 or 4 days using demineralized water to re-set moisture to 25% (w/w). After growing for 6 weeks, all plants were harvested as described for the soil conditioning phase, and total dry weight was determined.

Trait data

Relative Growth Rate (RGR) was determined in the greenhouse (16h/8h light/dark) in soil consisting of approx. 40% mixed conditioned soil inoculum and 60% sterilized Jena soil. To quantify RGR the proportional biomass increase from one-week old plants, grown in cylinder pots of 3 cm diameter x 6 cm depth, to three week old plants, grown in pots of 7 x 7 x 7 cm was measured (Hendry and Grime 1993). One-week old specimen of all study species were harvested the same day. Three-week old specimen were harvested at 3 different dates (one replicate block per day), washed free of soil particles, and dried at 70°C for at least 48 hours. RGR was calculated as (log W2 - log W1)/(t2-t1) (Hendry and Grime 1993), where W1 is total dry weight biomass at t1 = 7 days and W2 is total biomass at t2 = 21, 23 or 24 days (three replicate blocks).

SLA and SRL were quantified in a mesocosm experiment, which was conducted outdoors in the Botanical Garden of Leipzig (Germany) in 2011. Each species was represented as five replicates in a randomized block design. Individual plants grew for 12 weeks in separate mesocosms of 60 cm height and 15 cm diameter, filled with a mixture of soil derived from experimental plots of the Jena Experiment and sand (20%). Sampling and measurements of SLA followed recommendations of Cornelissen et al. (2003). Roots where washed using tap water, fine sieves and forceps. Cleaned roots where scanned and measured using a flat-bed scanner and the software WinRizo (Regent Instruments Inc., Canada) to estimate root length. Root mass was measured after drying for 48 hours at 70°C. SRL was then calculated as root length to root dry mass ratio.

Arbuscular mycorrhizal fungi (AMF) colonization levels in roots were determined for a random subset of 28 out of the 48 species (7 graminoids, 10 legumes and 12 forbs). Root material from plants grown in conspecific conditioned soil (see ‘feedback phase’, p. 32) was rinsed free of soil and stored in 50% ethanol. The fungal structures in roots were stained with

Trypan blue using a standard protocol (Brundrett et al. 1996). In short, roots were cut into fragments of approx. 2 cm and cleared in KOH at 90°C for 20-50 minutes, depending on root thickness.

After rinsing with tap water, roots were acidified in 2% HCL for one hour and subsequently stored overnight in 0.01% Trypan blue in a 5:1:1 mixture of lactic acid, demineralized water and glycerol. Roots were de-stained and stored in 50 % glycerol for microscopic investigation. To this end, roots were dispersed in a Petri dish of 5 cm diameter with a counting grid and examined under a microscope with magnification 10 x 40. The AMF colonization percentage of the roots was estimated according to the grid line intersection method (McGonigle et al. 1990). Distinction was made between septate and non-septate hyphae, the latter representing AMF (Hudson 1991).

Data analyses

Plant dry weights in the three soil treatments were used to calculate two PSF values per experimental block as log (total dry weight in soil type X / total dry weight in soil type Y) (Brinkman et al. 2010) where for PSFsterilized: X= conspecific conditioned soil and Y= sterilized soil and for PSFmixed: X= conspecific conditioned soil and Y= mixed conditioned soil. We tested whether plant-soil feedback effects could be attributed to altered nutrient levels (NO_3^-, NH_4^+ and Olsen's P) as a result of different nutrient levels in the different plant species' inocula by testing the correlation between PSFsterilized and the difference in nutrient levels in the conditioned vs. sterilized soil treatment. In order to test how well PSFsterilized corresponds with PSFmixed, a Spearman's rank-correlation test between the two PSF values was performed. Spearman's correlation analyses were also performed between plant functional trait values and the two PSF values. Due to the fact that Spearman's correlation test cannot handle ties (present in AMF colonization data), we used linear models to test the relationships between AMF colonization level in conspecific soil and the two PSF values, and between AMF colonization level in conspecific soil and SRL. We reported on Spearman's rho correlation coefficients for comparability with the other relationships. All analyses were done with the statistical software R version 3.1.0 (R core team 2013).

Results

PSFsterilized and PSFmixed

Across all plant species PSF effects ranged from negative to positive: PSFsterilized varied from - 0.81 to + 0.63, with a mean of - 0.10 $\pm$ 0.05 SE, while PSFmixed ranged from - 0.22 to + 0.59, with a mean of - 0.09 $\pm$ 0.02 SE (Fig. 1a,b, Supp. Fig. 1). The variance of PSFsterilized was significantly higher than the variance of PSFmixed (Bartlett's test $P < 0.0001$) (Supp. Fig. 1). Overall, the mean of PSFsterilized across the 48 species was marginally ($P = 0.06$) and the mean PSFmixed was significantly ($P = 0.0003$) negative. There was a positive correlation between the ranking of PSFsterilized and PSFmixed ($r = 0.33$, $P = 0.02$, Fig 1c).

We found no correlation between PSFsterilized and the difference in nutrient levels between the conditioned and sterilized soil for NH_4^+ ($r = 0.15$, $P = 0.33$), NO_3^- ($r = -0.08$, $P = 0.62$) and P-Olsen ($r = 0.19$, $P = 0.22$). There was also no relation between PSFmixed and nutrient levels in the soil inocula: NH_4^+ ($r = 0.07$, $P = 0.64$), NO_3- ($r = 0.04$, $P = 0.76$) and P-Olsen ($r = -0.05$, $P = 0.73$) (data not shown).

Plant traits and PSFs

PSFsterilized was negatively correlated with specific root length (SRL) (rho = -0.45, $P = 0.001$) and positively correlated with AMF colonization percentage of roots in conspecific soil (rho: 0.52; $P = 0.003$ (linear model)) (Fig. 2a,b). However, there was no correlation with relative growth rate (RGR) (rho = -0.15, $P = 0.30$), or with specific leaf area (SLA) (rho = 0.05, $P = 0.75$) (Fig.2c,d).

PSFmixed was marginally negatively correlated with SRL (rho = -0.25, $P = 0.08$) (Fig. 3a). In contrast, PSFmixed was not correlated with AMF colonization percentage of roots in conspecific soil (rho = 0.32; $P = 0.14$ (linear model)), with RGR (rho = -0.12, $P = 0.43$), or with SLA (rho = -0.11, $P = 0.44$) (Fig.3b,c,d). AMF colonization percentage of roots in conspecific soil was negatively correlated to SRL (rho = -0.81; $P < 0.001$ (linear model)) (Fig. 4).

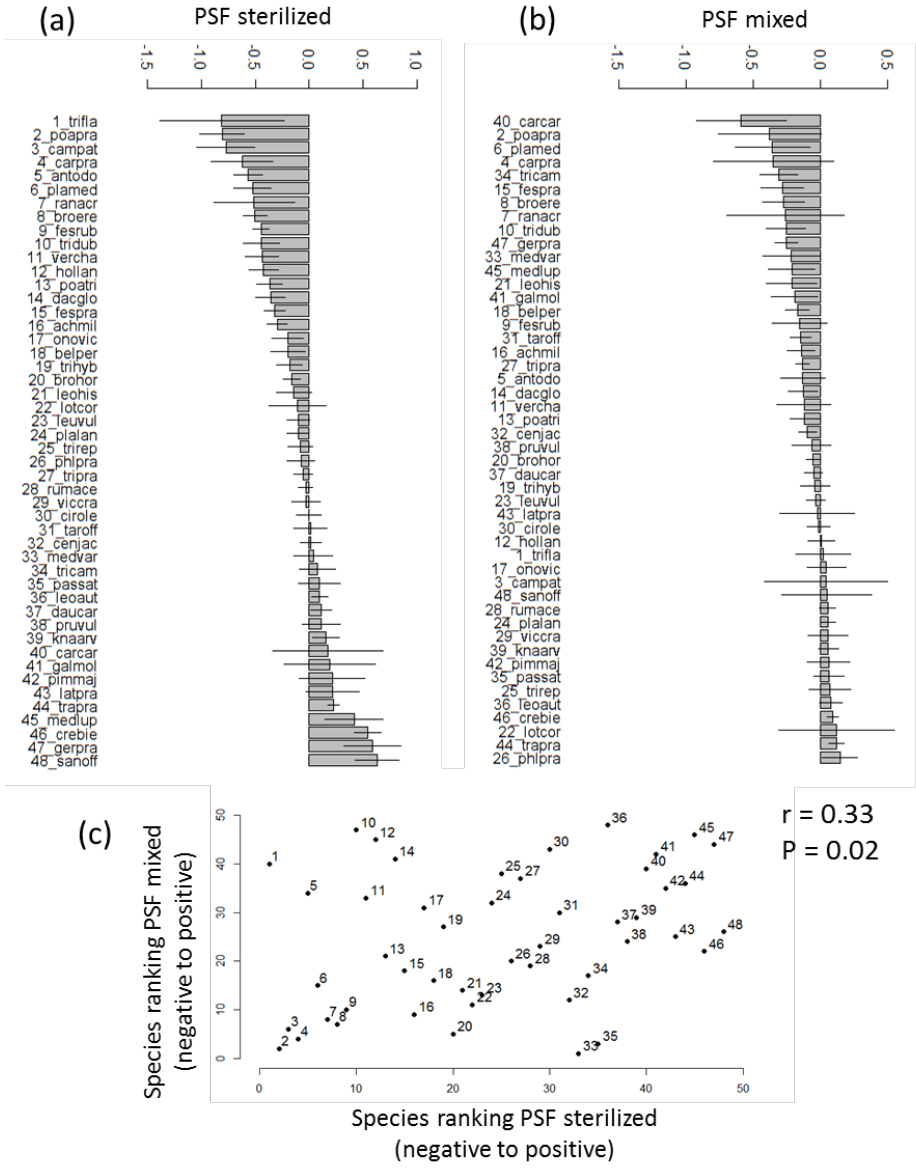


Figure 1 (a) PSFsterilized and **(b)** PSFmixed and **(c)** correlation of species rankings for PSFsterilized and PSFmixed of our 48 study species. PSFsterilized = log(biomass in conspecific soil) - log(biomass in sterilized soil); PSFmixed = log(biomass in conspecific soil) - log(biomass in mixed conditioned soil). Error lines indicate $\pm$ SE; label numbers (1-48) indicate the species ranking according to their PSFsterilized (in all panels), from most negative to most positive.

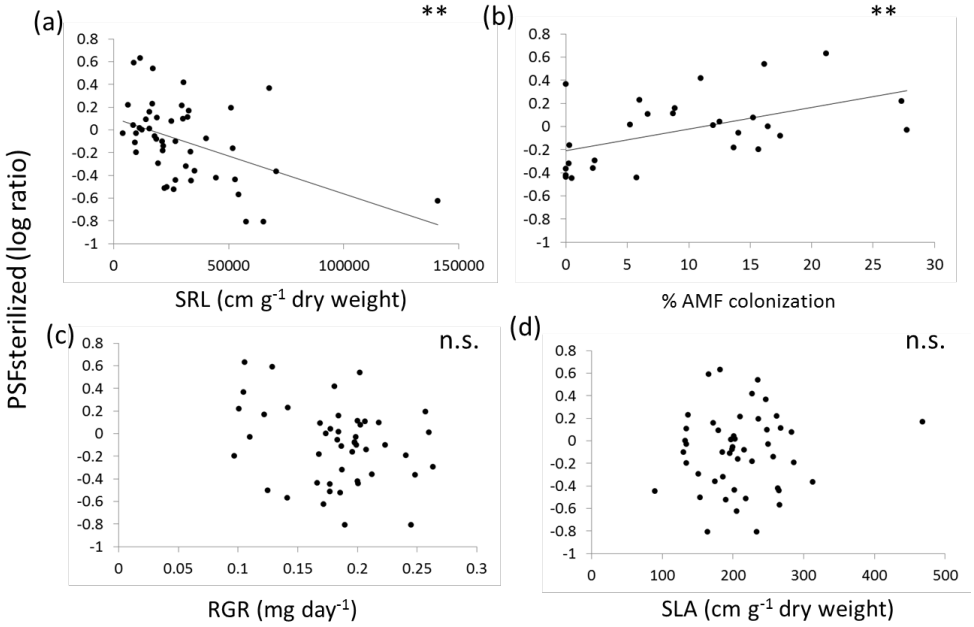


Figure 2 Relationship between PSFsterilized and **(a)** specific root length (SRL), **(b)** AMF colonization in conditioned soil (% root segments colonized by hyphae) **(c)** relative growth rate (RGR) and **(d)** specific leaf area (SLA). PSFsterilized = log(biomass in conspecific soil) - log (biomass in sterilized soil); number of asterisks denotes significance of Spearman's correlation test or general linear model (for AMF colonization) ($P < 0.01$ **, n.s. = not significant).

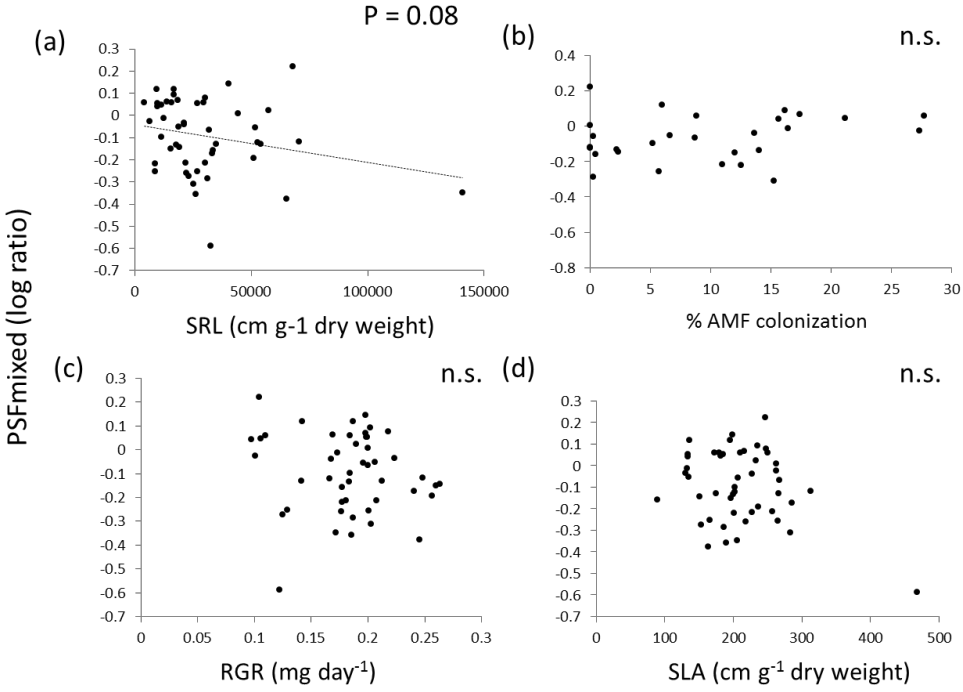


Figure 3 Relationship between PSFmixed and **(a)** specific root length (SRL), **(b)** AMF colonization in conditioned soil (% root segments colonized by hyphae) **(c)** relative growth rate (RGR), and **(d)** specific leaf area (SLA). PSFmixed = log(biomass in conspecific soil) - log(biomass in mixed conditioned soil); n.s. = not significant.

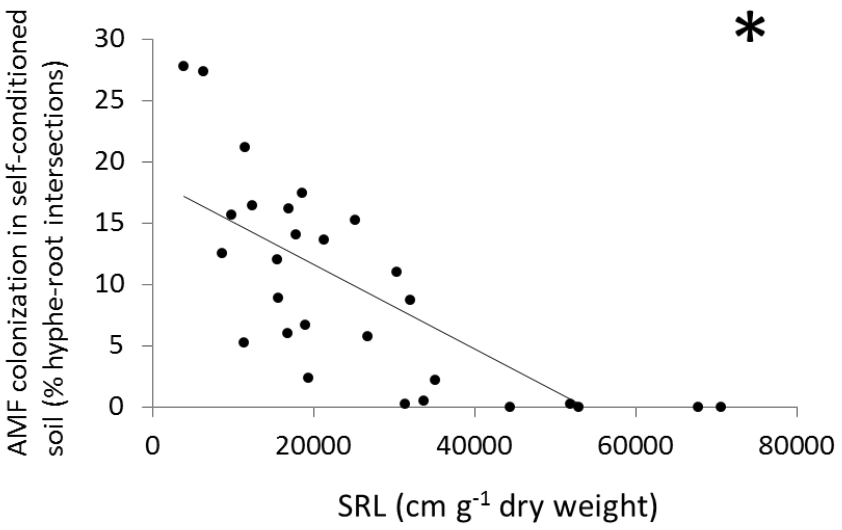


Figure 4 Relationship between specific root length (SRL) and AMF colonization in conditioned soil (% root segments colonized). Number of asterisks denotes significance of general linear model ($P < 0.05$ *).

Discussion

Overall, we found predominantly negative plant-soil feedback (PSF) across the 48 plant species, both for PSFsterilized and PSFmixed. The values of both PSF types ranged from negative to positive. There was a negative relation between both PSFs and specific root length (SRL). This relation was marginally significant for PSFmixed, and highly significant for PSFsterilized. The weaker relation with PSFmixed may be due to the fact that variation of PSFsterilized across species was larger than variation of PSFmixed across species, resulting in a stronger gradient. The larger variation of PSFsterilized furthermore indicates that the net effects on plant growth of all biota are stronger than the effects of host plant-specific soil biota. Moreover, it may well be that the morphological plant trait SRL is especially relevant for the strength of effects caused by more general root pathogens and mutualists, while those caused by more specialized organisms may come about via more species-specific root traits such as typical chemical defense compounds (Hawes et al. 2000).

Contrary to our predictions, we did not find significant correlations between PSFsterilized or PSFmixed and relative growth rate (RGR) or specific leaf area (SLA). We

expected to find negative correlations if there would be a trade-off between plant resource capture through investment in resource acquisition-related traits and vulnerability to natural enemies, with coordinated responses for both aboveground and belowground plant organs (e.g., Mooney 1972, Herms and Mattson 1992, van der Putten 2003, Rasmann et al. 2011). In our study, aboveground traits (i.e. SLA) and whole-organism traits (i.e. RGR) did not correlate with PSF, as opposed to root-related traits (SRL and AMF colonization percentage). These results are well in line with recent work on the predictive capacity of aboveground and belowground plant traits for microbial composition and microbial processes, where root traits but not shoot traits showed significant effects (Legay et al. 2014).

We found the percentage of root infection by AMF in conspecific conditioned soils to be significantly positively related to PSFsterilized. Together with our finding that PSFsterilized was significantly and PSFmixed was marginally related to SRL, we suggest that SRL is a key plant trait mediating the feedback effect of AMF on plant growth. This interpretation is supported by previous findings showing that mycorrhizal benefits to plants are generally higher when SRL is lower (Smith and Read 2010). A recent meta-analysis (Maherali 2014), however, challenged this common idea by showing that there is no consistent correlation between different measures of root coarseness and mycorrhizal growth response. Nevertheless the few studies available indicated a negative correlation between SRL and level of infection with, and plant growth response to, AMF. It has to be noted that imposed association between a plant species and a specific mycorrhizal community as in studies with commercial inoculum can yield biased results regarding mycorrhizal responsiveness in natural systems (Maherali 2014). In our study, we determined mycorrhizal colonization of plants growing in soil that contained a natural pool of soil biota species including AMF, collected from the Jena Experiment field site. Testing plant responses in these soils, as we did, might be more indicative for the actual benefits of the plant-AMF association for plants, because all plants were exposed to their natural AMF assemblages while also interacting with other soil biota (Graham et al. 1991, Hoeksema et al. 2010, Cortois and De Deyn 2012).

We conclude that plant species with thin roots experience stronger negative interactions with soil biota in their environment than plants with thick roots. This result supports our expectation that plants with fast growth characteristics, which includes high SRL, have a more negative PSF than plants with slow growth characteristics. We found a positive correlation between the species ranking of PSFsterillized and PSFmixed. This correlation implies that different ways of testing plant-soil feedback effects generally may

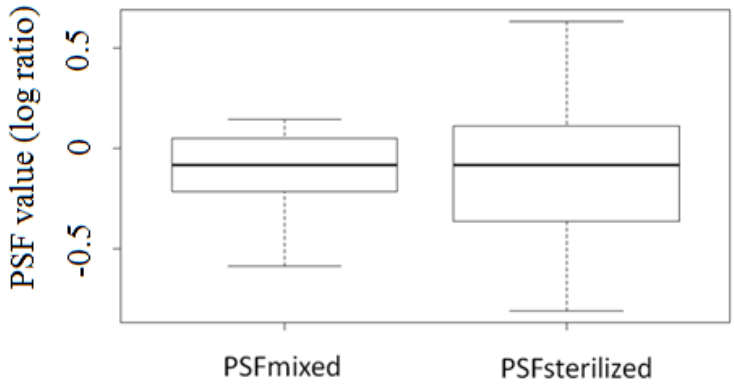
give comparable results. Moreover, the comparable ranking suggests that plants suffering from soil-borne pathogens benefit more from growth in soil from other plant species than plant species that benefit from symbionts. In the field, therefore, plant species that are sensitive to soil pathogens might be more at advantage in plant communities with strong spatio-temporal dynamics than plant species that have net benefit from symbionts. Our findings that plant traits (SRL and mycorrhizal colonization rate) have predictive power for the direction and magnitude of PSFsterilized, and that PSFsterilized has similar species ranking as PSFmixed, opens up new perspectives to link PSFs as they occur in nature (van der Putten et al. 2013) to plant ecological strategy theory (e.g., Grime 1977), thereby placing species-specific PSFs in a broader plant ecological and evolutionary framework.

