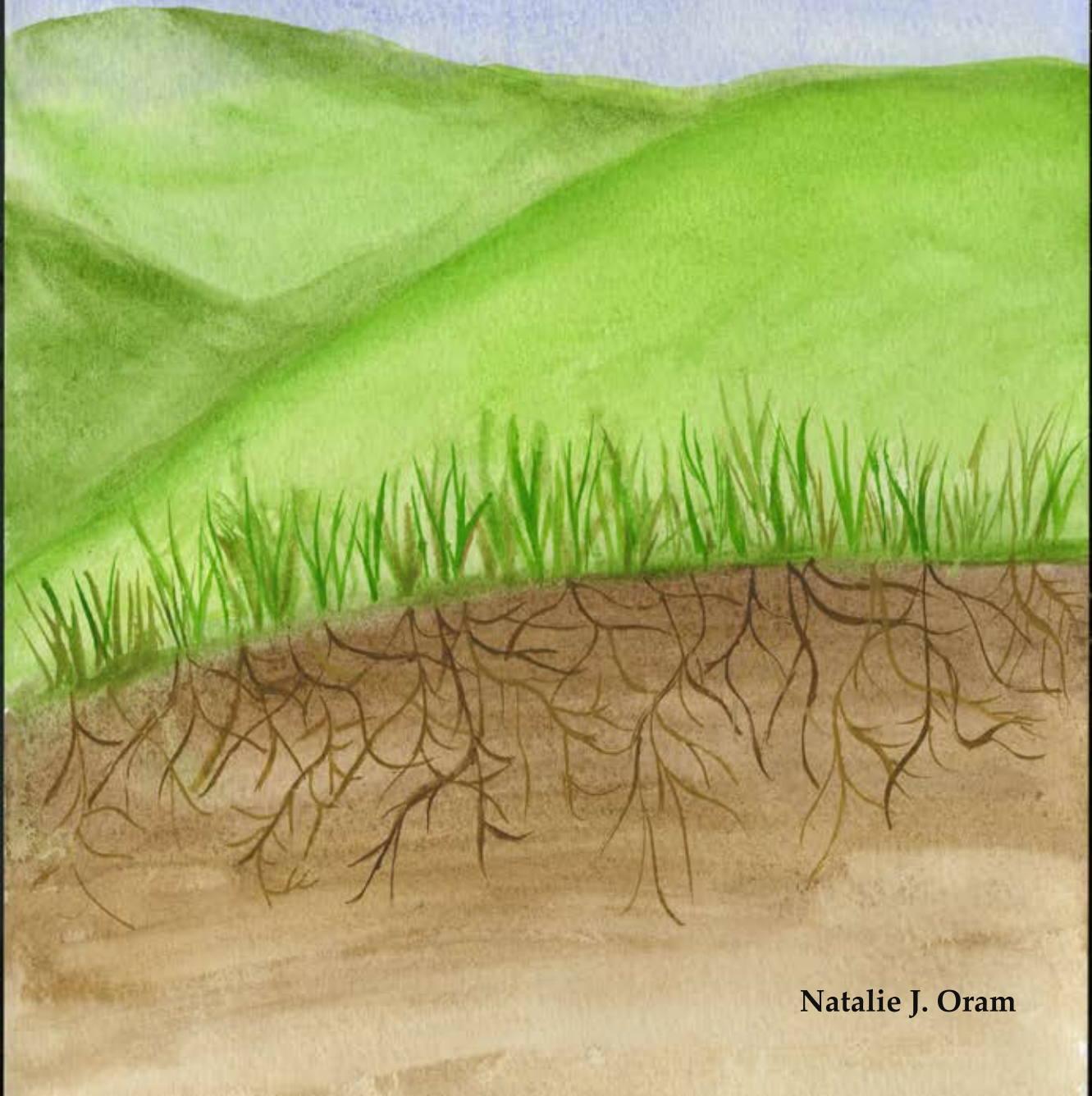


Root interactions in a diverse grassland

The role of root traits in belowground productivity and decomposition



Natalie J. Oram

Thesis committee

Promotor

Prof. Dr D. Kleijn
Professor of Plant Ecology and Nature Conservation
Wageningen University & Research

Co-promotor

Dr J. van Ruijven
Assistant professor, Plant Ecology and Nature Conservation Group
Wageningen University & Research

Other members

Prof. Dr E. Hoffland, Wageningen University & Research
Prof. Dr J.H.C. Cornelissen, VU Amsterdam
Prof. Dr M.J. Wassen, Utrecht University
Dr C. Veen, Netherlands Institute of Ecology (NIOO – KNAW), Wageningen

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*There is a pleasure in the pathless woods,
There is a rapture on the lonely shore,
There is society, where none intrudes,
By the deep Sea, and music in its roar:
I love not Man the less, but Nature more.*

- Lord Byron (Childe Harold's Pilgrimage, 1850)

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Chapter 1 General Introduction