Acknowledgments

We are grateful to Amalia Castro and Nasir Uddin for practical assistance. This work was supported by the Deutsche Forschungsgemeinschaft (FOR 1451) and by NWO-ALW VIDI through financial support to GBDD (grant nr 864.11.003), and NWO additional fund (grant nr 832.13.009) to WvP.

Supplementary information

Supplementary Figure 1 Boxplots of PSFsterilized and PSFmixed. PSFsterilized = log(biomass in conspecific soil) - log(biomass in sterilized soil); PSFmixed = log(biomass in conspecific soil) - log(biomass in mixed conditioned soil).



Supplementary Table 1 Six-letter abbreviation and full name of study species.

abbreviation	full name	abbreviation	full name
Ach mil	*Achillea millefolium*	Med lup	*Medicago lupulina*
Ant odo	*Anthoxanthum odoratum*	Med var	*Medicago varia*
Bel per	*Bellis perennis*	Ono vic	*Onobrychis viciifolia*
Bro ere	*Bromus erectus*	Pas sat	*Pastinaca sativa*
Bro hor	*Bromus hordeaceus*	Phl pra	*Phleum pratense*
Car car	*Cardamine pratensis*	Pim maj	*Pimpinella major*
Car pra	*Carum carvi*	Pla lan	*Plantago lanceolata*
Cen jac	*Centaurea jacea*	Pla med	*Plantago media*
Cir ole	*Cirsium oleraceum*	Poa pra	*Poa pratensis*
Cre bie	*Crepis biennis*	Poa tri	*Poa trivialis*
Dac glo	*Dactylis glomerata*	Pru vul	*Prunella vulgaris*
Dau car	*Daucus carota*	Ran acr	*Ranunculus acris*
Fes pra	*Festuca pratensis*	Rum ace	*Rumex acetosa*
Fes rub	*Festuca rubra*	San off	*Sanguisorba officinalis*
Gal mol	*Galium mollugo*	Tar off	*Taraxacum officinale*
Ger pra	*Geranium pratense*	Tra pra	*Tragopogon pratensis*
Hol lan	*Holcus lanatus*	Tri cam	*Trifolium campestre*
Kna arv	*Knautia arvensis*	Tri dub	*Trifolium dubium*
Lat pra	*Lathyrus pratensis*	Tri fla	*Trisetum flavescens*
Leo aut	*Leontodon autumnalis*	Tri hyb	*Trifolium hybridum*
Leo his	*Leontodon hispidus*	Tri pra	*Trifolium pratense*
Leu vul	*Leucanthemum vulgare*	Tri rep	*Trifolium repens*
Lot cor	*Lotus corniculatus*	Ver cha	*Veronica chamaedrys*
Luz cam	*Luzula campestris*	Vic cra	*Vicia cracca*

Chapter 4

Phylogenetic distance influences plant-soil feedback between plant species

Roeland Cortois, Dan Flynn, Gerlinde B. De Deyn, Wim H. van der Putten

To be submitted

Abstract

Plant-soil feedback (PSF) is increasingly recognized as a key mechanism driving spatio-temporal dynamics in plant communities. Many studies have shown that most plant species perform better in soils that have been influenced by other plant species; however, the mechanisms underlying these general responses have received relatively little attention. In order to provide a more general mechanistic understanding on the role of PSF in plant community dynamics, we examined if PSF between plants may depend on the phylogenetic relatedness between the interacting plant species. We hypothesized that distantly related species would benefit from avoiding their own negative PSF, whereas closely related species would benefit from each other's positive PSF. In order to test these hypotheses we grew 11 Western European grassland plant species with various degrees of phylogenetic distance between the 11 focal species and the species that conditioned their soil. Overall, plants with a negative PSF performed equal or better in soils from distantly related species. Plant species with a neutral or positive PSF generally performed worse in soil from more distantly related plant species. We conclude that negative PSFs may promote co-existence of more distantly related plant species, whereas positive PSF may promote stronger phylogenetic clustering in plant communities. We propose that further understanding of the role of PSF in plant community dynamics requires incorporation of phylogenetic relatedness and, ultimately, eco-evolutionary dynamics in plant-soil interactions.

Introduction

Reciprocal interactions between plants and soil organisms contribute to plant-soil feedbacks (PSFs), which have been identified as a major driver of spatio-temporal dynamics of plant communities (van der Heijden et al. 2008, Bever et al. 2010, van der Putten et al. 2013). Soil organisms can exert strong effects on plant performance and vary in mode of action. For example pathogens cause plant damage which suppresses plant growth and mutualistic symbionts and decomposer organisms provide plant growth limiting nutrients so that they generally promote plant growth (Wardle et al. 2004, Raaijmakers et al. 2009). Plant species promote specific microbial taxa in the rhizosphere (e.g., Westover et al. 1997, Bezemer et al. 2010), thereby creating plant species-specific biotic 'legacies' in the soil, which can persist over multiple plant generations (Kulmatiski and Beard 2011). Adaptive capacities in both plants (Lankau et al. 2009) and soil organisms (Lau and Lennon 2012) suggest that PSF may have an evolutionary role in the selection of plant and microbial traits (Schweitzer et al. 2014). This raises the question how plant phylogeny is related to the strength and direction of PSF between species (i.e. interspecific PSF). However, little is known about whether such a relation may exist, and empirical tests are needed.

In species-poor plant communities, such as occurring in early stages of primary succession, plant performance will be mainly influenced by PSF of conspecifics (van der Putten 2003). However, in mixed plant communities, PSF interactions involve multiple plant species growing in soil patches with a legacy of conspecific and of various heterospecific plant individuals (Brandt et al. 2013). The consequences for the performance of a specific plant species may depend on whether the soil has a predominant legacy of its own (conspecific), or of other (heterospecific) plant species. Numerous studies show that most plant species perform better when growing in soil with a legacy of heterospecific plants relative to performance in soil with a legacy of conspecific plants (Kulmatiski et al. 2008). This suggests that plant species will disappear from their current patches and will proliferate in patches with a legacy of other plant species. (e.g., van de Voorde et al. 2011)

An important and unaddressed question is how the variation in PSF effects may contribute to eco-evolutionary dynamics in plant communities (Bardgett and van der Putten 2014). Variation in PSF can be predictive for spatial patterns of species abundances in plant communities (Liu et al. 2012), overyielding of species in soils with a legacy of particular heterospecific plants (Kulmatiski et al. 2012) and the potential outcomes of exotic plant introductions (performance in non-native range) (Callaway et al. 2013, Suding et al. 2013).

Several studies have reported high prevalence of evolutionary conserved host-ranges of plant pathogens (e.g., Parker and Gilbert 2004, Gilbert and Webb 2007). Such evolutionary conservatism is expected to occur for root inhabiting mutualists as well (e.g., Hart et al. 2003, Maherali and Klironomos 2007, Kiers et al. 2010), although these are expected to have wider plant host ranges than pathogens (Richardson et al. 2000). Evolutionary conservatism of host ranges implies that phylogenetic distance between preceding and succeeding plant species may matter for their interaction via PSF, because phylogenetic distance may be predictive for the degree to which plant species may influence each other via PSF. Until now, no studies have tested effects of phylogenetic distance between plant species on PSF between preceding ('conditioning') and succeeding ('focal') plant species. In a recent study, Anacker et al. (2014) tested the effect of phylogenetic relatedness between 57 plant species on similarity of their responses to soil biota/soil legacies; however, they did not examine the effect of phylogenetic distance between response plants and the plant species that created the soil biotic legacy.

In order to examine the relation between phylogenetic distance and PSF, we conducted a controlled greenhouse experiment with 11 plant species that naturally co-occur in Central-Western European grasslands. We tested biomass production of these 11 focal plant species in soils with a legacy of conspecifics or of other species of known phylogenetic distances between the focal plants and soil-conditioning plants. The range of focal plant species included a variation of PSF responses to conspecific soil ranging from positive to negative. We tested the hypotheses that plant species suffering from conspecific PSF benefit more from feedback of distantly related than from closely related plant species and that plant species that benefit from positive conspecific PSF suffer most from feedback from distantly related plant species.

Material and methods

Soil origin

In September 2010, soil was taken from the top 30 cm of the Jena Experiment, a large ongoing grassland biodiversity experiment in Germany (Roscher et al. 2004). We conditioned the soil by growing 49 different plant species individually for two months in 1.5 liter pots filled with 1500 g of sieved (mesh size 1 cm) soil, consisting of a mixture of 80% sterilized

(using gamma-radiation) field soil and 20% unsterilized field soil, based on dry weight (w/w). There were eight replicate pots of each species. These so-called conditioned soils were lumped per plant species and homogenized with sieved sterilized field soil so that the mixture contained 35% conditioned soil; as a control we used sterilized soil inoculum. Then, new individuals (8 replicates) of the same plant species were grown in these soils, in pots of 550 ml filled with 550 g of soil. After two months, plants were harvested entirely and soils were stored at 4 ^{0}C for 12 months. These double conditioned soils were used to perform the growth experiment in which we test the relationship between PSF and phylogenetic distances.

Selection of focal species

We selected 11 focal species to represent three groups of plant species based on the sign of their conspecific PSF, spanning a PSF range from slightly positive to strongly negative. The quantification of the conspecific PSF was based on their biomass production in sterilized background soil inoculated with conspecific soil compared to sterilized soil inoculated with sterilized conspecific soil inoculum (chapter 3). We selected 5 plant species with significant negative conspecific PSF, 3 species with the response close to zero, and 3 species that had a significant positive response.

Selection of soil-conditioning species

Estimates of phylogenetic distances between the plant species were quantified using divergence time between the 49 plant species according to Allan et al. (2013). For each focal species we identified the plant species that was most closely related to the focal plant, and 2 or 3 additional ranges of increasing phylogenetic distance, in such a way to make sure that phylogenetic distance levels between the focal species and the species that conditioned the soil (see 'Soil origin') were similar for all focal test species (Supp. Table 1). For all species, soil conditioned by conspecifics was used to represent phylogenetic distance level 0. In total we used 31 soil types to span 4 levels of phylogenetic distance between the species that conditioned the soil and the responding species, for each of our 11 focal species.

Experimental set-up and biomass harvest

Pots were filled with 550 g of soil consisting of conditioned soil mixed with 65% sterilized soil as described above in 'Soil origin'. Plants were germinated on moist glass beads in a germination chamber and subsequently transferred to a climate room at 4°C, which kept them all in the same post-germination stage until planting. The plants were grown in the greenhouse (16h/8h light/dark) and were watered every second day to constant weight to maintain 25% moisture level (w/w). Every combination of response plant – soil-conditioning plant was replicated eight times, and each replicate was placed randomly in a block. Each of the eight blocks was placed on a separate trolley in the greenhouse. There were 416 pots in total. After five weeks of growth, plants were harvested per block (1 to 2 blocks per day). Aboveground biomass was clipped and dried at 70°C for minimally 72 h. Roots were shaken free of adhered soil, rinsed with tap water, dried at 70°C for minimally 72 h and weighed.

Data analysis

We tested the effects of phylogenetic distance between focal and soil-conditioning plant, feedback group of the focal plant species (negative, neutral or positive), and their interaction, on plant biomass production using linear mixed effect models, with focal plant identity as random factor. We then tested whether the type of PSF (negative, neutral, or positive) of the focal species significantly affected the relation between plant biomass and the phylogenetic distance between focal and the soil-conditioning plant species by using the slope of the regression line as response variable and the type of PSF as fixed factor. Analyses were performed in R version 3.1.2 (R core group 2014).

Results

Across all plant species, biomass was significantly affected by the phylogenetic distance (PD) between focal and soil-conditioning plant species ($P < 0.0001$), however this effect also interacted with the plant-soil feedback (PSF) group (positive, neutral, or negative PSF) of the focal plant species ($P< 0.001$) (Table 1). There was no main effect of the PSF group of the focal plant on plant biomass ($P = 0.93$). The relations underlying the interactive effect between PSF group and the effect of phylogenetic distance between the soil conditioning plant species and the focal plant species become evident in Fig. 1a,b,c. The slope of the relation tended to be zero to positive for species with negative conspecific PSF, while for species with neutral to positive conspecific PSF the slope was significantly lower than zero and most negative for plants with positive PSF (Fig. 2).

Table 1 Linear Mixed Effects model outcome, testing for main effects of phylogenetic distance (PD) and feedback group of focal plant (PSF), and interaction effects between PD and PSF, on total biomass of the focal plants. Focal plant identity was specified as random variable.

	numDF	denDF	F-value	p-value
Intercept	1	381	34.317	<0.0001
Phylogenetic distance (PD)	1	381	18.156	<0.0001
Feedback of test plant (PSF)	1	9	0.008	0.9308
P x PSF	1	381	15.638	0.0001

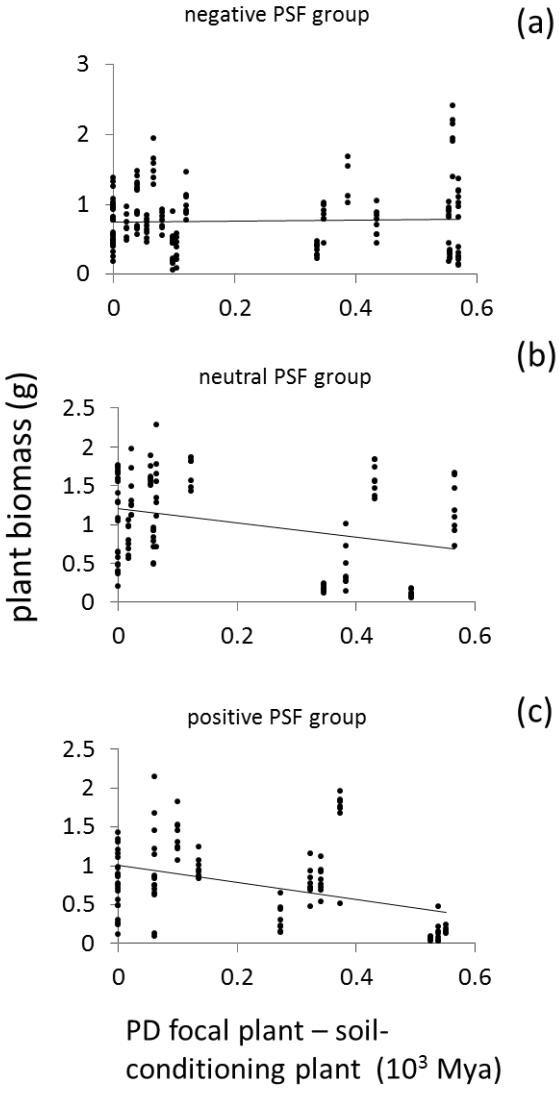


Figure 1 Relationship between phylogenetic distance (10^3 million years ago since species divergence) and total dry biomass (g) of focal plants with (**a**) a negative, (**b**) a neutral and (**c**) a positive response to conspecific conditioned soil. Each dot represents one plant individual; dots that are vertically aligned represent one focal species - soil-conditioning species combination. Solid lines are trend lines for ease of interpretation, not indicating the significance of the relationship.

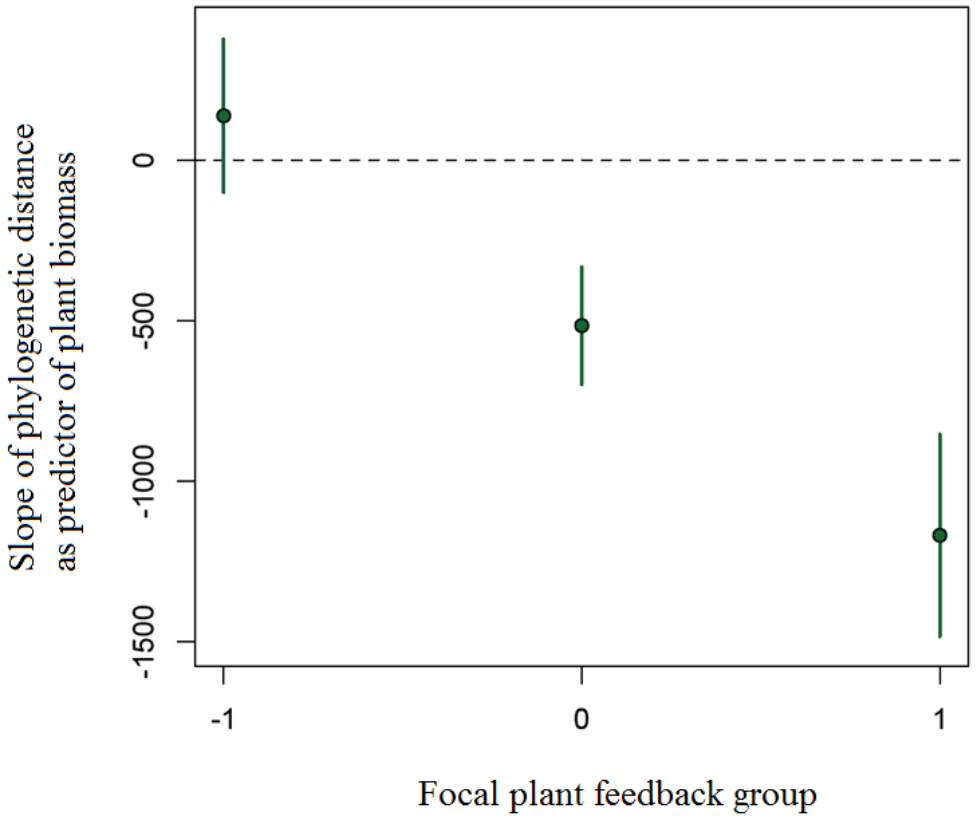


Figure 2 The mean and 95% confidence intervals (based on model fit) of the slopes of the regression between phylogenetic distance and focal plant biomass for the different feedback groups (negative: -1, neutral: 0 and positive response to conspecific conditioned soil: 1).

Within each PSF group some variation between individual species is notable (Fig. 3a,b,c). Amongst the group of species with negative PSF the grasses *Festuca rubra* and *Bromus erectus* displayed a more positive relation between phylogenetic distance with the plant species that conditioned the soil and biomass production (Fig. 3a). The negative relation between phylogenetic distance and biomass production for the neutral PSF species was most notable in the legume species *Trifolium pratense* (Fig. 3b), while for the species in the negative PSF group the negative relation was clearest in the forbs *Tragopogon pratensis* and *Crepis biennis* (Fig. 3c).

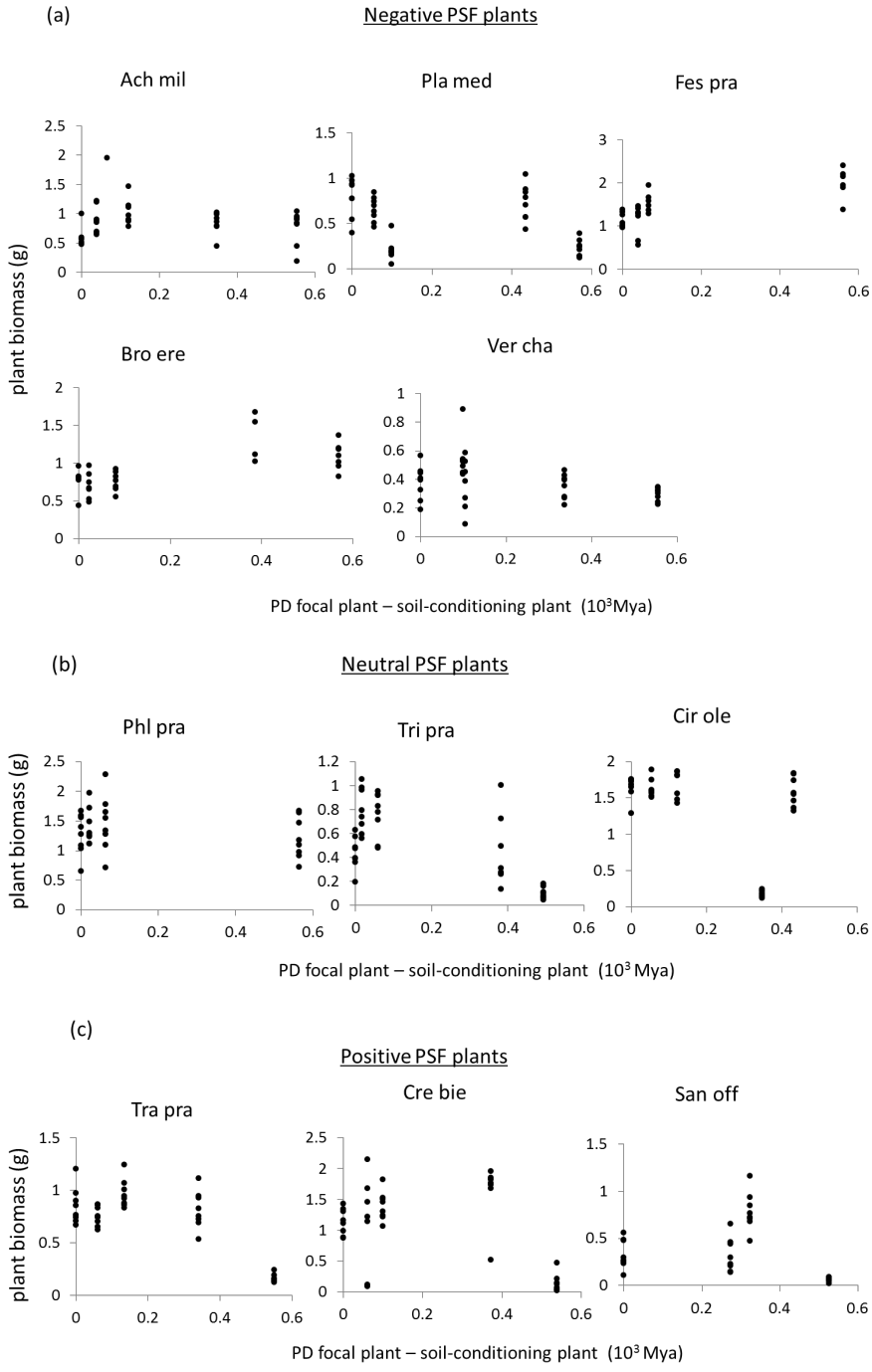

(a)
Negative PSF plants
Ach mil
Pla med
Fes pra
Bro ere
Ver cha
plant biomass (g)
PD focal plant – soil-conditioning plant (10³Mya)
(b)
Neutral PSF plants
Phl pra
Tri pra
Cir ole
plant biomass (g)
PD focal plant – soil-conditioning plant (10³ Mya)
(c)
Positive PSF plants
Tra pra
Cre bie
San off
plant biomass (g)
PD focal plant – soil-conditioning plant (10³ Mya)

Figure 3 Relationship between phylogenetic distance (10^3 million years ago since species divergence) and total dry weight biomass (g) of focal plants with (**a**) a negative, (**b**) a neutral and (**c**) a positive response to conspecific conditioned soil. Each dot represents one plant individual; dots that are vertically aligned represent one focal species - soil-conditioning species combination. Ach mil = *Achillea millefolium*, Pla med = *Plantago media*, Fes Pra = *Festuca pratensis*, Bro ere = *Bromus erectus*, Ver cha = *Veronica chamaedris*, Phl pra = *Phleum pretense*, Tri pra = *Trifolium pretense*, Cir ole = *Cirsium oleraceum*, Tra pra = *Tragopogon pratensis*, Cre bie = *Crepis biennis*, San off = *Sanguisorba officinalis*.

Discussion

There is increasing awareness that phylogenetic relationships among co-occurring plant species can have profound effects on plant community assembly and dynamics (e.g., Cavender-Bares et al. 2009, Allan et al. 2013, Cadotte et al. 2013). Phylogenetic relatedness has been found to influence plant-soil feedback (PSF) interactions between resident and colonizing species in field settings, with generally improved performance with greater phylogenetic distance (e.g., Webb et al. 2006, Liu et al. 2012). However, so far no study has explicitly tested whether phylogenetic relatedness among plant species may predict their PSF interactions.

In the present study, we found that the phylogenetic distance between eleven focal grassland species and the species that had previously conditioned the soil significantly predicted plant performance. Crucially, we found a strong interactive effect between phylogenetic distance and PSF group, meaning that the cost or benefit of growing in soil with a legacy of more distantly related plant species depends on whether the responding plant species show negative, neutral or positive response to soil legacies of conspecifics.

Our finding that the effect of phylogenetic relatedness depended strongly on the direction of the species' response to own soil biota may explain the results of a recent meta-analysis, showing, across more than 1000 experimental tests of plant-soil feedback, that phylogenetic distance between pairs of plant species was a very poor predictor of the strength and direction of indirect PSF effects between those species (Mehrabi and Tuck 2015). This apparent lack of effects of phylogenetic distance was consistent across types (e.g., grass vs. herb), life cycles (annual, biennial, perennial) and native/exotic status of the focal plants (Mehrabi and Tuck 2015). Here, we show that the magnitude of PSF effects in relation to phylogenetic distance between the conditioning and responding plant species can strongly

depend on the direction of the response of a plant to conspecific soil. The occurrence of opposing responses depending on the PSF group of plant species considered may have precluded clear main effects of phylogenetic distance in the study of Mehrabi and Tuck (2015).

Surprisingly, our results suggest that especially beneficial interactions between plants and soil microbes are phylogenetically preserved among plant species, given that species with a positive response to conspecific soil legacies showed a strongly reduced performance when growing in soil with a legacy of more phylogenetic distantly related species. It is generally assumed that host plant-specificity of microbes is especially large for pathogens (e.g., Gilbert and Webb 2007), but much less so for plant mutualists like arbuscular mycorrhizal fungi (AMF) (e.g., Richardson et al. 2000).

To date, only a few studies used the phylogeny of plants to understand the occurrence and role of plant-soil feedback. Among 57 plant species, Anacker et al. (2014) showed that the average plant species' response to soil legacy effects created by conspecifics relative to a range of heterospecific species were phylogenetically conserved. In that study, however, the role of the relatedness between the focal species and the species that conditioned the soil was not investigated. Hence, Anacker et al. (2014) tested the role of phylogenic relatedness in explaining interspecific differences of average PSF responses, while in our study, we specifically compared plant responses to conspecific soil with the response to heterospecific soil of known level of relatedness to the focal species. Thereby we mimicked interactions that take place at local scale during plant species interactions that may lead to increased dominance or species replacement in natural grassland (Bonanomi et al. 2005).

Our approach is especially useful to understand the role that phylogeny may play in the spatial and temporal dynamics of plant communities via effects of PSFs, because interactions take place between plant individuals so that the pairwise relatedness between the species that create a legacy and the species that respond to the legacy is expected to be more important than average PSF values of species (Webb et al. 2002, Cavender-Bares et al. 2009, Allan et al. 2013).

Temporal and spatial patterns of plant community dynamics have indeed been found to relate to intraspecific variation of plant responses to soil legacies, which were in part due to the level of relatedness between established and colonizing plants (Liu et al. 2012). These authors suggested that the observed phylogenetic overdispersion (co-occurring species are less related than expected by chance) of tree species in the subtropical forest they studied was likely the consequence of phylogenetic conservation in host-range of soil-borne pathogenic

fungi. Phylogenetic overdispersion is often interpreted as evidence for strong competition between closely related species (limiting similarity) or environmental filtering on ecologically important convergent traits (Webb et al. 2002). As indicated by our empirical study and that of Liu et al. (2012), PSF might be another mechanism that explains links between phylogenetic community structure and community dynamics by promoting co-existence of species with dissimilar plant species-specific pathogens. On the other hand, we found that plant species with positive responses to conspecific soil legacies perform worse on soil with a legacy of more distantly related species suggesting that these plant species would occur in local monoculture patches. We conclude that interspecific plant interactions through PSF depend on phylogenetic distance between species and the sign of the feedback plant species experience from conspecific soil; plant species that have negative PSF in own soil may benefit from growing in soil from distantly related species, whereas, clearly, plants that have positive PSF in conspecific soil suffer from growing in soil from distantly related species.

Acknowledgements

We thank Henk Martens for practical assistance. This work was supported by the Deutsche Forschungsgemeinschaft (FOR 1451) and by NWO-ALW VIDI through financial support to GBDD (grant nr 864.11.003), and NWO additional fund (grant nr 832.13.009) to WvP.

Supplementary information

Table 1 Overview of focal and soil-conditioning plant species, the latter ordered from low to high phylogenetic distance to the focal plant. PD = phylogenetic distance, 10^3 million years ago since species divergence

Focal species	Soil-conditoning species	PD level	PD (10^3 Mya)
Achillea millefolium	*Achillea millefolium*	0	0
	Leucanthemum vulgare	1	0.03881803
	Centaurea jacea	2	0.1208175
	Sanguisorba officinalis	3	0.34726232
	Poa pratensis	4	0.55411991
Bromus erectus	*Bromus erectus*	0	0
	Bromus hordeaceus	1	0.0221666
	Phleum pratense	2	0.08108573
	Luzula campestris	3	0.3864856
	Centaurea jacea	4	0.569408
Cirsium oleraceum	*Cirsium oleraceum*	0	0
	Centaurea jacea	1	0.05421796
	Taraxacum officinale	2	0.12167148
	Rumex acetosa	3	0.34638378
	Vicia cracca	4	0.43158894
Crepis biennis	*Crepis biennis*	0	0
	Tragopogon pratensis	1	0.06131863
	Achillea millefolium	2	0.13446863
	Onobrychis viciifolia	3	0.37289046
	Bromus erectus	4	0.53944235
Festuca pratensis	*Festuca pratensis*	0	0
	Dactylis glomerata	1	0.03963859
	Trisetum flavescens	2	0.06637089
	Cirsium oleraceum	4	0.56114511
Phleum pratense	*Phleum pratense*	0	0
	Poa trivialis	1	0.03392825
	Festuca rubra	2	0.06345864
	Leucanthemum vulgare	4	0.56566114
Plantago media	*Plantago media*	0	0
	Plantago lanceolata	1	0.05424804
	Veronica chamaedrys	2	0.09836662
	Tragopogon pratensis	3	0.3385153
	Vicia cracca	4	0.4345118
Poa pratensis	*Poa pratensis*	0	0
	Poa trivialis	1	0.01159907
	Holcus lanatus	2	0.05831567
	Lotus corniculatus	4	0.56005341

Sanguisorba officinalis	*Sanguisorba officinalis*	0	0
	Lotus corniculatus	1	0.27361342
	Leontodon autumnalis	3	0.32279147
	Phleum pratense	4	0.52665151
Tragopogon pratensis	*Tragopogon pratensis*	0	0
	Crepis biennis	1	0.06131863
	Taraxacum officinale	2	0.13506676
	Sanguisorba officinalis	3	0.34121306
	Poa trivialis	4	0.55175978
Trifolium pratense	*Trifolium pratense*	0	0
	Trifolium hybridum	1	0.01647339
	Lathyrus pratensis	2	0.05932049
	Carum carvi	3	0.3829549
	Luzula campestris	4	0.49406773
Veronica chamaedrys	*Veronica chamaedrys*	0	0
	Plantago media	1	0.09836662
	Plantago lanceolata	2	0.10401132
	Achillea millefolium	3	0.3360717
	Festuca pratensis	4	0.55557511

Chapter 5

Plant species with neutral soil feedback contribute most to plant community overyielding

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To be submitted

Abstract

Recent studies have proposed that positive relationships between plant species richness and plant community productivity is driven by reduced belowground pathogen pressure with increasing plant species richness. Consequently, plant species with negative plant-soil feedback (PSF) should produce more biomass in species-rich plant communities than in monocultures. However, this idea seems to contradict conclusions from studies in well-established plant communities demonstrating that plant species with negative PSF remain subordinate. Here, we tested how PSF of 46 plant species from the Jena biodiversity experiment relates to the contribution of these species to plant community biomass overyielding and to the complementarity effect. We found a positive trend between plant species-specific PSF and relative yield. This opposes our hypothesis, which predicted a negative relationship. Moreover, monoculture biomass was unrelated to PSF while in the 60-species mixtures, species-specific PSF was positively correlated with species-specific biomass. The community-averaged PSF also correlated positively with complementarity effects. These results suggest that species with negative PSF benefit least from growing in species mixtures and contribute least to community level overyielding. Moreover, our results indicate that complementarity in mixtures is larger when composed of plant species with neutral to positive PSF than when composed of plant species with strong negative PSF. We propose that the role of PSF in diversity-functioning relationships is significant and warrants further mechanistic testing, especially in community context.

Introduction

In grassland biodiversity studies, feedback interactions between plants and soil biota are increasingly being acknowledged as an important driver of the commonly found positive relation between plant diversity and productivity (van der Heijden et al 2008, Cardinale et al. 2012, Eisenhauer 2012). Similarly as for aboveground pathogen pressure on plants, which tends to decrease with increasing plant species richness of the community (e.g., Kranz 1990, Garret and Mundt 1999, Mitchell et al. 2002, Rottstock et al. 2014), pathogen 'dilution' effects in species diverse plant communities may also occur for belowground plant pathogens (Maron et al. 2011, Schnitzer et al. 2011, Kulmatiski et al. 2012, Hendriks et al. 2013). The reduced effect of both aboveground and belowground plant antagonists in increasingly diverse plant communities may be explained by host plant density-dependent impact of plant antagonists because increased host plant density can lead to disproportional increase of antagonists (Mordecai 2011).

Plant species are interacting with a wide array of pathogens, mutualistic symbionts, and decomposer organisms, and their net interactions have been conceptualized as plant-soil feedback (PSF) (Bever et al. 1997). PSF implies that plants continuously create species-specific biotic and/or abiotic legacies in the soil, for example by promoting different subsets of the resident pool of soil biota (e.g., Westover et al. 1997, Hausmann and Hawkes 2010, Bezemer et al. 2010). These altered soil biotic communities can then feedback to plant community structure by affecting plant performance in a species-specific way (Bever et al. 1997). Thus far, the majority of plant species tested produce more biomass when grown in soil with a legacy of other (heterospecific) plant species than in soil with a legacy of their own (conspecific) species (Kulmatiski et al. 2008). This suggests that in mixed plant communities, plants improve growing conditions of other species, while deteriorating conditions for their own offspring (Kulmatiski and Kardol 2008, Hendriks et al. 2014), which is commonly known as a Janzen-Connell effect (Janzen 1970, Connell, 1971, Packer and Clay 2000).

Thus far, the potential of belowground pathogen dilution to enable increased productivity of species-rich plant communities has been examined only at the level of total community productivity in relatively short-term mesocosm or greenhouse experiments (Maron et al. 2011, Schnitzer et al. 2011, Kulmatiski et al. 2012, Hendriks et al. 2013). Moreover, PSFs have been determined usually in the absence of intra- or interspecific competition (Kulmatiski and Kardol 2008). However, the effects of PSF on plant performance are known to be strongly dependent on the competitive environment of the focal plant,

because soil feedback effects can enhance or decrease when tested together with competition from the species that conditioned the soil (e.g., Casper and Castelli 2007, Hendriks et al. 2013). Kulmatiski et al. (2012) determined species-specific PSFs in the absence of competition, and showed that negative PSF related to high overyielding in mixtures. In the same study, the authors validated these modeling results in a greenhouse study with plant mixtures consisting of three species, showing that species with the most negative PSF had the highest relative yield in mixture versus monoculture. Overall, these results suggest interaction of PSF effects and the competitive effects plant species exert on each other, indicating that effects of PSF on plant performance may strongly depend on plant-plant interactions both via modified soil biotic communities and competition for resources (e.g. Casper and Castelli 2007, Petermann et al. 2008, Shannon et al. 2012, Jing et al. 2015).

To examine how species-specific PSFs among a large set of grassland plant species are associated with species-specific contributions to plant community overyielding in the field (Loreau and Hector 2001), we used 49 plant species of the long-term biodiversity experiment in Jena (Roscher et al. 2004). We determined 'direct' versus 'indirect' PSFs (Bever 1994) of all 49 plant species in the greenhouse and correlated these PSF values to species-specific productivity in monocultures and species-rich plant communities of the Jena Experiment, and with their relative yields (RY; i.e. the differential performance of a species in species-rich assemblages vs. in their monoculture). These three analyses were done for 46 of 49 species, as in the long-term field experiment, one species disappeared as a monoculture, one species disappeared from the species-rich plant communities, and one species was a strong outlier in the analysis (see 'methods'). For testing the relation between PSF and plant community complementarity we used all 49 species.

'Direct' PSFs comprise plant responses to conspecific soil legacies and can be expected to reflect effects of plant-soil interactions on plant performance in monocultures. 'Indirect' PSFs capture plant growth responses to soil legacies of heterospecific plants and can be expected to reflect effects of plant-soil interactions on plant performance in the species-rich assemblages. Strong negative PSFs in monocultures may become less detrimental in species-rich assemblages and positively affect species productivity (Kulmatiski et al. 2012). However, these assumptions do not take interspecific competition into account, and species with negative PSF may become subordinate in well-established grasslands (e.g., Klironomos 2002). Therefore, it remains unclear whether in mixtures plant species with strong negative PSF will show high or low relative yields (Loreau and Hector 2001) compared to plants with more neutral PSF.

Given the expectations that plant species with negative PSF remain subordinate in diverse plant communities (Klironomos 2002) and that monocultures of plants with strong negative PSF will deteriorate faster over the years than plants with more neutral PSF, we expected that PSF and productivity in both species-rich assemblages (H1) and in monocultures (H2) are positively related. We further expected that PSF and relative yield of individual plant species in 60-species mixtures are negatively related (H3), thereby assuming that the dilution of biota that cause negative PSF in diverse communities overrules the expected high dominance of plant species with a neutral to positive PSF in species mixtures. Finally, we expected that the complementarity effect of plant species mixtures is negatively related to the community weighted mean PSF values (H4), indicating increased benefit of the release of species-specific negative soil legacies when growing together in mixtures.

Material and methods

Study system and experimental approach

We conducted a plant-soil feedback (PSF) experiment under controlled conditions in a greenhouse (16h/8h light/dark) using a two-phase approach of a soil-conditioning and a plant feedback phase. The plant species comprised grassland species from the Jena Experiment, a long-term biodiversity study located in Thuringia, Germany (50° 55' N, 11° 35' E). The experiment has been established in 2002. Experimental treatments in the field include monocultures and plant mixtures sown with 2, 4, 8, 16, and 60 species (Roscher et al. 2004). All plant species are typical for mesophilic meadows of Central-Western Europe, including legume and non- legume forbs and C3 grasses. In the conditioning phase, 49 out of the pool of 60 plant species were grown individually for 8 weeks in a 20% mixture (w/w) of living soil and sterilized soil originating from the Jena experimental field site and sterilized by gamma irradiation. In the feedback phase, all 49 plant species were grown in soil conditioned by individuals of their own species (hereafter called 'conspecific' soil), and in a mixture of soil conditioned by all 49 plant species (hereafter called 'mixed conditioned' soil), which served as reference treatment to quantify the strength and direction of PSF.

Plant-soil feedback experiment

To start the conditioning phase approx. 700 kg of soil was collected in October 2010 from the top 20 cm of the east border lane of the Jena field experimental site, which was managed as

mown grassland since establishment of the experiment. The soil is a Eutric Fluvisol developed from loamy sediments with a pH ranging from 7.1 to 8.4 (Roscher et al. 2004). Immediately after collecting, the soil was shipped to the Netherlands Institute of Ecology at Wageningen, The Netherlands, and stored at 4°C for one month. The soil was sieved through a mesh (size: 1 cm) to remove stones, large root fragments and large soil fauna. After sieving, the soil was manually homogenized. Eighty percent of this soil was sterilized by gamma irradiation (average of 25 kGray), and stored in sealed plastic bags at room temperature until further use. The non-sterilized soil was stored in a container at 4°C for approx. 3 weeks until it was used to prepare the soil mixture and fill the pots.

In December 2010, seeds of all 49 plant species were surface-sterilized by soaking in 5% or 25% household bleach (5% sodium hypochlorite solution) for 30 seconds. 25% household bleach was used when 5% bleach was not a sufficient concentration to prevent fungal infection of seeds. The sterilized seeds were rinsed with demineralized water and placed in a growth cabinet (16h/8h 22°C/16°C light/dark) on water-saturated glass beads in a closed transparent plastic box. Based on Roscher et al. (2004), prior to germination, some seeds were scarified or treated with gibberelic acid (Sigma Chemical co., St. Louis, USA). Despite pre-germination treatments, we failed to germinate 11 of 60 species (see Supp. Figure 1). After germination, which took 3-12 days, seedlings were transferred to a climate room at 4°C and 16h/8h light/dark day/night conditions, which kept them all in the same post-germination stage until planting. End of December, 416 pots of 1.5 liter each were filled with 1500 g of soil, consisting of a mixture of 80% sterilized soil and 20% unsterilized soil (w/w).

Pots were arranged in the greenhouse (16h/8h light/dark) in 8 replicate blocks. Because of variation in germination time, planting of the different species was spread over two weeks. Two seedlings per species were planted per pot and pots were spatially randomized within blocks. Soil moisture level was kept within a range of 15-30% water content (w/w) by watering pots to constant weight every second or third day with demineralized water. After two months of growth, we harvested the plants and collected the soil from each pot individually in order to use the soil in the feedback phase. During harvesting, aboveground biomass was clipped and dried at 70°C for minimally 72 hrs. Roots were shaken free from adhered soil before rinsing with tap water drying at 70°C for minimally 72 h.

Soil from each plant species was stored separately in plastic bags and kept at room temperature by day and 4°C at night during the 9 harvest days. Cross-contamination of soils from different plant species during harvest was avoided by cleansing all used material in 70%

ethanol in between working steps. A 50 ml composite sample was taken from all plant species-specific soils and dried at 40°C during 72 h in order to analyze nutrient concentration and moisture content. Plant available P was determined according to Olsen et al. (1954). Soil mineral N was extracted by shaking 10 g (dry weight) soil with 50 ml 1 M KCl for 2h. NH_4^+ and NO_3^- were determined calorimetrically in the KCl extract.

All 49 conditioned soils from phase 1 were split into two equal parts. One part was left untreated and the second part was used to prepare mixtures of all soils based on equal dry weight proportions. The two soils were kept separate and mixed with sterilized Jena soil at a rate of 65% sterilized soil and 35% living inoculum soil (w/w). Thus, we obtained two soil treatments: 'conspecific conditioned' soils, which contained inoculum soil conditioned separately by the 49 plant species and 'mixed conditioned' soil, which contained equal proportions of inoculum soil from all 49 plant species. To test the plant growth response to these soils, seeds of all plant species were germinated as done for the conditioning phase and planted in pots of 550 ml filled with either 550 g conspecific conditioned or mixed conditioned soil. Treatments were carried out in 8 replicates, using block as replicate, resulting in an experimental design of 49 plant species x 2 soil types x 8 replicates = 784 pots. All seedlings that died in the first week after planting were replanted and pots were spatially randomized per block. Plants were watered every 3 or 4 days per block with demineralized water to a moisture content of 25 % (w/w) based on weighing. After growing for 6 weeks, all plants were harvested as described for the soil conditioning phase.