The roots of the diversity-productivity relationship

Nearly 160 years ago, Darwin and Wallace hypothesized diverse mixtures to be more productive than monocultures because “co-existing species differ ecologically” (Darwin 1859; Purvis & Hector 2000). More recently, experimental manipulations of plant diversity confirm this pattern, showing a positive diversity-productivity relation¹ aboveground (Hector *et al.* 1999; Tilman *et al.* 2001; van Ruijven & Berendse 2005; Marquard *et al.* 2009; Reich *et al.* 2012; Cardinale *et al.* 2012) and belowground (Reich *et al.* 2004; Fornara & Tilman 2008; Mueller *et al.* 2013; Cong *et al.* 2014; Ravenek *et al.* 2014). There is mounting evidence that the root causes of the positive diversity-productivity relationship are belowground. For instance, due to niche differentiation (Levine & HilleRisLambers 2009; Turnbull *et al.* 2013), resource partitioning (McKane, Grigal & Russelle 1990; McKane *et al.* 2002; von Felten & Schmid 2008), and higher nutrient use efficiency (van Ruijven & Berendse 2005). What drives these processes is still unclear. An alternative, but likely congruent, line of research shows that plant-soil feedbacks underlie the plant diversity-productivity relation, due to the accumulation of species-specific soil pathogens at low plant diversity, which can drastically reduce productivity (Schnitzer *et al.* 2011; Maron *et al.* 2011). Recently, selection for niche differentiation between species through character displacement has been found to contribute to positive biodiversity effects (Zuppinge-Dingley *et al.* 2014).

The diversity-productivity relation illustrates what is now consensus, that species diversity is crucially important for ecosystem functioning- the efficiency by which ecosystems recycle nutrients, capture resources, produce biomass (Tilman 1999; Hooper *et al.* 2012; Cardinale *et al.* 2012), and withstand climate extremes (Isbell *et al.* 2015). It is then highly concerning that global biodiversity is being lost at an unprecedented rate (Butchart 2010), and has accelerated over the past decades due to human related activities such as urban expansion, energy production, and agriculture (Cardinale *et al.* 2012). Indeed, we may be in the throes of the Earth’s sixth mass extinction (Barnosky *et al.* 2011). To understand how and to what extent biodiversity loss will compromise ecosystem

¹ Terms in bold are defined in the glossary (Box 1.1)

functioning, the belowground drivers of the positive diversity-productivity relation need to be further elucidated.

Partitioning the effects of biodiversity

The positive effect of plant diversity on productivity is coined the 'net effect' of biodiversity. The net effect is defined as the difference between the observed yield of a diverse community from the expected yield (the monoculture yields of the component species, weighted by their initial relative abundance in mixture) (Loreau and Hector 2001). Using the additive partitioning method, this net effect can be broken into two non-exclusive components, selection effects and complementarity effects (Box 1.2, Loreau and Hector 2001). Selection effects are positive when species that are productive in monoculture have higher relative yields in a mixture than species which are less productive in monocultures. Relative yield is the difference between a species' observed yield in mixture, relative to its expected yield. The selection effect is sometimes equated with the sampling effect. However, unlike the selection effect, the sampling effect is the increasing probability of including a highly productive species with increasing plant diversity. Further, a sampling effect does not necessarily lead to positive biodiversity effects. For this to occur, the productive species must become dominant (increase in abundance) in the mixtures. Complementarity effects are positive if all species in a mixture have a higher relative yield on average than expected. Selection and complementarity effects are a mathematical derivation, which can inform hypotheses on the ecological mechanisms underlying the net effect of biodiversity. However, ecological conclusions cannot be drawn directly. It is important to note that the complementarity effects referred to in this thesis are not equivalent to 'resource complementarity' or 'complementary interactions' between plant species.

In experimental biodiversity manipulations, both selection and complementarity effects have been reported to contribute to net effect of biodiversity aboveground (Cardinale *et al.* 2007). Complementarity effects are generally attributed to positive interactions between species in mixtures, such as niche differentiation or resource partitioning (Loreau & Hector 2001). Over time, the contribution of complementarity effects to net biodiversity effects increases, compared to selection effects, suggesting that positive

complementarity effects are not transient (Fargione *et al.* 2007; Cardinale *et al.* 2007; van Ruijven & Berendse 2009; Marquard *et al.* 2009; Craven *et al.* 2016). The strengthening of complementarity effects has been proposed to be due to increased input and retention of nitrogen in a nitrogen limited system (Fargione *et al.* 2007). However, experimental evidence for the mechanisms underlying complementarity effects is limited.

Belowground, the strength of complementarity and selection effects are largely unknown, due to the methodological constraint of identifying species specific roots in mixtures. Thanks to a molecular technique (Mommer *et al.* 2008) which determines the relative abundance of species-specific root biomass in mixtures, belowground selection and complementarity effects are no longer in the dark. Currently, the single study which determined selection and complementarity effects belowground found that positive complementarity effects facilitated a positive net biodiversity effect in a four-species mixture (Mommer *et al.* 2010). In field biodiversity experiments, belowground selection and complementarity effects have yet to be unearthed. Determining how these effects contribute to the positive net effects of plant diversity on productivity gives insight into possible mechanisms that contribute to the diversity-productivity relation. For instance, predominant belowground complementarity effects lends support to the hypothesis that niche differentiation could underlie the diversity-productivity relation.