Data analysis

Species-specific PSF effects were calculated per experimental block (N = 8) as log (total dry weight in 'condpecific conditioned' soil / total dry weight in 'mixed conditioned' soil) (Brinkman et al. 2010) and averaged per species. This PSF value reflects the strength and direction of host-specific soil effects on plant performance (Bever 1994). We tested whether our inoculation approach successfully excluded potential abiotic plant-soil feedback effects by testing the correlation between 'direct' versus 'indirect' PSF and the concentrations of plant available P, NO_3^- and NH_4^+ in the plant species-specific soils at the end of the conditioning phase (Kardol et al. 2006).

We correlated the PSF of each species with its average aboveground dry weight biomass in the field using Pearson product-moment correlation tests, separately for

monocultures and 60-species mixtures. Plant biomass data in the field experiment were collected each year in spring and summer (for details, see Marquard et al. 2009). We used the average yearly biomass per species, averaged over the years 2004, 2005, 2006 and 2008, i.e. not including the first two years of the experiment as that period was considered as the establishment phase. Field biomass data were log-transformed prior to the statistical analyses to meet assumptions of the Pearson product-moment correlation test. Relative yields (RYs) were calculated for each species to contrast performances in mixtures and monocultures across all species by correlating the log (average biomass in 60 species mixtures / average biomass in monoculture) with PSF using Spearman's Correlation analysis. *Campanula patula* was not present anymore in 60 species plots and *Carum pratensis* had disappeared from the monoculture plots, so that these two plant species were excluded from the above analyses.

To test the relation between species-specific PSF and the complementarity effect of the mixed plant communities, we correlated the average PSF value of the species constituting a specific assemblage (based on presence) with the size of the complementarity effect (Loreau and Hector 2001) of that assemblage. The complementarity effect values were derived from the partitioned biodiversity effect values of all the plant communities, averaged over the years 2005-2008 (Marquard et al. 2009). First we calculated per plot the average PSF value of the constituting species weighted according to the sown proportion of each species (Van de Voorde et al. 2011), using all species for which PSF data were available (49 species). We correlated these values with the complementarity effect of the community in each plot, the calculation of which includes the 11 plant species for which we did not have PSF data (Marquard et al. 2009). There were 16 plots containing 2 species, 16 plots containing 4 species, 16 plots containing 8 species, and 14 plots containing 16 species. To statistically test the relation between average PSF and complementarity of a plot, we used Spearman's correlation test for all diversity levels together and performed separate Spearman's correlation tests for the different diversity levels. All statistical analyses were performed in R (R core team 2014).

Results

The average PSF value of the 49 plant species ranged from – 0.59 to + 0.22. These values correspond to a reduction of biomass production by 45% and an increase of biomass production by 25%, respectively, in 'conspecific conditioned soil' compared to 'mixed conditioned soil'. In total, 31 plant species had a negative, and 18 plant species had a neutral

to positive PSF value. Plant-available nutrient concentrations in the soils after the conditioning phase were not related to the PSF values (P-Olsen: r = -0.08, P = 0.61; NH_4^+: rho = -0.02, P = 0.87; NO_3^-: rho = -0.03, P = 0.87; data not shown), showing that there was no relation between PSF and nutrient depletion in the conditioning phase.

The plant species-specific PSF values were not related to the log-transformed aboveground biomass, neither in the 60 species mixtures (r = 0.20; P = 0.18), nor in monoculture (r = -0.004, P = 0.98). However, after removing the outlier *Luzula campestris* (indicated in Figs 1 and 2) a significant positive relation between plant species-specific PSF and their aboveground biomass in 60 species mixtures was found (r = 0.35, P = 0.02) (Fig. 1a). In contrast, there was no relationship between PSF and aboveground biomass for the monocultures, also not after removal of the outlier *L. campestris* (r = 0.24, P = 0.11) (Fig. 1b).

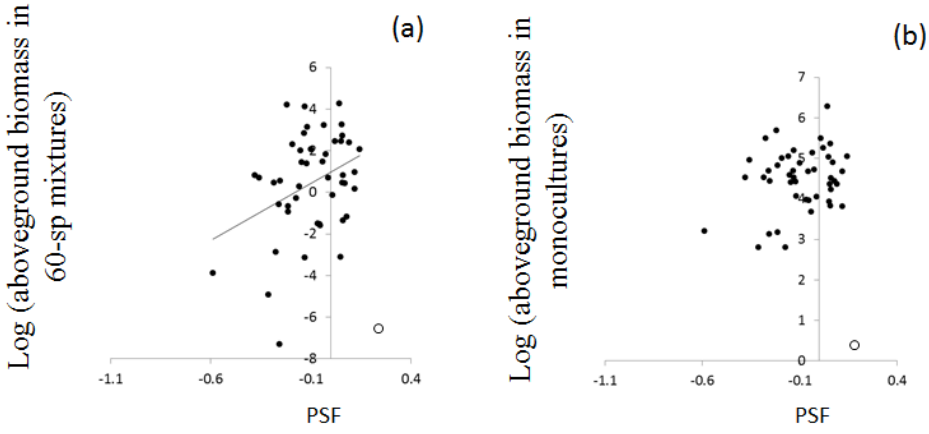

Figure 1 Relationship between plant-soil feedback (PSF) as log(plant dry weight on conspecific conditioned soil / plant dry weight on mixed conditioned soil) of 48 grassland species and their mean log-transformed average standing biomass from 2004-2008 in 60-species plots (a) and in species monocultures (b) of established plant communities of the Jena experiment. The empty circle in (a) and (b) depicts the outlier *Luzula campestris*, which was excluded from analysis. The solid line indicates a significant positive relationship (r = 0.35, P = 0.02).

Opposite to our expectation we found a positive trend between the relative yield of plant species in 60-species mixtures and their PSF value (Spearman's correlation, rho = 0.26, P = 0.08), suggesting that plant species that performed relatively well in mixtures compared to in monoculture had a higher PSF than the ones that performed poorly in mixtures relatively to their performance in monocultures (Fig. 2).

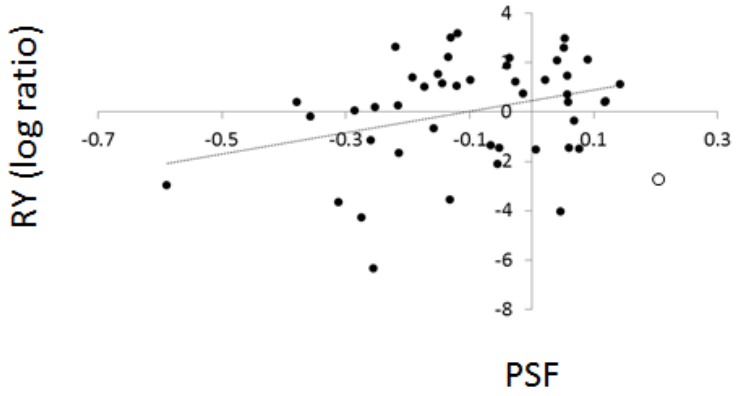

Figure 2 Relationship between plant-soil feedback as log(plant dry weight on conspecific conditioned soil / plant dry weight on mixed conditioned soil) (PSF) of 46 grassland species and their relative yield (RY) as log(average biomass in 60 sp. plots * 60) – log(average biomass in monoculture) in the Jena experiment. *Luzula campestris* (indicated by empty circle) is not included in this analysis. Dotted line indicates a positive trend (Spearman´s rank correlation: rho = 0.26, P = 0.08).

Across all 2, 4, 8 and 16 plant species communities, the complementarity effects of the plant assemblages were significantly positively related to their community-weighted mean PSF (rho = 0.30, P = 0.003) (Fig. 3). When the diversity levels in the field plots were analyzed individually, the complementarity effect was significantly positively correlated to average PSF in the 8-species plots (rho = 0.64, P = 0.005) and marginally significant in the 4-species plots (rho = 0.43, P = 0.08), but not in the other richness levels (2-species: P = 0.92, 16-species: P = 0.25) (Fig. 3).

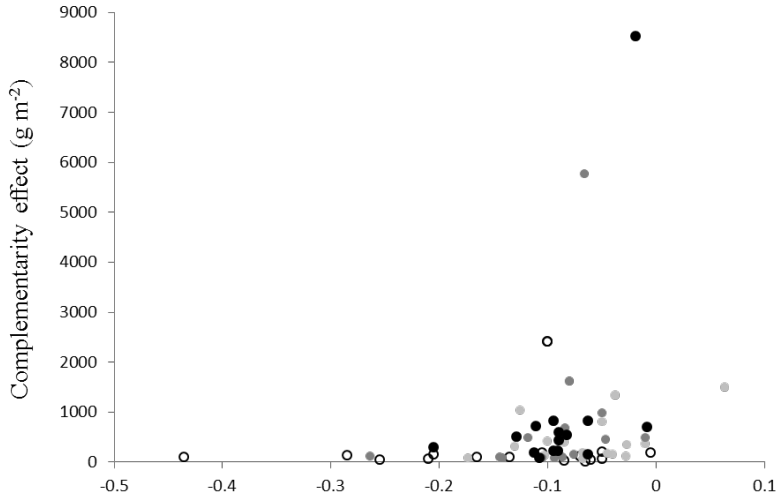

Figure 3 Relationship between average plant-soil feedback (PSF) as log(plant dry weight on conspecific conditioned soil / plant dry weight on mixed conditioned soil) of the species constituting a specific assemblage in the Jena experiment (each data point represents one assemblage) and the mean complementarity effect of that assemblage over the years 2005-2008. Open circles represent two-species mixtures, light gray circles four-species mixtures, dark grey circles eight-species mixtures and black circles represent sixteen-species mixtures. Spearman's rank correlation: rho = 0.135, P = 0.003.

Discussion

Plant species with strong negative plant-soil feedback (PSF) have been proposed to benefit from growing in species-rich plant communities, thereby driving overyielding of diverse plant communities (Kulmatiski et al. 2012). However, this proposal appears to be inconsistent with studies of well-established grasslands showing that plant species with strong negative PSF remain subordinate to plants with neutral to positive PSF (Klironomos 2002, Mangan et al. 2010). The main aim of the present study was to test how species-specific PSF effects, as determined in the greenhouse in the absence of competition, relate to species relative performances in monocultures compared to species-rich plant communities. We used plant species and soil from the long-term Jena Experiment (Roscher et al. 2004).

In support of our first hypothesis, we found a positive relationship between the plant-soil feedback (PSF) value of a plant species and its average aboveground biomass in the most diverse plant assemblages of the Jena Experiment. Therefore, our results show that plant species with the least negative (or most neutral) PSF values contribute most to total biomass in the species-rich plant assemblages. We note that eleven plant species that occurred in the 60-species plots were missing from our PSF dataset, but given that the biomass of these missing plant species in those plots covered a wide range from low to high species-specific biomass (Supp. Fig. 1), it is unlikely that the absence of these species from our PSF dataset created bias in our analyses. Our finding that plant species with neutral to positive PSF values contributed more to plant community biomass than plant species with more negative PSF values is in support of plant species with strong negative PSF being subordinate (Klironomos 2002). A similar conclusion was drawn for tree species in tropical forests (Mangan et al. 2010). However, in the latter two studies, species abundance was analyzed, which is not necessarily indicative of their contribution to community biomass. Moreover, a recent study in semi-arid grasslands did not find a relationship between PSF and the plant species' relative abundance based on frequency of occurrence (Reinhart 2012). Therefore, limited evidence exists that may support the generality of this positive relationship between PSF and plant species abundance in mixed plant communities, and further investigation is warranted both at the level of plant abundance as well as at the level of biomass production.

Contrary to our second hypothesis, we did not find a relationship between the PSF of plant species and their biomass in monoculture. We had expected biomass in monocultures to be lower for species with negative PSF, which was not the case. However, a recent study on productivity of the monocultures in the Jena Experiment over the years 2003-2011 (Marquard

et al. 2013) showed no clear patterns of decline of the biomass of specific species in successive years. The authors also found no consistent year-to-year biomass changes in monocultures compared to species-mixtures, so that no consistent relation between monoculture biomass decline and the performance of those species in mixtures was apparent. A possible mechanistic explanation of the lack of relationship between our PSF values and monoculture performance in the Jena field experiment is that over the years the soil food-web developed a top-down control of soil-borne diseases, such as has been shown for suppression of soil-borne diseases in continuous monoculture cropping of the same species (Weller et al. 2002).

In our study, which is the first to relate individual PSF values from controlled greenhouse conditions to plant performance in the field in a longer-term biodiversity experiment, increasing negative PSF was not associated with more, but rather with less species-specific overyielding in mixed plant assemblages. This suggests that in species mixtures in the field, the dominance of plants with a neutral to positive feedback limits the realized overyielding of plants with a negative PSF and that, consequently, their importance for community level overyielding may be less substantial than proposed (Kulmatiski et al. 2012). Overall our results are more in line with the finding in the Cedar Creek biodiversity experiment that plant species with the strongest density-dependent decline of foliar fungal attack in species-rich plant communities showed lowest relative yields (HilleRisLambers et al. 2004). Given that negative PSF effects also operate in a host density-dependent way (Bever et al. 1997, Mordecai 2010) the mechanisms explaining low relative yields of plant species with strong negative PSF in our study might be similar to those in the study of Hille Ris Lambers et al. (2004). However, the reported patterns to date are correlative and the actual underlying causal mechanisms remain to be determined.

Our finding that the size of the complementarity effect of a plot was positively correlated with the community-weighted mean PSF value of the component plant species further supports the idea that plant community overyielding is not driven mostly by the release from detrimental effects of soil-borne enemies. Rather, high complementarity effects were found in plots where most plant species have a close to neutral soil feedback. This finding suggests that complementarity effects in species mixtures may not be primarily due to reduced negative PSF in plant species mixtures (e.g., Schnitzer et al. 2011), but that other mechanisms, like resource partitioning among co-occurring plant species, may be more important (Tilman et al. 1997, Loreau and Hector 2001)

In conclusion, we show that plant species with the most negative PSF achieve the

lowest relative yields in and contribute least biomass to species-rich assemblages. Our findings suggest that plant-soil feedback interactions act as a driver of the relative dominance of plant species in well-established plant communities. Plant species with neutral PSF contributed most to overyielding and absolute yield in mixed plant communities. Therefore, our data do not support the hypothesis that increased productivity with increased plant species diversity is mainly due to relaxation of negative plant-soil feedback.

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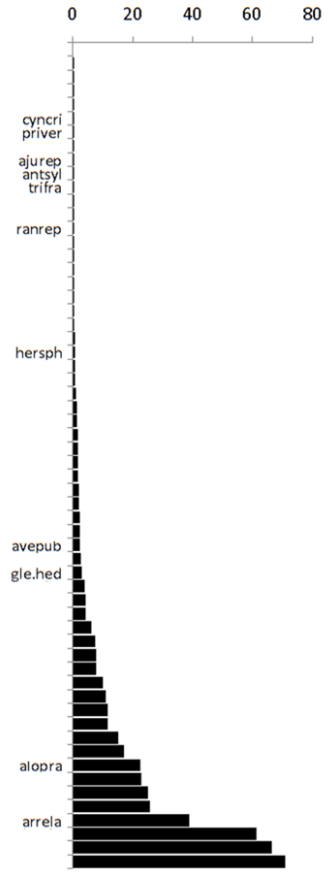
Supplementary information

Supplementary figure 1 Average aboveground dry biomass (g m^{-2}) over the years 2004-2008 of all plant species present in the 60-species plots of the Jena experiment. Species for which we did not obtain PSF data, due to failed germination, are indicated with their abbreviated name. (cyncri = *Cinosurus cristatus*, priver = *Primula veris*, ajurep = *Ajuga reptans*, antsyl = *Anthriscus sylverstris*, trifra = *Trisetum fragiferum*, ranrep = *Ranunculus repens*, hersph = *Heracleum sphondylium*, avepub = *Avenula pubescens*, glehed = *Glechoma hederacea*, alopra = *Alopecurus pratensis*, arrela = *Arrhenaterum elatius*).



Chapter 6

Mechanisms underlying abundance and diversity responses of nematode communities to plant diversity

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To be submitted

Abstract

Plant community diversity can affect the abundance and species richness of soil organisms, but the underlying mechanisms remain largely unexplored. We used structural equation modelling (SEM) to test mechanistic linkages between plant community diversity and soil nematode community composition (abundance and taxon richness) across all nematode feeding groups. Nematode community composition was determined in soil samples collected from the long-term Jena grassland biodiversity experiment. The abundance of individuals in all nematode feeding groups, except for predatory nematodes, increased with plant species and functional group richness. Abundance of plant feeding nematodes related positively to shoot C:N ratio, whereas microbial feeders responded positively to shoot biomass of the plant community. Predatory nematode abundance responded positively to numbers of plant feeders. Taxon richness of plant feeders and predatory nematodes related positively to plant functional group richness. Taxon richness of microbial feeding nematodes related positively to plant species, as well as functional group richness, which could be explained via C:N ratio of the plant tissue. Densities of plant feeding nematodes per unit root mass decreased with increased plant diversity, suggesting reduced top-down control of plant productivity. We conclude that abundances of plant feeding, microbial feeding and predatory nematodes are all promoted by plant species diversity, however, each by a different mechanism. Plant diversity also drives nematode diversity, however, by other mechanisms than nematode abundance.

Introduction

Many studies over the last two decades have demonstrated that plant diversity loss may reduce primary productivity and other ecosystem functions (Hooper et al. 2005, Cardinale et al. 2012). Altered plant community diversity can have cascading effects on the abundance, diversity and activity of higher trophic level organisms (Balvanera et al. 2006, Scherber et al. 2010, Ebeling et al. 2014), which in turn can affect rates of important ecosystem processes such as herbivory and decomposition (e.g., Ebeling et al. 2014). Until recently, most studies on plant diversity effects on higher trophic level organisms have focussed predominantly on aboveground interactions. The number of studies that analyze relationships between plant diversity and belowground community composition is rapidly increasing (e.g., Zak et al. 2003, De Deyn et al. 2004a, Viketoft et al. 2009, Eisenhauer et al. 2010, Eisenhauer et al. 2011, De Deyn et al. 2011). Despite this increasing focus on the belowground world, the underlying mechanisms by which plant diversity influences belowground communities have been poorly resolved (Bardgett and Wardle 2010). Most aboveground-belowground studies to date have been based on relatively short-term experiments, while belowground communities often show long time lags in their responses to plant community manipulation treatments (Eisenhauer et al. 2012).

Plant community biomass, as well as plant community diversity and composition, all have been identified as potential drivers of belowground community composition (Bardgett and Wardle 2010). The relative importance of these different factors for belowground community composition may depend on the functional group (e.g., trophic level or feeding type) of soil biota that is considered (e.g., Wardle et al. 1999, De Deyn et al. 2011). Furthermore, it has been recognized that lower trophic levels of soil biota, such as plant feeders, can be more responsive to changes in plant species identity or diversity than organisms from higher trophic levels in the soil food web (Wardle et al. 2003, De Deyn et al. 2004a, Viketoft et al. 2009, Scherber et al. 2010). However, it remains largely unexplored how the diversity and abundance of different trophic groups of soil organisms, such as plant feeders, microbial feeders, omnivores and predators, respond to the various underlying plant community properties (Scherber et al. 2010).

Analyzing all different taxa present in soil communities is complicated because of the sheer diversity in soil (Bardgett and van der Putten 2014). However, there are some phyla of soil biota, such as nematodes, that include a wide variety of feeding types and trophic groups that can be extracted from soil all together. This makes it possible to study diversity-related

plant community effects on abundance and composition within and between belowground trophic levels (Bongers 1990, Yeates 1993, Kardol et al. 2010).

Plant functional groups can promote specific nematode feeding groups (Wardle et al. 1999, Viketoft et al. 2009, Sohlenius et al. 2011), presumably because of differences in resource quality (Orwin et al. 2010). However, also plant species within the same plant functional group can host very different nematode communities (De Deyn et al. 2004a, Viketoft et al. 2005, Sohlenius 2011). Therefore, plant species or functional group diversity effects on nematode community composition may be due to plant quantity or plant quality, or both, but it is not well understood how these different mechanisms operate and whether or not they are similar or different for plant feeding, microbial feeding and predatory nematode feeding types.

The aim of the present study was to determine how plant species and functional group richness influence belowground nematode community composition and structure. Using structural equation modeling (SEM), we tested different *a priori* hypotheses regarding plant and soil parameters that may mediate the effect of plant community structure on the abundance and diversity of different nematode feeding groups (plant feeders, microbial feeders, omnivores and predators). We performed our study in the long-term Jena Experiment (Roscher et al. 2004) where plant monocultures and mixtures from 2, 4, 8, 16 and 60 species had been established in the field eight years before our sampling. A previous study of nematode communities in the Jena experiment (Eisenhauer et al. 2011), 3 and 5 years after establishing the experiment, pointed at increases in nematode taxon richness but no effects on nematode abundances with increasing species richness of the plant community. Here, we elaborate on the approach of Eisenhauer et al. (2011) by explicitly testing for various potential pathways linking plant community diversity to nematode abundance and diversity of different trophic groups.

We tested the hypothesis that the abundance and taxon richness of plant, bacterial and fungal feeders, omnivores and predators increases with higher species and functional group richness of plant communities. We investigated how increasing plant biomass with higher plant species and functional group richness (Marquard et al. 2009, Ravenek et al. 2014) may explain the findings, assuming predominant bottom-up control of soil biota by plant resource input in both detritus-based and living plant-based components of the soil food web (Wardle 2002). We used SEM in order to test whether the mechanistic linkages between plant community diversity-related parameters and soil nematode abundance and taxon richness depend on the nematode feeding group and trophic level. SEM tests the fit of data to *a priori*

formulated hypotheses when assuming a particular organization among variables (Shipley 2000, Grace 2006). SEM allows for testing multivariate hypotheses in which some plant and nematode variables can act as predictor and response variables at the same time (e.g., Veen et al. 2010).

We expected to find an indirect positive relationship between plant community diversity and abundance and taxon richness of microbial feeding nematodes, via increased microbial biomass (Lange et al. 2014) and increased organic matter content of the soil (Steinbeiss et al. 2008). Plant feeder abundance was expected to increase with increasing plant community diversity via increasing plant aboveground biomass (Marquard et al. 2009) and to decrease via increasing C:N ratio of the plant tissue (Abbas et al. 2013). Similarly, plant feeder taxon richness was expected to be positively related to plant community diversity. Finally, we predicted plant community diversity to have an indirect positive effect on the abundance of predators via increases in the abundance of the other nematode feeding groups. Specifically for plant feeding nematodes, we examined how their numbers per unit root dry mass per soil volume depended on plant species and functional group richness; decreasing nematode densities per unit root mass with increased plant richness would suggest reduced plant exposure to nematode herbivory when plant community diversity increases.

Material and methods

Study site and soil sampling

We performed our study in the long-term grassland biodiversity field experiment at Jena, Germany (50° 55’ N, 11° 35’ E). The experimental field site is located on the floodplain of the river Saale and has been established in 2002 on former fertilized arable land that had been used for the production of wheat and vegetables. Soil is Eutric Fluvisol developed from loamy sediments. The experimental treatments include monocultures, mixtures of all 60 plant species in the species pool, and plant species mixtures of 2, 4, 8 and 16 species. Functional group richness also varies near-orthogonally with species richness from 1 to 4, comprising grasses, tall herbs, small herbs, and legumes. All 60 plant species are typical for mesophilic meadows of Central-Western Europe.

Further details on experimental design and field conditions have been provided by Roscher et al. (2004). In September 2010, we collected soil samples in the 82 vegetated main plots of the

experiment. Five soil cores of 2 cm diameter and 15 cm depth were taken from all plots: four cores at the corners of a 1 m^2 square and one core in the center of the square; the square itself was placed at min. 50 cm from the plot edges. These five soil samples were mixed and homogenized so that there was one sample per plot. Soil samples were transported to the Netherlands Institute of Ecology at Wageningen and kept at 4°C for maximally two weeks until nematode extraction. Nematodes were extracted from 100 g of fresh soil using Oostenbrink elutriators (Oostenbrink 1960) to separate the nematodes from the heavier soil particles after which the floating nematodes were collected on a stack of four sieves (one sieve of 75 µm and three sieves of 45 µm mesh size). The nematodes on the sieves were rinsed off onto a double cotton filter that was placed in 100 ml tap water for 24 hours at room temperature to let the nematodes migrate through the cotton filter into the water. All nematodes in the 100 ml nematode suspension were concentrated in 2 ml water after which the nematodes were fixed by diluting the suspension with 4 ml hot and 4 ml cold 4% formalin. Total nematode abundances in each sample were counted in 1 ml (i.e. 10% of the total sample), and 150 nematodes were identified to family or genus level using an inverted microscope. Samples from three out of 82 plots got lost (one 16-species plot, one two-species plot and one monoculture plot (*Trifolium repens*), leaving 79 samples for data analysis. Nematode taxa were assigned to feeding groups according to Yeates et al. (1993) (supplementary Table S2). A soil subsample was weighed fresh and again after drying at 105°C to determine soil moisture levels and to be able to express nematode densities per 100 g dry soil.

Plant and soil parameters

We compiled a data set of plot-level plant and soil parameters from published and non-published data sets of the Jena Experiment. Root biomass in 2011was determined by Ravenek et al. (2014). In short, standing root biomass was collected from 0 to 40 cm depth in all 1-, 2-, 4-, 8-, 16-, and 60-species plots. Three soil cores of 3.5 cm diameter were taken from every plot. Cores were stored cool at 4°C until further handling. The bulk material of the pooled cores was weighed and subsequently washed for root material. Remaining soil particles were removed by hand. Roots were dried at 60 – 70 ° C before weighing.

Aboveground plant community biomass of all plots was harvested during peak standing biomass in late May and August. This was done by clipping the vegetation in 2-4

rectangles of 0.1 m^2 at 3 cm above the soil surface. The harvested biomass was separated per species sown in the plot, cleaned from weeds and dried at 70°C for 72 h. The dry aboveground biomass was weighed per species per plot, and summed per plot (for details, see Weigelt et al. 2010).

The C and N concentrations of aboveground plant tissue were determined as described in Abbas et al. (2013). In short, aboveground biomass was harvested in 2010 in late May prior to mowing. Plants were clipped at 3 cm above ground level in four rectangles of 20 x 50 cm^2. Sample location was selected randomly, leaving out the outer 70 cm of the plot. Biomass was dried at 70°C for at least 48 h. The concentrations of C and N were measured by analyzing the mixture of pooled plot biomass using an Elemental Analyzer (EA, Vario EL III, Elementar, Germany).

Microbial biomass in soil was determined as soil microbial carbon biomass per gram dry soil (µg C microbial · g dry $soil^{-1}$) based on rates of oxygen use and CO_2 production. In short, O_2 consumption of soil microorganisms in fresh soil equivalent to 3.5 g dry soil was measured over a period of 24 h at 22°C using an electrolytic O_2-microcompensation apparatus (Scheu 1992). Substrate-induced respiration (Anderson and Domsch 1978) was determined by adding D-Glucose to saturate catabolic enzymes of the microorganisms according to preliminary studies (4 mg D-glucose · g dry $soil^{-1}$ solved in 400 µL deionized water). Maximum initial respiratory response (MIRR; [µL O_2 g^{-1} dry soil h^{-1}]) was calculated as the mean of the lowest three O_2 consumption values within the first 10 h after glucose addition. Microbial biomass (µg C · g dry $soil^{-1}$) was calculated as 38 × MIRR (Beck et al. 1997).

Soil organic matter content was determined as described in Steinbeiss et al. (2008). In short, three samples (diameter: 4.8 cm, depth of 30 cm) were taken in the core area of each plot in April 2008. Subsequently, samples were dried at 40 ^{0}C. All soil samples were passed through a sieve with a mesh size of 2 mm. The samples were further sieved using 1mm mesh size according to common root removal methods (Allard et al. 2005, Ostonen et al. 2005, Stevens and Jones 2006). Total carbon concentration was analyzed on ball-milled subsamples (time 4 min, frequency 30 s^{-1}) by an elemental analyzer at 1150^{0}C (Elementar analysator vario Max CN, Elementar Analysen systeme GmbH, Hanau, Germany). To determine the organic carbon concentration, either the carbonate or the organic compounds need to be removed (Bisutti et al., 2004). Inorganic carbon concentration was measured by elemental analysis at 1150 ^{0}C after removal of organic carbon for 16 h at 450^{0}C in a muffle furnace (Hirota and Szyper 1975, Keefe 1994). Organic carbon concentration was then calculated as the difference between both measurements.

Data analysis

Prior to all analysis, we excluded 3 out of 79 plots from the data set in order to meet the assumptions of the General Linear Models (GLMs) that we constructed (see further). These outlier plots occurred across the community diversity gradient (Supp. Table S1) Effects of (log-transformed) species richness and functional group richness level of plant communities on overall community composition of nematode feeding groups (plant feeders, bacterial feeders, fungal feeders, omnivores and predators) were tested using nonparametric Multivariate Analysis of Variance (MANOVA) using the function Adonis in the R package Vegan (Oksanen et al. 2013). Nonparametric MANOVA was used because multivariate normality could not be achieved. Subsequently, we performed separate GLM's, testing for linear effects of plant species or functional group richness level on each of the five nematode feeding groups. The critical P value was adjusted for multiple testing of significance (Bonferroni). Nematode abundance data were log-transformed to meet GLM assumptions. In order to test whether the feeding pressure of plant feeding nematodes that could be expected in the subsequent year was linearly related to species and functional group richness of the plant community, we used root mass data of 2011 (Ravenek et al. 2014). These data were used to calculate the log-ratio of the number of plant feeding nematodes per dry mass of roots in the top 20 cm of the experimental plots. This proxy for nematode feeding pressure on plant roots was used as dependent variable in GLMs, testing for linear effects of (log-transformed) species richness and functional group richness of plant communities.

We constructed Structural Equation Models (SEM) to analyze via which pathways plant species richness and plant functional richness affected abundances and taxon richness of plant feeders, microbial feeders and predators. We started SEM by including all pathways from plant species or functional richness to nematode abundance or taxon richness. We compared the model-implied and observed variance-covariance matrix to test the model fit to the data using a maximum likelihood estimation method. By stepwise removal of non-significant paths from the initial model we selected the model that best fitted our data.

Results

Plant species richness significantly affected the nematode abundances across the nematode feeding groups (plant feeders, fungal feeders, bacterial feeders, omnivores and predators) (MANOVA $F_{1,74}$ = 11.25; P = 0.001). Similarly, functional group richness of the plant community significantly affected the nematode abundances (MANOVA $F_{1,74}$ = 8.01; P = 0.001). Analysis of the different nematode feeding groups (using Bonferroni correction for multiple testing; critical P-value = 0.01) revealed that the abundance of plant feeding nematodes (regression slope: 0.15 $\pm$ 0.04; P = 0.0008), of fungal feeders (regression slope: 0.26 $\pm$ 0.06; P < 0.0001), of bacterial feeders (regression slope: 0.20 $\pm$ 0.07; P < 0.003), and of omnivores (regression slope: 0.28; P = 0.0009) increased with increasing species richness. However, there was no relation between plant species richness and the abundance of predators (regression slope: 0.008; P = 0.95) (Fig. 1, Supp. Table S3).

Similarly, the abundance of plant feeding nematodes (regression slope: 0.12 $\pm$ 0.04; P = 0.004), of fungal feeders (regression slope: 0.23 $\pm$ 0.06; P = 0.0005), and of omnivores (regression slope: 0.22; P = 0.005) increased with increasing functional group richness of the plant communities. The abundance of bacterial feeders increased marginally with functional group richness (regression slope: 0.14 $\pm$ 0.06; P = 0.03), whereas the abundance of predators was not related to plant functional group richness (regression slope: 0.20; P = 0.09) (Fig. 2, Supp. Table S4).

The number of plant feeding nematodes per gram dry root (log ratio) decreased significantly with plant species richness (regression slope: - 0.21 $\pm$ 0.08; P = 0.010), but not with functional group richness (P = 0.19) of plant communities (Fig. 3a,b).

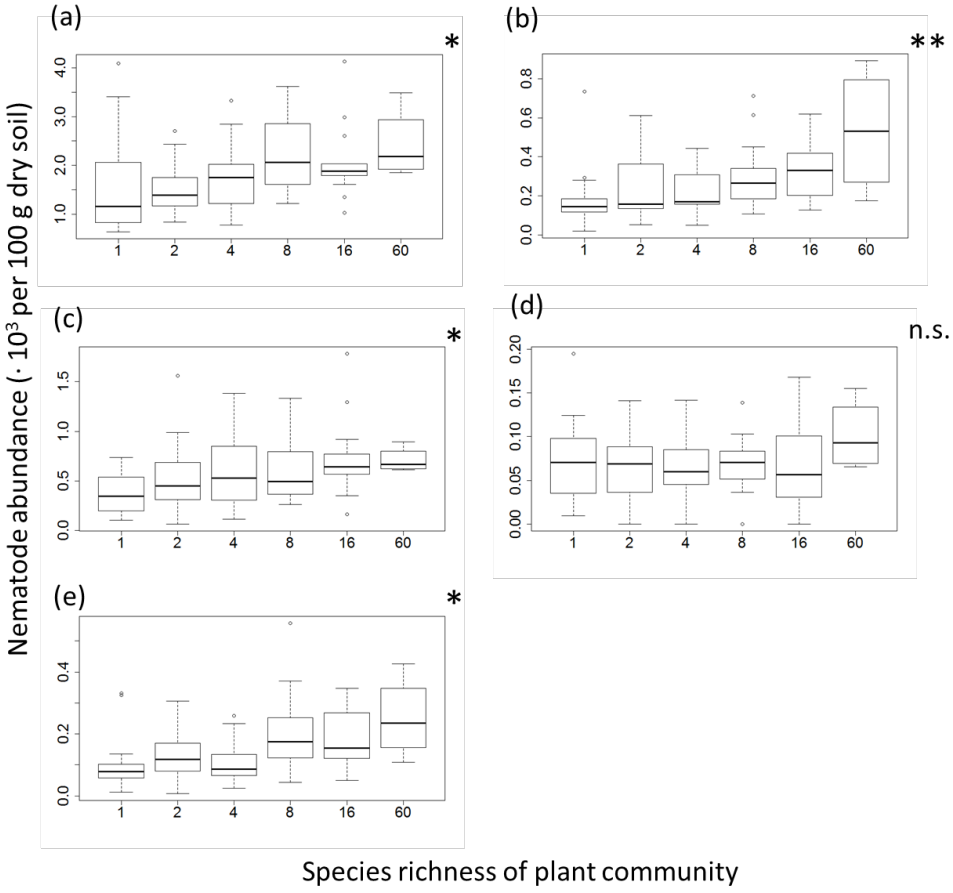

Figure 1 The effect of plant species richness (1, 2, 4, 8, 16, 60) of plant communities on the abundance (divided by 10^3) of (**a**) plant feeding nematodes, (**b**) fungal feeding nematodes, (**c**) bacterial feeding nematodes, (**d**) predatory nematodes and (**e**) omnivorous nematodes. Number of asterisks above each subpanel denotes significance of relationship ($P < 0.01$ *, $P < 0.0002$ **, n.s. = not significant, Bonferroni correction: $K = 5$; critical $P = 0.01$).

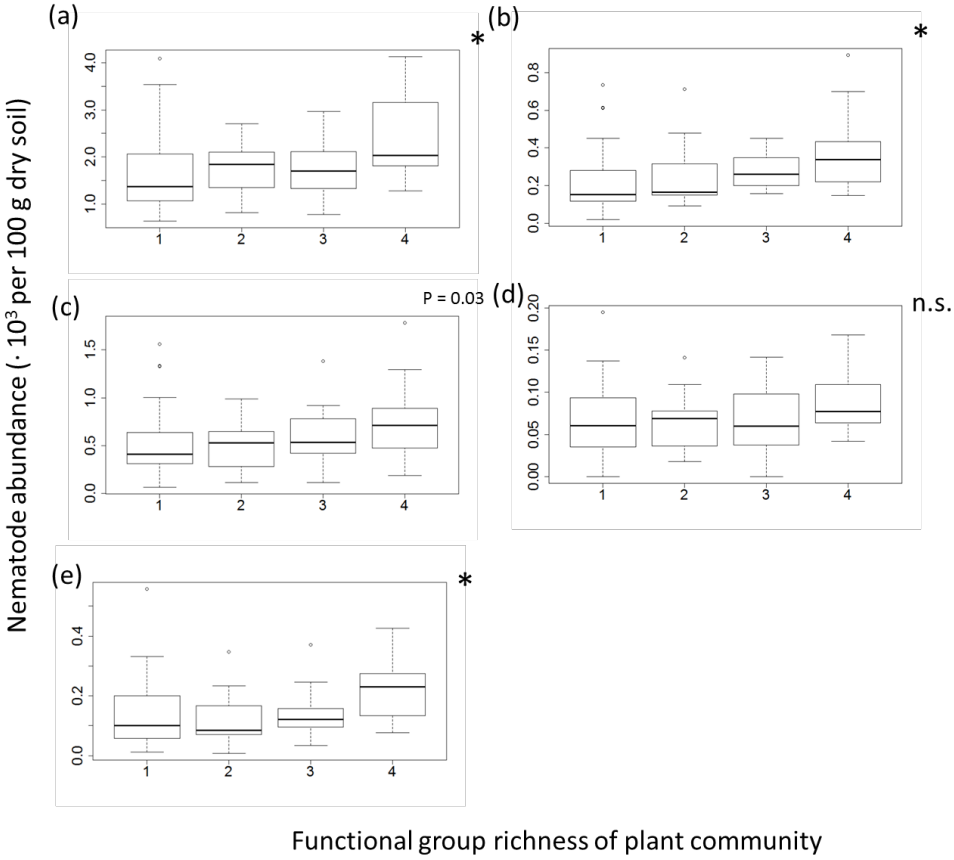

Figure 2 The effect of plant functional group richness (1, 2, 3, 4) of plant communities on the abundance (divided by 10^3) of (**a**) plant feeding nematodes, (**b**) fungal feeding nematodes, (**c**) bacterial feeding nematodes, (**d**) predatory nematodes and (**e**) omnivorous nematodes. Number of asterisks above each subpanel denotes significance of relationship ($P < 0.01$ *, n.s. = not significant, Bonferroni correction: K = 5; critical P = 0.01).

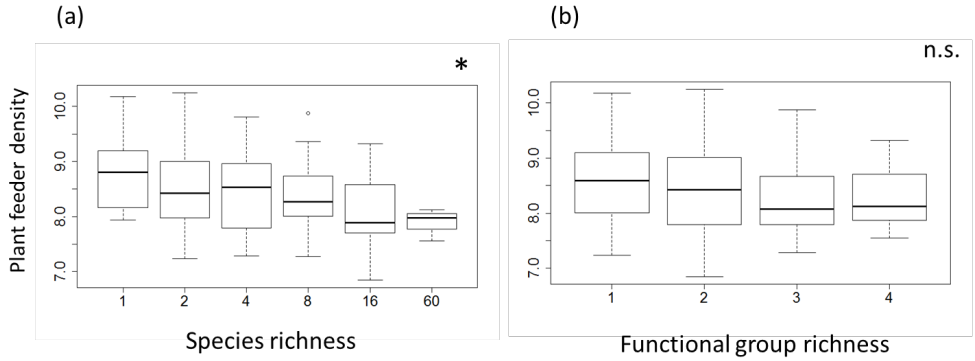

Figure 3 Log-ratio of the number of plant feeders and dry root mass per 100 g dry soil as affected by (**a**) species richness and (**b**) functional group richness of plant communities. Number of asterisks denotes significance of relationship ($P < 0.05$ *, n.s. = not significant).

Structural equation models

The positive effect of plant species richness on the abundance of microbial feeders was mediated via increased shoot biomass, while for plant feeders this was mediated via higher plant C:N ratios (Fig. 4a). Although microbial biomass increased with species richness of the plant community and organic matter content of soil, higher microbial biomass did not translate into higher abundance of microbial feeders. The abundance of predators increased with the abundance of plant feeding nematodes, but not with microbial feeder abundance. Similarly, plant functional group richness was positively related to the abundance of microbial feeders via increasing plant biomass and plant feeding nematodes via increasing plant C:N ratios (Fig. 4b). In contrast, taxon richness of microbial feeding nematodes increased with plant species richness via enhanced C:N ratios, but not by increased shoot biomass, whereas taxon richness of plant feeding nematodes was explained both via increased C:N ratios and shoot biomass (Fig. 4c). Functional richness of the plant communities also had a positive influence on taxon richness of microbial feeding nematodes via increased C:N ratios, whereas it had a direct positive effect on taxon richness of plant feeding and predatory nematodes (Fig. 4d).

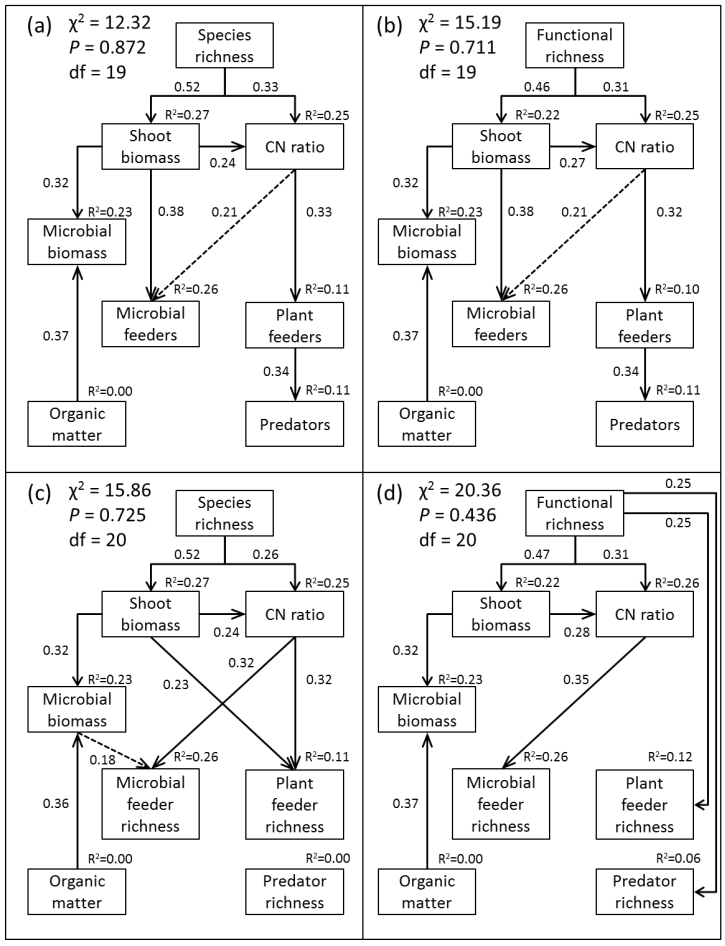

Figure 4 Model results of the SEM analyses showing the influence of plant species richness on (**a**) nematode abundances and (**c**) nematode taxon richness, and of plant functional group richness on (**b**) nematode abundances and (**d**) nematode taxon richness. χ^2 and P are the test results from the comparison between the model-implied and observed variance-covariance matrices, with $P > 0.05$ indicating that there is no difference between model-implied and observed variance-covariance matrices. Square boxes display variables included in the model: species richness (number of plant species per plot); functional richness (number of plant functional groups per plot); shoot biomass (g dry weight per m^2 in 2010); organic matter (percentage of soil organic matter); microbial biomass (µg C microbial per g dry soil); C:N ratio (ratio of C to N in shoot tissue in 2010); microbial feeders (number of microbial feeding nematodes per 100 g dry soil); plant feeders (number of plant feeding nematodes per 100 g dry soil); predators (number of predator nematodes per 100 g dry soil); microbial feeder richness (number of taxa of microbial feeding nematodes); plant feeder richness (number of taxa of plant feeding nematodes); predator richness (number of taxa of predator nematodes). Solid arrows represent significant relationships at $P < 0.05$, dashed arrows represent relationships at $P < 0.10$. R^2-values associated with the response variables indicate the proportion of explained variation by the relationship with the other variables. Values associated with the arrows represent standardized path coefficients.

Discussion

In the present study, we investigated the effects of species and functional group diversity of plant communities on the community composition and structure of nematodes in a long-term grassland biodiversity experiment (Jena Experiment). In our discussion of the results, we refer to 'plant community diversity' in cases where the effects of species and functional group diversity on nematode abundances had the same direction, which was common for many of the results. Previous similar studies have often discriminated between effects of plant biomass (quantity) versus plant species identity or diversity (quality) as potential drivers of abundance and composition of plant-associated biota communities. However, direct underlying mechanisms (e.g., Ebeling et al. 2014) most often remained unaddressed. Here, we sought to identify mechanisms underlying quantity vs. quality effects of plant community diversity by testing *a priori* hypothesized mechanistic pathways between plant community diversity and the abundance and species richness of different feeding groups of nematodes.

Effects of plant community diversity on different feeding groups

In support of our first hypothesis, we found that the abundances of all nematode feeding types, except for predatory nematodes, were positively related to both species and functional group richness of plant communities, suggesting bottom-up control of nematode abundances by plant community diversity. Plant community diversity had uniformly strong effects on nematode abundance across trophic levels, except for predatory nematodes. This is not in support of the suggestion that plant diversity effects decrease with increasing trophic level (Scherber et al. 2010). The weak response of predatory nematodes to plant community diversity could have been caused by their very low abundance (see Supp. Table 1). Furthermore, as predatory nematodes presumably mainly fed on plant feeders (see SEM results), their actual trophic level might be at comparable trophic distance to the plants as those of microbial feeding and omnivorous nematodes. Other plant diversity experiments showed either no effects of plant community diversity on nematode abundances, or effect sizes declined with increasing trophic position of nematodes (De Deyn et al. 2004a, Viketoft et al. 2009). Sohlenius et al. (2011) demonstrated that differences of nematode communities between plant communities increased with sampling year, indicating belowground time lags of plant community manipulations. Indeed, there is increasing awareness that relatively slow belowground responses to plant community manipulation are to be expected (Scherber et al.

2010, Eisenhauer et al. 2012), which may explain the lack of plant community diversity effects on nematode communities in relatively early stages of the Jena Experiment and other field experiments (e.g., Korthals et al. 2001, Gastine et al. 2003). This phenomenon is also reflected in our data, showing stronger responses of nematode abundances to plant community diversity after eight years than the effects reported by Eisenhauer et al. (2011) three and five years after establishment of the Jena Experiment. The density of plant feeding nematodes expressed as abundance per unit root dry mass declined with species richness of the plant communities, but not with functional group richness. This suggests that the nematode community shifts from an herbivory-based to a detrital-based food web when plant species richness increases (Eisenhauer et al. 2011). One factor that could underlie this finding is that there is a certain degree of host plant species-specificity of plant feeding nematode species. Plant species identity has indeed been reported to be an important predictor of nematode community composition (e.g., De Deyn et al. 2004a), which may lead to dilution of host species-specific plant feeding nematodes in plant species-rich communities.

Underlying mechanisms

We performed Structural Equation Modeling (SEM) to disentangle whether the positive effect of plant community diversity on nematode abundances and species richness was determined via pathways related to plant quality (e.g. tissue C:N) or quantity (e.g. plant biomass) (Wardle 2002). In contrast to our hypothesis that the abundance of microbial feeders would be positively related to microbial biomass, we found that microbial feeder abundance was directly positively affected by shoot biomass and not via pathways involving microbial biomass. This was despite the fact that microbial biomass increased with shoot biomass. Various factors may have contributed to the lack of a relationship between soil microbial biomass and the abundance of microbial feeding nematodes. First, it could be that the abundance of microbial feeding nematodes was not bottom-up controlled in our grassland system, but that other factors limited their abundance. Second, total microbial biomass does not necessarily relate to the productivity of microbes, but may reflect top-down control of microbial biomass by microbial feeding nematodes.

In contrast to our predictions that plant community diversity affects the abundance of plant feeding nematodes positively via increased aboveground biomass and negatively via increases in C:N ratio of plant tissue, we found no support for these pathways. The only pathway in our SEM model that could explain the increased abundance of plant feeding

nematodes with increasing plant community diversity was an increase of C:N ratio of aboveground plant tissue. These findings suggest that the abundance of plant feeding nematodes was, similarly as for microbial feeding nematodes, not mainly controlled by total resource availability, but (for plant feeders) by plant traits that are correlated to aboveground C:N ratio. High C:N itself is not likely to have caused high plant feeding nematode abundance, because community-level herbivory rates are expected to decrease with decreasing nutritional value of consumed biomass (e.g., Cebrian et al. 2009). Similarly to our results for nematode plant feeders, Ebeling et al. (2014) reported a SEM analysis of aboveground herbivorous invertebrates in the Jena Experiment showing that their abundance increased with increasing C:N ratio of aboveground plant tissue and not via aboveground biomass. The authors speculated that an increase in habitat volume (stem material) with increasing stem height in diverse plant communities (see also Abbas et al. 2013) caused the increased abundance of aboveground invertebrate herbivores. In our case, this latter mechanism is not a potential explanation for our finding of a positive association between C:N ratio and plant feeding nematode abundance. However, it is intriguing that this counter-intuitive pattern occurs for both above- and belowground plant feeders, suggesting that plant biomass quantity *per se* is not a strong predictor for invertebrate herbivory in plant communities.

Conclusions

In the present paper, we demonstrated that after eight years, experimental manipulation of both species and functional group diversity of plant communities resulted in strong bottom-up effects of plant diversity on nematode abundances in all but one feeding groups (predatory nematodes). This finding is in contrast to the ideas that cascading effects of plant community diversity on belowground biota are generally weak, and that these effects dampen with increasing trophic level. Using structural equation modeling (SEM), we showed that (*a priori* hypothesized) mechanistic pathways underlying the observed relationships between plant community diversity and nematode abundances in different feeding groups could not be explained by direct effects of resource quantity for either microbial feeders (approximated by microbial biomass) or plant feeders (approximated by plant aboveground biomass). Microbial feeder abundance was directly positively affected by plant biomass, while the abundance of plant feeders was directly positively influenced by increased C:N ratio of aboveground biomass. As results of a SEM are merely correlative, our results do not reveal mechanisms

directly, but they suggest that bottom-up control of plant community diversity on abundances of nematode feeding groups predominantly involve mechanistic linkages related to plant quality and/or effects of plants on the soil habitat.

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Supplementary information

Supplementary TableS1 Abundances and number of taxa of plant feeding, fungal feeding, bacterial feeding, predatory and omnivorous nematodes per 100 g dry soil in the three outlier plots, not included in the analyses.

Abundances	**B3A13**	**B3A17**	**B3A20**
Plant feeders	6748	1667	1141
Fungal feeders	130	12	196
Bacterial feeders	216	36	478
Predators	173	0	110
Omnivores	87	134	123
Taxa			
Plant feeders	5	4	6
Fungal feeders	5	1	2
Bacterial feeders	4	2	7
Predators	3	3	2
Omnivores	2	1	2

Plant species richness of outlier plots: B3A13: 4 sp, B3A17: 1 sp, B3A20: 8 sp.

Plant functional group richness of missing plots: B3A13: 1 FG, B3A17: 1 FG, B3A20: 2 FG.

Supplementary TableS2 Observed nematode taxa and their assignment to feeding groups (according to Yeates et al. 1993). The bacterivores plus fungivores together represent the microbial feeding nematodes.

Bacterivores	**Plant feeders**	**Omnivores**	**Predators**	**Fungal feeders**
Alaimus	*Anguinidae*	*Campydoridae*	*Aporcelaimidae*	*Aphelenchoididae*
Aulolaimus	*Criconematidae*	*Dorylaimidae*	*Discolaimidae*	*Diphterophora*
Bastiana	*Dolichodoridae*	*Qudsianematidae*	*Mononchidae*	*Leptonchidae*
Cephalobidae	*Helicotylenchus*	*Thornenematidae*	*Trischistoma*	
Cylindrolaimus	*Heterodoridae*			
Diplogasteridae	*Oxidyrus*			
Monhysteridae	*Paratylenchus*			
Panagrolaimidae	*Pratylenchus*			
Paramphidelus	*Rotylenchus*			
Plectidae	*Trichodoridae*			
Prismatolaimus	*Tylenchidae*			
Rhabditidae				
Theratocephalidae				

Supplementary TableS3 Abundances and number of taxa of plant feeding, fungal feeding, bacterial feeding, predatory and omnivorous nematodes per 100 g dry soil per species richness level of the plant community (mean ± SE; N = 15 for monocultures and 4 species plots, N = 14 for 2 species plots, N = 16 for 8 species plots, N = 12 for 16 species plots and N = 4 for 60 species plots).

	Plant species richness					
Abundances	**1**	**2**	**4**	**8**	**16**	**60**
Plant feeders	1635 ± 297	1562 ± 146	1759 ± 184	2234 ± 209	2085 ± 218	2427 ± 372
Fungal feeders	188 ± 46	245 ± 43	225 ± 29	303 ± 44	327 ± 40	533 ± 162
Bacterial feeders	386 ± 54	539 ± 100	623 ± 103	596 ± 82	739 ± 114	710 ± 64
Predators	74 ± 14	67 ± 11	65 ± 10	66 ± 9	65 ± 13	102 ± 21
Omnivores	108 ± 26	127 ± 20	108 ± 18	209 ± 36	183 ± 27	252 ± 67

Taxa	**1**	**2**	**4**	**8**	**16**	**60**
Plant feeders	5.4 ± 0.4	5.1 ± 0.4	5.7 ± 0.3	5.5 ± 0.4	6.2 ± 0.3	5.8 ± 0.3
Fungal feeders	2.0 ± 0.0	1.9 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.0 ± 0.0
Bacterial feeders	3.6 ± 0.5	4.9 ± 0.3	5.6 ± 0.3	5.3 ± 0.5	5.3 ± 0.3	5.5 ± 0.6
Predators	1.5 ± 0.1	1.9 ± 0.2	1.6 ± 0.2	1.5 ± 0.2	1.7 ± 0.3	1.8 ± 0.3
Omnivores	2.4 ± 0.2	1.9 ± 0.2	2.2 ± 0.2	2.3 ± 0.2	2.2 ± 0.2	3.0 ± 0.4

Supplementary TableS4 Abundances and number of taxa of plant feeding, fungal feeding, bacterial feeding, predatory and omnivorous nematodes per 100 g dry soil in relation to functional group (FG) richness level of the plant community (mean + SE; N= 31 for 1 FG, N= 19 for 2 FG, N= 10 for 3 FG and N= 16 for 4 FG).

	Plant functional group richness			
Abundances	**1**	**2**	**3**	**4**
Plant feeders	1704 ± 169	1775 ± 125	1777 ± 206	2415 ± 219
Fungal feeders	224 ± 33	254 ± 36	282 ± 31	373 ± 52
Bacterial feeders	525 ± 68	511 ± 59	629 ± 103	738 ± 101
Predators	63 ± 8	63 ± 8	64 ± 14	91 ± 9
Omnivores	138 ± 22	121 ± 19	148 ± 28	219 ± 25
Taxa	**1**	**2**	**3**	**4**
Plant feeders	1.0 ± 5.2	2.0 ± 05.7	3.0 ± 6.2	4.0 ± 5.8
Fungal feeders	2.0 ± 0.0	1.8 ± 0.1	2.1 ± 0.1	2.1 ± 0.1
Bacterial feeders	4.3 ± 0.3	5.4 ± 0.3	5.9 ± 0.4	5.2 ± 0.4
Predators	1.4 ± 0.1	1.8 ± 0.2	1.5 ± 0.3	2.1 ± 0.1
Omnivores	2.3 ± 0.1	1.9 ± 0.1	2.0 ± 0.2	2.7 ± 0.2

Chapter 7

General discussion

Life in the soil is indispensable for plant growth and productivity (Wardle et al. 2004, van der Heijden et al. 2008). However, soil organisms can both promote and suppress plant growth (Wardle 2002, Buée et al. 2009). In the context of the biodiversity-ecosystem functioning debate (Cardinale et al. 2012), it is generally thought that functioning of belowground communities improves with increasing plant diversity, that is, diverse (species-rich, functionally diverse, genetically diverse) plant communities support belowground communities that allow for greater plant productivity (Eisenhauer 2012).

The central aim of the research presented in this thesis was to enhance predictability of plant-soil interactions at the level of individual plants (chapters 2, 3 and 4) and at the level of plant communities (chapter 5).

In chapter 3, I demonstrated that plant functional traits can be used to predict interspecific variability of plant-soil feedbacks (PSFs). Therefore, my research may provide a conceptual framework to place relationships between PSFs and plant traits in the context of plant ecological strategies. In chapter 4, I identified phylogenetic relatedness between species as a promising predictor of indirect PSF (van der Putten et al. 2013), which may provide an additional explanation for the positive effect of plant phylogenetic diversity on ecosystem functioning (Cadotte et al. 2008). In chapter 5, I demonstrated that plant species with increasingly negative direct PSF (measured in the greenhouse) contributed least to community-level overyielding of species-rich plant assemblages in the field. Finally, in chapter 6, I showed that the abundances of different nematode feeding groups in the Jena Experiment were strongly affected by plant community diversity, but these effects could not be explained by food availability for the different nematode groups.

Now, I will discuss and integrate the findings of my thesis.

Breaking new ground: the role of plant traits in PSFs

Given that genotypes with the highest fitness over time are favored by natural selection, the ubiquity of negative PSFs among plant species elicits the obvious question: why do most plant species build up more negative than positive interactions with soil organisms, while some species are able to maintain net positive PSF? In order to answer this question from an evolutionary perspective, it is necessary to gain insight in the ecological trade-offs that allow for the coexistence of plant species showing negative PSF and plant species that show less negative or positive PSF.

From the onset of PSF studies, it has been clear that plant species may escape their negative direct PSF by growing in soil with a legacy of other plant species (Bever 1994). Nevertheless, negative PSF interactions with own soil biota, which reduce plant survival, growth, and finally reproductive output, should be selected against; so the questions remain: what are the advantages of plants with negative PSF compared to plants with less negative PSF, and conversely, at what price (in evolutionary currency) do plant species develop and maintain positive PSF?

There is large consensus that plants show trait syndromes that are associated to a spectrum of 'slow' (resource acquisitive, well defended) to 'fast' (resource conservative, poorly defended) plant ecological strategies (Reich 2014). The incorporation of PSFs in this plant ecological strategy framework can be assisted by analyzing what plant traits are related to PSF variation among plant species. In **chapter 3**, I tested the overall hypothesis that the 'slow' to 'fast' plant ecological strategy spectrum will also be reflected in the interactions between plants and intimate plant symbionts belowground. Among 48 plant species of the Jena Experiment, I analyzed the relationship between species-specific PSF and the values of four plant traits related to the 'slow' to 'fast' spectrum (specific leaf area, SLA; relative growth rate, RGR; specific root length, SRL; and colonization level or roots by arbuscular mycorrhizal fungi, AMF). I determined PSF in two ways: comparing biomass production of plants grown in soil conditioned by conspecifics with that of plants grown in sterilized soil (named PSFsterilized) or in a mixture of the 48 different conditioned soils (named PSFmixed). These soils were all mixed with a uniform sterilized background soil to minimize abiotic differences among treatments. PSFsterilized reflects net effects of all soil biota, whereas PSFmixed reveals effects of relatively specialized soil organisms. With PSFsterilized, I quantified the degree to which plants are susceptible to antagonistic

interactions with soil microbes and, conversely, promote beneficial interactions; hence, I expected interspecific variation of PSFsterilized to be strongly associated with plant traits that relate to the 'slow' to 'fast' ecological spectrum. I indeed provided support for the idea that plant trait values characteristic for a 'fast' ecological strategy are associated with negative PSF. I based this conclusion on the finding of a strong negative correlation between a plant's PSFsterilized value and its SRL, indicating that thick-rooted plants had positive PSFsterilized and thinner rooted plants had increasingly negative PSFsterilized (or, more precisely: plants with high vs. low dry weight investment per unit root length, respectively). Indeed: high SRL is characteristic for 'fast' plants, while low SRL is characteristic for 'slow' plants (Reich 2014). One examined aboveground and one examined whole-organism plant trait (SLA and RGR, respectively) showed no relation with PSFsterilized. This was surprising, because I expected to find negative correlations if there would be a trade-off between plant resource capture through investment in resource acquisition-related traits and vulnerability to natural enemies, with coordinated responses for both aboveground and belowground plant organs. Although plant traits are often found to be inter-correlated, the coordination of plant trait expression on a whole-plant level is by no means absolute, as ecological niches – which are tightly coupled with ecological strategies – are multi-dimensional (Kraft et al. 2015).

It should not come as a surprise that interspecific PSF variation was in the first place related to trait values of roots, where the interactions between plants and soil organisms take place. Plants need to limit root accessibility to soil pathogens, but also need to maximize root uptake capacity of water and nutrients. A plant's SRL may influence these processes, as thin roots may be prone to root antagonists (Bauerle et al. 2007) but good in acquiring plant resources (Ryser and Lambers 1995). Moreover, I suggest that AMF may mediate the positive relation between SRL and PSFsterilized, as I found that thick-rooted species had high colonization levels of arbuscular mycorrhizal fungi (AMF). Therefore, I conclude that negative PSFsterilized is associated with vulnerability of plants to pathogens, while positive PSFsterilized is associated with plant dependency on AMF.

Coming back to the question of what the advantages are of plants with negative PSF compared to plants with less negative PSF, my study suggests that plant species with negative PSF adopt an ecological strategy that is aimed at fast resource acquisition, which is generally found in ruderal, short-lived plants (Grime 1977). The advantage of these species to 'slow' plants would then be that they are good colonizers of disturbed habitat, which can be expected to be relatively free of soil biota causing negative PSF, as these build up with increasing time of plant growth in a specific soil patch. By contrast, plant species that develop and maintain a

positive balance of interactions with soil biota are expected to be relatively strong competitors, able to persist for a longer time in a specific vegetation patch (competitor strategy in the scheme of Grime (1977). Positive PSFs may be even more typical for stress-tolerant plant species (*sensu* Grime 1977), as these plants may heavily rely on microbial mutualists for nutrient acquisition and defense against antagonists (e.g., Rodriguez et al. 2008). Answering the question of what are the evolutionary costs of maintaining positive PSFsterilized, the 'price they pay' may be partly covered by the fact that they need to invest carbon in maintaining a mutualistic relationship with AMF or other growth-promoting root symbionts (Mooney 1972).

Important to note is that the species pool of the Jena Experiment does not include typical ruderal plants; they are mostly typical for the C-S-R (competitive-stress tolerant-ruderal) (intermediate) strategy in the scheme of Grime (1977). Thus, greater variability of both PSF values and trait values is to be expected when extending the approach I used to a wider range of plant ecotypes, which may translate in stronger patterns, also involving the other examined plant traits.

Another important finding of chapter 3 is that plant species that ranked low for PSFsterilized (i.e. have strongly negative net effects of own soil biota) tended also to rank low for PSFmixed (i.e. they grew better in soil that was conditioned by other plant species than in own soil). This finding also fits with the interpretation given above regarding the relation between PSF and plant ecological strategies: it suggests that plant species with strong negative PSF are indeed adapted to 'break new ground', i.e. to colonize disturbed vegetation patches with a legacy of mainly heterospecific plants, or thrive during early successional stages of vegetation (van der Putten et al. 1993, Kardol et al. 2006).

Above, I discussed my findings from the perspective of plant ecological strategy theory. However, using plant traits as a proxy for species-specific PSFs also has a more practical advantage. Species-specificity of PSF has been shown to be ecosystem-specific (Bezemer et al. 2006, Casper et al. 2008), which means that the ranking of species from negative to positive PSF (see chapter 3) may not only be dependent on the identities of co-occurring species, but also on the environmental (soil) characteristics of the plant community under study (Ehrenfeld et al. 2005). If future studies are able to predict the ranking of species-specific PSF based on plant traits, it may be possible to indirectly infer effects of interspecific PSF differences on plant community dynamics (e.g., Klironomos 2002, Anacker et al. 2014), using plant traits as a proxy for PSF.

Predicting PSF between plants by their phylogenetic relatedness

In chapter 3, I focused on the interspecific variability of PSF interactions (above). I discussed how the average species-specific PSF value of a species may affect and predict its ecological dynamics. However, the full incorporation of PSF as a factor influencing plant ecological dynamics requires us to take a closer look at the intraspecific variability of indirect PSFs (van der Putten et al. 2013), as the PSF effects that a plant experiences in a plant community are not spatially uniform in a vegetation, but depend on the soil legacy formation in the specific soil patch where a focal plant establishes (Hendriks et al. 2014). For example, van de Voorde et al. (2011) demonstrated that, during secondary succession of abandoned agricultural fields in The Netherlands, the surrounding plant species of the early successional plant species *Jacobaea vulgaris* changed over time, and plant species that co-occurred with *J. vulgaris* later in succession exerted an increasingly negative effect on *J. vulgaris* via their indirect feedback to *J. vulgaris* performance. In a subtropical forest, Liu et al. (2012) found evidence that phylogenetic overdispersion of co-occurring tree species was the result of indirect PSF being less negative when caused by decreasingly related species (determined as seedling mortality, affected by pathogenic soil fungi).

To date, two factors have been identified as potential predictors of the size and direction of indirect PSF between plant species. The first factor is successional position of the focal plant: plant species are likely to receive weaker indirect negative feedback (or none) from plant species that are typical for an earlier successional stage than the focal plant, while early successional focal plants suffer from direct negative feedback (van der Putten et al. 1993, Kardol et al. 2006). The second factor that has been shown to influence indirect feedback is the phylogenetic relationship between a focal plant and the plant that formed the soil legacy (i.e. the soil-conditioning plant) (e.g., Webb et al. 2006, Díez et al. 2010, Liu et al. 2012). Here, host plant species-specificity of the biota that cause PSF is expected to be an important factor explaining variation of indirect PSFs between plants (Bever 2003).

Indirect PSF of a plant species A on another plant species B involves specificity in the soil biotic legacy that plant A creates, and specificity in the response of plant B to this particular biotic community (Fig. 1). In Fig. 1, step (1) shows the specific imprint of plant species A on the community of soil microbes in its rhizosphere, which is depicted by 0, - and + signs, indicating whether the effect of a microbe on plant growth of A is neutral, negative or positive, respectively. Step (2) shows the subset of these microbes that can associate with

plant species B, which may be dependent on the phylogenetic distance between A and B, as rhizosphere microbes are expected to show phylogenetically conserved plant host-ranges (e.g., Liu et al. 2012). In the example of figure 1, the pathogens of plant A exhibit low compatibility with plant B, while A's growth-promoting microbes are compatible with B. Finally, step (3) depicts the actual effect of the microbes that can associate with B on plant growth of B, which is dependent on for example responsiveness to mycorrhiza and tolerance to pathogens.

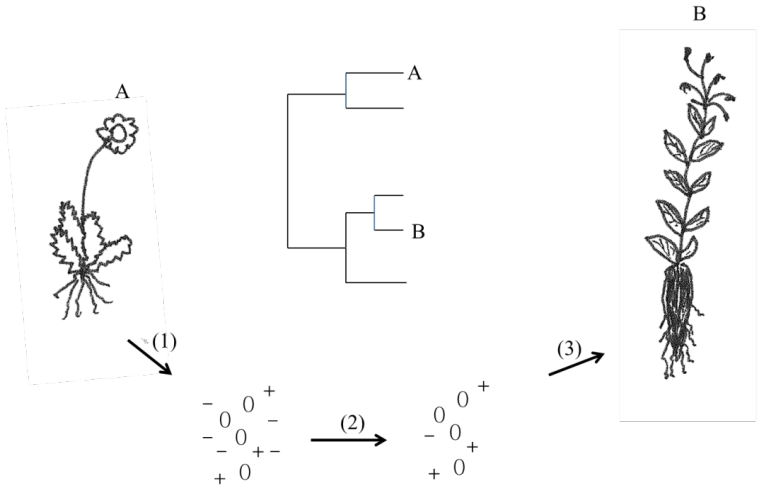

Figure 1 Conceptual model of indirect plant-soil feedback, showing the role of phylogenetic relatedness between plant A and plant B in influencing the indirect feedback effect of A on B via affecting the abundance and taxonomical identity of soil microbial antagonists (-), commensal microbes (0) and plant growth-promoting microbes (+). See main text for explanation.

I will now use this conceptual model to briefly discuss the results I obtained in **chapter 4**. In this study, I chose 11 focal plant species to represent negative, neutral and positive PSF plants, based on their PSFsterilized value (see chapter 3). The results of my phylogenetic PSF experiment showed that the relatedness of focal plants and soil-conditioning plants (31 species in total) significantly affected the biomass production of focal plants, but crucially, only when the direct feedback of the focal plant was taken into account; with increasing phylogenetic distance between focal and soil-conditioning plant, negative PSF plants showed no different or slightly better growth, neutral PSF plants showed on average worse growth, and positive PSF plants showed on average much worse growth. This finding may suggest that especially mutualists (+) had a phylogenetically restricted host plant range in my study.

However, a mechanistic understanding of my findings would have required the identification of microbes in rhizospheres and soils; in particular, in order to discriminate between step (1) and (2) as factors explaining interspecific feedbacks, rhizosphere biota have to be identified, ideally in the rhizosphere of plant A, the conditioned soil of plant A, and the rhizosphere of plant B. For example, AMF may have a wide plant host-range in terms of compatibility, but if a specific soil-conditioning plant does not promote AMF because its dependency on AMF is very low, the indirect feedback of this plant to a mycorrhiza-dependent plant species may be strongly negative. In chapter 5, I found that the negative response of positive PSF plants to distantly related plants often coincided with the fact that these distantly related plants were grasses. I speculate that the low association of these grasses with AMF (see chapter 3) could have led to poor performance of positive PSF plants in soils that were conditioned by grasses. Although this is a merely speculative interpretation, it illustrates my point that, possibly, we should not only consider phylogenetic ranges of root symbionts to explain effects of phylogeny between plants on their indirect PSF interactions, but also phylogenetic signal in the tendency of plants to interact with specific functional groups of soil microbes (e.g., AMF and pathogens). In summary, the role of 'specificity' of interactions between plants and roots symbionts in influencing indirect PSF involves species-specificity of the build-up of antagonists and mutualists by the soil-conditioning plant (step 1), the degree to which these organisms are compatible with the plant that responds to the legacy (2), and the responsiveness of the responding plant in terms of growth, survival and fitness (3).

PSF and biomass overyielding in the Jena Experiment

My main aim in **chapter 5** was to determine the relationships between the average direct versus indirect PSFs of 46 plant species, as determined in a short-term PSF experiment, and their biomass production in monocultures and 60-species mixtures of the Jena Experiment, of which the latter included all our study species. In the greenhouse, I quantified PSFs by conducting a short-term experiment covering eight weeks of soil conditioning and subsequent assessments of plant biomass after another six weeks of growth in the feedback phase. This is a typical duration of greenhouse experiments examining PSFs using the inoculation approach (Kulmatiksi and Kardol 2008), in which the variation of abiotic conditions (e.g., nutrient levels) among treatments is minimized in order to separate these from the effects of intimate

root symbionts on plant growth (Troelstra et al. 2001, Brinkman et al. 2010). As I showed in chapter 2, results from short-term pot studies of plant-soil interactions cannot be translated straightforwardly to potential outcomes of these interactions in the field. Such translation requires for example experiments that bridge between greenhouse and field.

Recent evidence suggests that plants benefit from growing in diverse communities because antagonistic interactions between plants and soil microbes decrease with increasing plant species diversity (Maron et al. 2011, Schnitzer et al. 2011). However, these studies left the question open of how PSF operates as a mechanism of facilitation among plant species, as the performance of individual plant species was not considered in those two studies. Moreover, Maron et al. (2011) not only showed overall positive effects of fungicide application on plant community productivity in low diverse assemblages, but at the single plant species level, both negative and positive effects of fungicides on plant growth were reported. Therefore, the latter two studies might need to be considered in the first place as experimental tests of the effect of plant species diversity on interactions between plants and intimate root symbionts. The longer term effects of feedback between plants and soil organisms were not assessed in these studies, as PSF involves reciprocal interactions between plant community structure and community structure of soil organisms over time.

The effect that short-term species-specific PSF may have on the fate of different plant species in well-established plant communities has been examined in a few studies. While Reinhart (2012) showed that there was no relationship between PSF and species abundances in three Prairie plant communities, two earlier studies showed strong positive correlations between PSF determined under controlled conditions and plant abundance in the field (Klironomos 2002, Mangan et al. 2010). Plant species with a negative direct versus indirect PSF, as assessed in a short-term experiment, were found to be less abundant than plant species with a more neutral or positive direct versus indirect PSF. Irrespective of the mechanisms underlying this positive association between PSF and plant abundance, species-specific overyielding of plants that are characterized by negative PSF might be impeded because they remain subordinate.

Recently, Kulmatiski et al. (2012) suggested that plant species with the most negative PSF will overyield most in mixed plant communities, because they are expected to strongly benefit from growing in soil of species-diverse plant communities. In **chapter 5**, I found that species with the most negative PSF had the lowest productivity in species-rich grasslands of the Jena Experiment. This result is in line with Klironomos (2002) and Mangan et al. (2010), who both found that plants with negative PSF had low abundance in mixed plant

communities. The relative yield of these species could still be high, if their biomass performance in monocultures would be even lower. Surprisingly, however, monoculture performance was not related to PSF in our study, while negative PSF is expected to develop rapidly in monocultures. These patterns of biomass production in monocultures and 60-species communities resulted in a positive relation between species-specific relative yields and PSF, which was contrary to my hypothesis. Furthermore, the complementarity effect in plant communities (which reflects the average relative yield across co-occurring plant species in a plot) was positively related to the average PSF value of the species comprising the assemblages. As complementarity includes both facilitation and niche complementarity mechanisms, high complementarity might be expected when plants with a neutral PSF grow together; I speculate that this would allow for the development of dense vegetation, which allows for strong effects of species complementarity through niche overlap (Tilman et al. 1997). Clearly, this suggestion requires further investigation.

I propose that the release from negative PSFs is not such a strong driver of positive species richness-productivity relationships in grasslands as suggested by recent studies (Schnitzer et al. 2011, Maron et al. 2011, Kulmatiski et al. 2012). While dilution of host plant species-specific pathogens causing negative PSF may still provide an additional explanation for observed positive diversity-productivity relationships in plant communities (Maron et al. 2011, Schnitzer et al. 2011, Kulmatiski et al. 2012, Hendriks et al. 2013), my results suggest that these positive relationships mainly arise when plants grow together that have a neutral PSF in the first place, and not so much because negative PSF effects decline in diverse plant communities. Much more evidence is needed to test this idea.

Plant diversity effects on the structure of nematode communities

Up till now, I discussed short-term PSF interactions between plants and microbial root symbionts (e.g., root pathogens and mycorrhizal fungi). These interactions involve highly dynamic feedbacks between plants and microbes in the rhizosphere, and can strongly affect plant performance from one to the next plant generation. A large number of recent studies, including the research described in this thesis, have shown the important role of short-term PSFs in driving relationships between plant community diversity and ecosystem functioning. It should however be kept in mind that root symbionts function within a specific soil abiotic setting, of which the conditions (e.g., soil aeration, moisture content, plant nutrient

concentrations) are also shaped by the activity of soil organisms, albeit these soil biota effects need to be conceived on longer time scales than short-term PSF interactions (Bardgett et al. 2005, Kardol et al. 2013, chapter 2 of this thesis).

In short-term PSFs, biotic interactions in the rhizosphere may have stronger plant species-specificity (Bever 2003) than in the case of *indirect pathway* interactions, which are mainly confined to the detrital pathway (Wardle et al. 2004). However, this detritus-consumer food web is for a large part fueled by root exudates of individual plants (e.g., Bonkowski 2004, Bais et al. 2006), and recent studies increasingly show that decomposition of litter can also be quite plant species-specific (Makkonen et al. 2012). Consequently, the distinction between the *direct* and the *indirect* pathway (Wardle et al. 2004), in terms of plant species-specificity, is fading. This is also reflected by increasing awareness that the characteristics of individual plants drive the structure and functioning of belowground communities, both in detritus- and live plant-based food webs (Bardgett and Wardle 2010). To date, however, the direct mechanisms that underlie effects of plant community characteristics on community structure and functioning of soil organisms involved in the *indirect pathway* are poorly known.

In **chapter 6**, my main aim was to gain insight in the mechanisms that drive relationships between plant diversity (species and functional group richness) and the community composition of nematode communities in the Jena Experiment. I focused on nematodes, because these organisms take up several key positions in the soil food web: fungal, bacterial and plant feeders, and omnivores and predators, are present in virtually all terrestrial ecosystems (Ferris et al. 2001).

In my study, I observed that the abundance and (to a lesser extent) taxon diversity of all feeding groups, except predatory nematodes, increased with both species and functional diversity of the plant community.

As both aboveground and belowground plant biomass in the Jena Experiment increases with higher plant community diversity (Marquard et al. 2009, Ravenek et al. 2014), and because plants are the main primary carbon source for soil organisms (Wardle 2002), one explanation for my results would be that bottom-up control of nematode abundances is caused by increased plant resource quantity. However, structural equation modeling (SEM), in which I incorporated various soil and plant parameters (i.e. aboveground standing plant biomass, C:N ratio of aboveground plant tissue, organic matter content of soil and microbial biomass in soil), demonstrated that plant feeder abundance could not be explained by plant biomass, and microbial feeder abundance could not be explained by microbial biomass. Instead, the

abundance of microbial feeders was positively related to plant biomass, and plant feeder abundance was positively related to increased C:N ratio of aboveground biomass. While these results are no direct evidence of a lack of bottom-up control of microbial or plant feeding nematodes by the availability of their food sources, they do suggest that other variables, not related to availability, but potentially to quality/composition, of food resources may determine nematode abundances.

In a recent study, Scherber et al. (2010) performed SEM on a long-term dataset compiling abundance and diversity data of various below- and aboveground organism groups present in plant communities of the Jena Experiment. They identified species richness as a direct factor (surpassing effects of plant biomass) affecting the abundance and diversity of biota. Effects of species-diversity *per se* on nematode abundances have also been reported previously (e.g., Viketoft et al. 2009). However, these two studies, and in fact most similar studies, did not explain the underlying mechanisms of plant diversity. Without a knowledge of mechanisms, it would be difficult to understand why relationships between plant species richness and soil communities sometimes appear (Viketoft et al. 2009) and sometimes not (De Deyn et al. 2004a).

Finally, the results of chapter 6 also corroborate the finding of Eisenhauer et al. (2012) that belowground effects of plant community changes may develop with considerable time lags. I found much stronger effects of plant diversity on nematode abundances than the nematode study of Eisenhauer et al. (2011) in the same Jena Experiment, which was based on soil sampling 3 and 5 years after its establishment, while we sampled soil 8 years after establishment. In general, the often reported increasing strength of biodiversity-ecosystem functioning relationships over time in field experiments (Reich et al. 2012) is an emerging issue. However, as we still know relatively little about what factors structure belowground communities, the mechanisms underlying this observation remain elusive.

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SUMMARY

Because of ongoing human alterations of the biosphere, biodiversity is declining worldwide at an ever increasing rate. A few decades ago, this decline prompted the question of how biodiversity is linked to ecosystem functioning, which motivated setting up numerous experiments explicitly investigating these links. In terrestrial systems, most biodiversity studies have manipulated community diversity of plants, because plants play an irreplaceable role as primary producers. In my thesis, I present research conducted in the framework of a large grassland biodiversity experiment in Jena, Germany, named the Jena Experiment. In 2002, eighty two plots were established by sowing plant monocultures and species mixtures on the floodplain of the river Saale, on a former arable field. The experiment covers a gradient of 1, 2, 4, 8, 16, 32 and 60 plant species. This treatment was crossed with a functional group-richness gradient: the number of functional groups (grasses, legumes, short and tall herbs) was varied from 1 to 4 across the species-richness gradient.

The aim of my study was to deepen our understanding of the role of soil biota in driving linkages between plant community diversity and plant productivity. Relations and feedbacks between plant community composition and soil functioning have received increasing attention over the past two decades, motivated by the general awareness that soil biota may exert control over plant community dynamics and productivity. Not only are soil organisms responsible for the decomposition of organic material, they also intimately interact with plant roots, either acting as antagonists to plants or as plant growth-promoting symbionts. Reciprocal interactions between plant and soil communities are an important component of so-called 'plant-soil feedbacks' (PSFs). In the PSF loop, plant community composition drives changes in belowground communities and abiotic conditions, the changes of which subsequently alter plant community composition. Decomposition processes operate on a time scale that surpasses the life duration of individual plants, and it is generally thought that the community composition and activity of decomposers is not so much plant species-specific, but rather responds to plant community-level aspects of plant quantity and quality. In contrast, the community of intimate (microbial) symbionts that builds up in the rhizosphere during the lifetime of an individual plant, has often been reported to have a high degree of host plant species-specificity and to result in 'short-term' PSF interactions.

In the second chapter of my thesis, I commented on a study that applied the widely used experimental approach of inoculating live soils into sterilized background soil in order to

study the effects of root symbionts on plant growth, separated from abiotic soil variation across experimental treatments. I briefly reviewed the main challenges of this type of experiments, particularly the way in which we could extrapolate the findings from highly controlled greenhouse studies to field conditions. This sets the scene for all further chapters; I tried to make clear that we make many assumptions when translating results of controlled studies to natural systems, and that we should continuously and carefully consider these assumptions and aim for rigid hypothesis testing by cross-talking between different levels of ecological realism.

In chapter 3 of my thesis, I studied short-term PSFs in the greenhouse using soil obtained from the Jena Experiment field site. My aim was to determine, using 49 species of the Jena Experiment, the size and direction of individual PSFs and to test how these relate to plant traits. First, I grew individuals of all species for a few weeks in sterilized soil inoculated with field soil. In the subsequent feedback phase, I grew all plant species in sterilized soil inoculated with (I) species-specific inoculum (conspecific conditioned soil), (II) sterilized species-specific inoculum, or (III) a mixture of all 49 species-specific inoculums (mixed conditioned soil). I compared biomass production in conspecific conditioned soil to biomass production in sterilized soil (PSFsterilized) and in mixed conditioned soil (PSFmixed). PSFsterilized reflects the degree to which plants are susceptible to antagonistic interactions with soil microbes or, conversely, promote beneficial interactions. PSFmixed reveals effects of relatively specialized soil organisms.

I correlated these PSF values to the values of a selection of plant traits, and found that species with increasing specific root length (SRL), which corresponds to less dense (or thinner) roots, are increasingly susceptible to antagonistic interactions in conspecific conditioned soil (i.e they have strong negative PSFsterilized). With this finding, I made a first important step in placing PSFs in plant ecological strategy frameworks, as high SRL is typical for plants that adopt a 'fast' ecological strategy, being characterized by fast resource acquisition but poor defense against antagonists. Moreover, I found that thick-rooted plants were characterized by both positive PSFsterilized and high colonization rates of arbuscular mycorrhizal fungi. Thus, I proposed that mycorrhiza mediate the relation between root architecture and PSF. Finally, I showed that PSFmixed showed much weaker relationships with SRL and mycorrhizal colonization, but that species ranking of PSFmixed was similar to species ranking of PSFsterilized. This indicates that plants with increasingly negative net interactions in conspecific conditioned soil increasingly benefit from growing in mixed conditioned soil. This finding also fits with the idea that plants with negative PSF are adapted to fast

acquisition of resources and to escape their negative PSF by colonizing foreign soil patches.

In chapter 4, I used the double-conditioned soil of the experiment from chapter 3 to set up a phylogenetically explicit PSF experiment. In this experiment I aimed to test whether phylogenetic relatedness between pairs of plant species is related to the indirect PSF between them. I grew eleven focal plant species, chosen to represent plants that had negative, neutral and positive PSFsterilized, in soils that were conditioned by conspecifics and soils conditioned by three to four other species with a varying degree of phylogenetic relatedness to the focal plant species. I demonstrated that focal plants with a negative PSF showed no different or slightly better growth, focal plants with neutral PSF showed worse growth and focal plants with positive PSF showed much worse growth, with increasing phylogenetic distance between focal and soil-conditioning plant. These results were surprising, because especially focal plants with a negative PSF were expected to respond strongly positively to phylogenetic distance. Instead, I found that especially positive PSF plants are strongly negatively affected by their relatedness to soil-conditioning plants. The most important conclusion of this study is that the effect of phylogenetic relatedness on PSF interactions between plant species likely depends strongly on the tendency of the focal plant species to develop detrimental or beneficial interactions with soil microbes.

In chapter 5, I used the PSFmixed values of chapter 3 in a correlational analysis to test how short-term PSFs relate to species' performances in field based monocultures and species-rich (60 species) plant communities of the Jena Experiment. I showed that plants with the most negative PSF produced least biomass in the 60-species plant communities. This corroborates recent studies showing that plant species with negative PSF remain subordinate in plant communities. However, surprisingly a plant's performance in monoculture was not related to its short-term PSF, whereas we expected plants with negative PSF to quickly decline over time. As a result of these patterns, species-specific overyielding was positively related to species-specific PSF, indicating that community overyielding was mostly driven by plant species with a neutral PSF. Based on these results I argue that we need to further critically examine the role of PSFs in positive effects of species richness on plant community productivity.

Finally, in chapter 6 I examined feedback between plant and soil communities by examining nematode communities in the Jena Experiment and the role of plant community quantity and quality in driving nematode feeding group abundance and diversity. Nematodes take up key positions in the soil food web and are readily assigned to feeding groups. Across the whole plant community diversity gradient of the Jena Experiment, I found strong positive

effects of both plant species- and plant functional group-richness on nematode abundances of plant, bacterial and fungal feeders, as well as omnivores, but not for predators. Subsequently, I performed structural equation modeling (SEM) to test potential pathways linking plant and nematode communities. The SEM analysis showed that the positive effect of plant diversity on the abundance of microbial feeding nematodes (fungal plus bacterial feeders) could not be explained by the increase of microbial biomass (the trophic level just below plants) with increasing plant community diversity. Similarly, the abundance of plant feeding nematodes was not driven by the higher plant biomass in diverse plant communities. Instead, increased plant biomass was a significant link between plant diversity and the abundance of microbial feeding nematodes. In contrast, higher plant feeding nematode abundance was linked to plant diversity via the increased C to N ratio of aboveground plant biomass. Moreover, despite the increase of plant feeding nematode abundance, their density per unit root biomass decreased with increasing plant diversity, indicating a root feeder dilution effect. Overall, I demonstrated that the mechanisms underlying strong linkages between plant diversity and belowground nematode communities may not be regulated by simple bottom-up relations via resource abundance and are instead expected to be mediated via a diversity of pathways.

SAMENVATTING

Door de toename van menselijke invloed op de biosfeer neemt de biodiversiteit op aarde snel af. Deze afname heeft enkele decennia geleden geleid tot het stellen van de vraag welke verbanden er bestaan tussen biodiversiteitsverlies en het functioneren van ecosystemen. Deze vraag gaf op zijn beurt aanleiding tot het opzetten van experimenten om deze verbanden te onderzoeken. De rol van de bodem is in de meeste studies onderbelicht gebleven. In mijn onderzoek ben ik nagegaan in hoeverre terugkoppelingsinteracties tussen planten en bodem voorspelbare effecten hebben op de samenstelling en het functioneren van plantengemeenschappen.

In terrestrische ecosystemen hebben de studies naar de rol van biodiversiteit in het functioneren van ecosystemen vooral de effecten van diversiteit van plantengemeenschappen onderzocht, omdat planten een onvervangbare rol spelen als primaire producenten en zodoende productiviteit van ecosystemen bepalen alsmede de link vormen naar hogere trofische niveaus.

In mijn thesis presenteer ik onderzoek dat ik heb uitgevoerd in het kader van een groot grasland-biodiversiteitsexperiment in Jena, Duitsland, genaamd het Jena Experiment. In 2002 werden daar tweeëntachtig proefvlakken uitgezet en verschillende behandelingen aangebracht door het zaaien van monoculturen en soortenmengsels van wilde planten op de uiterwaarden van de rivier de Saale, een gebied dat tot de zestiger jaren in gebruik was als akker. Het Jena Experiment omvat een gradiënt van 1, 2, 4, 8, 16, 32 tot 60 ingezaaide plantensoorten. Deze behandeling van soortenrijkdom werd gekruist met een gradiënt van het aantal functionele groepen: uitgaande van de totale pool van 4 functionele groepen (grassen, vlinderbloemigen, lage en hoge kruiden) werd het aantal groepen gevarieerd van 1 tot 4 binnen de verschillende soortenrijkdom-niveaus. In dit onderzoek werd door de jaren heen gevonden dat met meer ingezaaide plantensoorten de plantenproductie van de plantengemeenschappen toenam. De grote openstaande vraag blijft echter: welke mechanismen kunnen dit resultaat verklaren?

Het doel van mijn studie was er op gericht om een beter begrip te krijgen van de rol die bodemorganismen spelen in de totstandkoming van verbanden tussen de diversiteit en productiviteit van plantengemeenschappen. Relaties en terugkoppelingen tussen de samenstelling van plantengemeenschappen en het functioneren van de bodem hebben de laatste twee decennia toenemende aandacht genoten. Hieraan ligt het breed gedragen idee ten grondslag dat bodemorganismen een sterke invloed hebben op de dynamiek en productiviteit van plantengemeenschappen. Naast het ontbinden van organisch materiaal en het vrijmaken

van voedingsstoffen in voor planten goed opneembare minerale vorm, vertonen bodemorganismen ook nauwe interacties met levende plantenwortels als ziekteverwekker, worteleter of groeibevorderaar, zoals mycorrhizaschimmels; hierbij kunnen ze de plant variërend van negatief tot positief beïnvloeden. De wederzijdse beïnvloeding van planten- en bodemgemeenschappen vormen een belangrijk onderdeel van zogenaamde ‘plant-bodem terugkoppeling', in het Engels ‘plant-soil feedback’ (PSF) genoemd. In het PSF proces veroorzaakt de samenstelling van plantengemeenschappen veranderingen in gemeenschappen van bodemorganismen en abiotische bodemfactoren, welke op hun beurt weer een weerslag hebben op de samenstelling van de plantengemeenschap. De afbraak van organisch materiaal gebeurt op een tijdschaal die de levensduur van individuele planten overschrijdt en er wordt verondersteld dat de samenstelling en activiteit van afbraakorganismen niet sterk reageert op individuele plantensoorten, maar eerder reageert op de gemiddelde kwaliteit en kwantiteit van plantenmateriaal op plantengemeenschapsniveau. Daartegenover staan de vele (microbiële) symbionten (‘samenlevers’) die in de onmiddellijke wortelomgeving en zelfs deels in de wortels van individuele planten leven; deze organismen worden vaak gekenmerkt door een hoge plantensoort-specificiteit, en hun activiteit resulteert in zogenaamde ‘korte-termijn PSFs’.

In het tweede hoofdstuk van mijn thesis becommentarieerde ik een studie die gebruik maakte van een wijdverbreide experimentele methode waarbij levende bodem wordt geïnoculeerd in een achtergrond van gesteriliseerde bodem om de effecten van bodemorganismen op plantengroei te bestuderen, terwijl de abiotische verschillen tussen de bahandelingen worden geminimaliseerd. Ik bediscussieerde de voornaamste uitdagingen van dit soort experimenten, in het bijzonder hoe we bevindingen van sterk gecontroleerde kasstudies kunnen extrapoleren naar veldsituaties. Deze discussie is relevant voor alle volgende hoofdstukken. Mijn voornaamste doel van dit commentaarstuk was om duidelijk te maken dat we vele aannames maken wanneer we resultaten van gecontroleerde experimenten extrapoleren naar natuurlijke systemen. Mijn stelling was dat we deze aannames kritisch moeten beschouwen en streven naar het robuust testen van hypothesen door het voortdurend vergelijken van resultaten uit experimenten met verschillende mate van ecologisch realisme.

In hoofdstuk 3 van mijn thesis bestudeerde ik korte-termijn PSFs in de kas, gebruik makend van bodem die afkomstig was van het Jena Experiment. Mijn doel was om de individuele PSF waarden van 49 plantensoorten van het Jena Experiment te bepalen en te onderzoeken hoe deze PSF-waarden verband houden met verschillende plantkenmerken. In een eerste fase liet ik individuen van alle soorten apart groeien in gesteriliseerde grond,

geïnoculeerd met bodem van het Jena Experiment. In een volgende fase liet ik alle plantensoorten opnieuw groeien in gesteriliseerde grond, geïnoculeerd met (I) soort-specifiek inoculum (conspecifiek-geconditioneerde bodem), (II) gesteriliseerd soort-specifiek inoculum, of (III) een mengsel van alle 49 soort-specifieke inocula (gemengd geconditioneerde bodem). Ik vergeleek de biomassaproductie in conspecifiek-geconditioneerde bodem met de biomassaproductie in gesteriliseerde bodem (PSFgesteriliseerd) en gemengd-geconditioneerde bodem (PSFgemengd). PSFgesteriliseerd weerspiegelt de mate waarin planten vatbaar zijn voor negatieve interacties met bodemmicro-organismen, en, omgekeerd, positieve interacties kunnen bevorderen. PSFgemengd toont ons de effecten van relatief gespecialiseerde bodemorganismen.

Vervolgens correleerde ik deze PSF-waarden met een aantal plantkenmerken. Ik vond dat planten met toenemende specifieke wortellengte (SWL) (hetgeen betekent dat de wortels relatief dunner zijn), in toenemende mate vatbaar zijn voor negatieve interacties in conspecifiek-geconditioneerde bodem (deze planten hebben dus sterk negatieve PSFgesteriliseerd). Met deze bevinding heb ik een eerste belangrijke stap gezet in het integreren van PSF in de theorie van ecologische strategieën van planten, aangezien een hoge SWL typisch is voor planten die een ‘snelle’ groeistrategie vertonen. Deze wordt gekenmerkt door een snelle verwerving van voedingsstoffen, water en andere groeifactoren, maar een zwakke verdediging tegen plantenvijanden. Ook vond ik dat planten met dikkere wortels een positieve PSFgesterilizeerd vertoonden en een hoge associatiegraad met arbusculaire mycorrhizaschimmels hebben. Ik stelde voor dat mycorrhizae het verband veroorzaken tussen wortelarchitectuur en PSF. Ten slotte toonde ik aan dat PSFgemengd veel zwakkere verbanden toonde met SWL en de associatiegraad met mycorrhizae, maar dat de soortrangschikking voor PSFgemengd vergelijkbaar was met de soortrangschikking voor PSFgesterilizeerd. Dit duidt aan dat planten met een toenemende mate van negatieve interacties in conspecifiek geconditioneerde bodem in toenemende mate profijt hebben van het groeien in gemengd geconditioneerde bodem. Deze bevinding past ook bij het idee dat planten met negatieve PSF aangepast zijn aan een snelle verwerving van natuurlijke hulpbronnen en aan het ontsnappen van negatieve PSF door het koloniseren van nieuwe stukjes grond zonder recente groeigeschiedenis van de plantensoort in kwestie.

In hoofdstuk 4 gebruikte ik de dubbel-gecoditioneerde gronden van hoofstuk 3 om een fylogenetisch gecontroleerd PSF experiment op te zetten. In dit experiment wilde ik testen of de paarsgewijze PSF tussen plantensoorten voorspeld kan worden door hun onderlinge fylogenetische afstand. Ik selecteerde 11 plantensoorten, waarvan een deel negatieve, een deel

neutrale en een deel positieve PSFgesteriliseerd vertoonden. Ik liet deze soorten groeien in bodems die waren geconditioneerd door soortgenoten en bodems die waren geconditioneerd door drie tot vier andere plantensoorten met een fylogenetische afstand tot de onderzochte soort variërend van nauw tot veel minder verwant. Ik vond dat de onderzochte plantensoorten met een negatieve PSF een zelfde of iets betere groei vertoonden in grond afkomstig van minder verwante plantensoorten. Daarenboven vertoonden planten met een neutrale PSF een slechtere groei, en planten met positieve PSF veel slechtere groei wanneer de fylogenetische afstand toenam tussen de onderzochte plant en de plant die de bodem conditioneerde. Daarentegen vond ik dat vooral positieve PSF planten zwakke groei vertonen op bodems die geconditioneerd waren door ver-verwante planten. De belangrijkste conclusie van hoofdstuk 4 is daarom dat de effecten van fylogenetisch afstanden tussen planten op PSF interacties in belangrijke mate af lijken te hangen van de neiging van de onderzochte plant om positieve dan wel negatieve interacties te ontwikkelen met bodemmicro-organismen.

In hoofdstuk 5 gebruikte ik de PSFgemengd waarden van hoofdstuk 3 in een correlatie-analyse, waarin ik het verband testte tussen korte-termijn PSF en soort-specifieke biomassa productie in monocultuur en soortenrijke plantengemeenschappen (60 soorten) van het Jena Experiment. Ik kon aantonen dat planten met de meest negative PSF de minste hoeveelheid biomassa produceerden in de 60-soorten mengsels. Dit stemt overeen met recente studies die tonen dat plantensoorten met negatieve PSF subdominant blijven in plantengemeenschappen, maar is in tegenspraak met de idee dat planten met een negatieve PSF relatief veel voordeel hebben bij het groeien in mengculturen. De biomassaproductie van een soort in monocultuur hield geen verband met haar korte-termijn PSF, terwijl ik verwachtte dat planten met negatieve PSF snel minder biomassa zouden produceren. Een gevolg van deze patronen was dat soort-specifieke meeropbrengst positief gerelateerd was aan soort-specifieke PSF. Dit betekent dat de meeropbrengst van mengculturen voornamelijk veroorzaakt werd door planten met neutrale PSF. Gebaseerd op deze resultaten besluit ik dat we de rol van PSF bij het ontstaan van een positief verband tussen plantendiversiteit en biomassaproductie verder kritisch moeten bekijken.

Ten slotte onderzocht ik in hoofdstuk 6 terugkoppelingen tussen planten- en bodem gemeenschappen door de samenstelling van gemeenschappen van bodemaaltjes in het Jena Experiment te relateren aan kwaliteit- en kwantiteitaspecten van de plantengemeenschappen. Bodemaaltjes vervullen sleutelposities in het bodemvoedselweb en zijn relatief gemakkelijk te classificeren in voedingsgroepen. Over de hele diversiteitsgradiënt van plantengemeenschappen in het Jena Experiment vond ik sterke effecten van zowel soorten- en

functionele groepsdiversiteit van planten op de aantallen planten-, bacterie- en schimmeleters, alsook op de aantallen alleseters, maar niet op de aantallen predatore nematoden. Ik voerde een Structural Equation Model (SEM) uit om verschillende mogelijke causale verbanden te testen die planten- en aaltjesgemeenschappen zouden kunnen verbinden. Deze SEM-analyse toonde dat het positieve effect van plantendiversiteit op de aantallen microbe-eters (schimmel- en bacterie-eters samen) niet kon worden verklaard door de toename van microbiële biomassa met toenemende diversiteit van de plantengemeenschap. Een gelijkaardige bevinding was dat de aantallen planteneters niet verklaard konden worden door de hogere plantenbiomassa in divers samengestelde plantengemeenschappen. In plaats daarvan vormde plantenbiomassa een significante schakel tussen plantendiversiteit en de aantallen microbe-etende aaltjes. Daarentegen ging het hogere aantal plantetende aaltjes in divers samengestelde plantengemeenschappen samen met een toename van de verhouding koolstof/stikstof in het bovengrondse plantenmateriaal. Bovendien nam ondanks de toename van de aantallen plantetende aaltjes hun dichtheid in de wortels (uitgedrukt als hoeveelheid aaltjes per massa-eenheid wortel) af met toenemende plantendiversiteit, hetgeen wijst op een verdunningseffect op deze wortelvoeders. In grote lijnen kon ik tonen dat de mechanismen die ten grondslag liggen aan de verbanden tussen plantendiversiteit en gemeenschappen van bodemaaltjes waarschijnlijk niet veroorzaakt worden door eenvoudige relaties gebaseerd op voedselaanbod, maar eerder door een verscheidenheid aan mechanismen, zoals bijvoorbeeld de kwaliteit van het voedselaanbod en van beschikbare habitats.

ABSTRACT

In my thesis project I studied the role of soil biota as possible drivers of linkages between plant community diversity and plant productivity. My study was carried out in the framework of a large grassland biodiversity experiment in Jena, the so-called Jena Experiment. In chapter 1 I explain how soil biota may exert control over plant community productivity by recycling organic material and by intimately interacting with plant roots, either acting as antagonists to plants or as plant growth-promoting symbionts. Reciprocal interactions between plant and soil communities are an important component of so-called 'plant-soil feedbacks' (PSFs). In the PSF loop, plant community composition drives changes in belowground communities and abiotic conditions, which can subsequently alter plant community composition and productivity. Such PSF interactions have been proposed to play a major role in plant community composition and functioning.

In the second chapter I review studies that use an experimental approach of inoculating live soils into sterilized background soils to study the effects of root symbionts on plant growth. I demonstrate that we make many assumptions when translating results of controlled studies to natural systems. I propose that we should continuously and carefully consider these assumptions and aim for rigid hypothesis testing by cross-talking between different levels of ecological realism.

In chapter 3 I test how plant traits relate to PSF using a 49 grassland plant species of the Jena Experiment. First, I grew individuals of all species for two months in sterilized soil inoculated with field soil. In the subsequent feedback phase, I grew all plant species for 6 weeks in sterilized soil inoculated with (I) species-specific inoculum (conspecific conditioned soil), (II) sterilized species-specific inoculum, or (III) a mixture of all 49 species-specific inoculums (mixed conditioned soil). Subsequently I compared biomass production in conspecific conditioned soil to biomass production in sterilized soil (PSFsterilized) and in mixed conditioned soil (PSFmixed). Species with increasing specific root length (SRL) were increasingly susceptible to antagonistic interactions in conspecific conditioned soil (i.e. they had strong negative PSFsterilized), while thick-rooted plants had both positive PSFsterilized and high colonization rates of arbuscular mycorrhizal fungi (AMF). Finally, I showed that species ranking of PSFmixed was similar to species ranking of PSFsterilized, indicating that plants with increasingly negative net interactions in conspecific conditioned soil increasingly benefit from growing in mixed conditioned soil. With these findings, I made a first important step in placing PSFs in plant ecological strategy frameworks: high SRL is typical for plants

that adopt a 'fast' growth strategy, characterized by fast resource acquisition but poor defense against antagonists and little reliance on AMF.

In chapter 4, I test the relation between phylogenetic relatedness and the feedback effect of one (soil conditioning) plant species to another (responding) plant species. This is named indirect PSF. I grew eleven focal plant species, chosen to represent plants that had negative, neutral and positive PSFsterilized, in soils that were conditioned by conspecifics and soils conditioned by three to four other species with a varying degree of phylogenetic relatedness to the focal plant species. I found that plant species with negative PSF had no different or slightly better growth when growing in soil conditioned by plant species with larger phylogenetic distance to the focal plant. In contrast, plant species with neutral PSF grew less well, and species with positive PSF even worse, in soil conditioned by plant species with increasing phylogenetic distance to the focal plant. I conclude that the effect of phylogenetic relatedness on PSF interactions between plant species may depend on the tendency of the focal plant species to develop detrimental or beneficial interactions with soil microbes.

In chapter 5, I use the PSFmixed values of chapter 3 in a correlational analysis to test how short-term PSFs relate to longer-term species' performances in the field, using established monocultures and species-rich (60 species) plant communities of the Jena Experiment. Based on some recently published studies I expected that plants with more negative PSFmixed would benefit most from growing in mixtures; these plant species were expected to overyield most in mixed plant communities. However, opposite to the expectation, plant species with the most negative PSF produced least biomass in the 60-species plant communities, whereas plant performance in monoculture was not related to its short-term PSF. I conclude that species-specific overyielding was positively related to species-specific PSF, and that community overyielding was mostly driven by plant species with a neutral to positive PSF.

Finally, in chapter 6 I examine the role of quality and quantity of plant biomass in driving nematode feeding group abundance and diversity. I found strong positive effects of both plant species- and plant functional group-richness on abundances of plant feeding, bacterial feeding and fungal feeding nematodes, as well as omnivores, but not for predators. Structural equation modeling (SEM) analysis showed that the positive effect of plant diversity on the abundance of microbial feeding nematodes (fungal plus bacterial feeders) could not be explained by increased microbial biomass. Similarly, the abundance of plant feeding nematodes was not driven by the higher plant biomass in species rich plant communities.

Instead, increased plant biomass explained the positive relation between plant species richness and the abundance of microbial feeding nematodes, while for plant feeding nematodes, increased C to N ratio of aboveground plant biomass appeared to explain the positive relation between the abundance of plant feeding nematodes and plant species and functional group richness. Importantly, the density of plant feeding nematodes per unit root biomass decreased with increasing plant diversity, indicating a root feeder dilution effect. I conclude that plant diversity does not explain nematode community composition primarily by simple bottom-up relations, but that other aspects, such as quality of resource and microhabitats quality, may play a role as well.

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CURRICULUM VITAE

Roeland Cortois was born July 30, 1987, in Vilvoorde, Belgium. He was raised in the beautiful village of Melsbroek, 12 kilometers north-east of Brussels. He completed secondary education in 2005 and started his Bachelor in biology at the university of Ghent, Belgium, that same year. He earned his master's degree in biology in 2010 and directly after moved to Wageningen, The Netherlands, to start his PhD project together with Wim van der Putten and Gerlinde De Deyn. The results of this research project are reported in this thesis. Throughout his life, Roeland has sought beauty, laughter and love. Up till now, he found a lot of these things, and therefore considers himself a lucky man.

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (4.5 ECTS)

- The curse of the black box (2012)

Post-graduate courses (7.5 ECTS)

- Principles of ecological genomics (2011)
- Nematode identification course (2011)
- Big five: identification of plant parasitic nematodes (2011)
- Molecular advances in ecology (2011)

Laboratory training and working visits (0.9 ECTS)

- Statistics; Facilities of University of Jena, Germany (2011)
- Discussion and work on paper; University of Zurich, Switzerland (2012)

Invited review of (unpublished) journal manuscript (1 ECTS)

- Plant and Soil: plant-soil interactions (2012)

Competence strengthening / skills courses (2.5 ECTS)

- Writing workshop for PhD students of Jena; Jena consortium (2012)
- Effective behaviour in your professional surroundings; WGS (2015)

PE&RC Annual meetings, seminars and the PE&RC weekend (0.9 ECTS)

- Biochar symposium (2010)
- Eco-informatics (2011)
- Biodiversity research at the crossroads (2014)

Discussion groups / local seminars / other scientific meetings (7.5 ECTS)

- NIOOO PhD Journal club (2010-2012)
- NIOO Seminars (2011-2014)

International symposia, workshops and conferences (8.5 ECTS)

- Wageningen soil meeting (2011)
- Annual meeting of British Ecological Society (2011-2012)
- NAEM Meetings; 2 poster presentations (2011-2013)
- Root microbiome; Greece (2012)

Supervision of MSc student

- Relation between plant traits and plant-soil feedback

The research presented in this thesis was conducted at the Department of Terrestrial Ecology at the Netherlands Institute of Ecology (NIOO-KNAW) in Wageningen. This is NIOO thesis 118.

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