Do root-root interactions facilitate complementarity effects?

The diversity- productivity relation has been hypothesized to occur due to a more complete use of limiting resources at higher diversity, due to species' *complementary* niches (niche complementary hypothesis, Tilman 1999). Support for this hypothesis has been found in long-term biodiversity experiments; resource use efficiency was greater in diverse plant communities (HilleRisLambers *et al.* 2004; van Ruijven & Berendse 2005). A principle assumption of this hypothesis is that species differ in their traits and/or growth strategies which leads to an increase in total nutrient uptake (e.g. Levine & HilleRisLambers 2009). Hence, a diverse community is likely to be more functionally diverse and better able to capture nutrients than a species-poor community. The frequent success of species approaches in explaining ecosystem functions, e.g. productivity, demonstrates that the assumption that trait variation is greater between than within

species holds to a certain extent. However, species' traits may better explain the processes underlying diversity- ecosystem functioning relations than the species' identity (Díaz & Cabido 2001). Roscher *et al.* (2012) showed that the community weighted mean and functional trait diversity related to resource acquisition and life history explained complementarity and selection effects better than plant species diversity. They concluded that diversity in nitrogen use strategies contributed to positive complementarity and net biodiversity effects (Roscher *et al.* 2012). Aboveground plant traits are extensively considered in trait based approaches (e.g. Craine *et al.* 2002a; Cadotte *et al.* 2009; Roscher *et al.* 2013; Kunstler *et al.* 2016). These approaches frequently consider independent measures of species traits, weighted by the relative abundance of species in mixtures, to test if trait dominance or diversity can explain community productivity or biodiversity effects. However, the fate of plant-plant interactions belowground can influence nutrient uptake and growth. Therefore, the effects of community weighted mean or diversity of root traits is important to consider in explanations of plant community productivity, and complementarity effects.

Differences in the root trait vertical root distribution between species, or functional groups (Berendse 1982; Casper & Jackson 1997), have been hypothesized to facilitate spatial niche differentiation (Parrish & Bazzas 1976; Levine & HilleRisLambers 2009; Skinner & Comas 2010; Belter & Cahill 2015), leading to greater nutrient use efficiency, complementarity effects and productivity in diverse communities (Hooper 1998; van Ruijven & Berendse 2005; de Kroon *et al.* 2012). However, support for this hypothesis is limited and inconclusive. Increases in vertical root distribution (i.e. a greater proportion of deep roots) with plant diversity could signal vertical root segregation. Reports from the Jena Experiment and the Wageningen Biodiversity Experiment have found that community vertical root distribution does not change with plant diversity (Cong *et al.* 2014; Ravenek *et al.* 2014). In contrast, at the Cedar Creek Experiment, root biomass below 30 cm increased with increasing plant diversity, and related to increases in above- and belowground productivity (Mueller *et al.* 2013). These conflicting results signal more research is required to elucidate whether the mean or diversity of species' vertical rooting depths in a community could underlie the diversity-productivity relationship.

Consideration of vertical root distribution at the species level is required to elucidate whether it contributes to above- and belowground productivity.

Plant species may differ in their vertical root distribution inherently, or through plasticity, in response to biotic and abiotic factors. An inherent trait value results from genetic controls, i.e. the value of a specific trait when the plant is in the 'control' environment. Roots grow in a complex environment, and therefore, the 'true' inherent vertical root distribution in this context is both difficult to determine, and perhaps not ecologically meaningful (i.e. an inherent root distribution may not exist as roots may always adjust to their environment). In the context of this thesis, a species' inherent vertical root distributions is its vertical root distributions in monoculture. Connecting inherent traits to an ecosystem function, such as productivity overcomes the methodological constraint of separating species-specific roots in mixture. It also tests if an independent measure of traits can predict the outcomes, in terms of ecosystem functions such as productivity, of belowground plant interactions in the field.

However, plant roots have been reported to be highly plastic (Hodge 2004). Plasticity is the extent to which a plant can alter its traits in response to abiotic and biotic stimuli in its environment. The environment in which roots reside is a complex one, with chemical and structural challenges (e.g. heterogeneous resource distribution and soil compaction). Roots have been shown to respond to patches of available nutrients by increasing root length, initiating lateral roots or increasing biomass (Fransen, de Kroon & Berendse 1998; Hodge *et al.* 1999; Hodge 2004; Kembel & Cahill 2005). Plants may also respond to neighbours belowground through root segregation: placing its roots away from its neighbour or aggregation: increasing root length or biomass near a neighbour's roots. Root segregation is in line with theories of niche differentiation, and has been found in a diverse grassland using DNA barcoding (Kesanakurti *et al.* 2011). In contrast, other studies in diverse grasslands have shown predominantly random root placement (Frank *et al.* 2010), root aggregation (Frank *et al.* 2015) or both aggregation and segregation Price *et al.* (2012).

Whether plants aggregate or segregate their roots can be mediated by two main factors: plant-induced changes in nutrient availability, or non-nutrient signals between plants (i.e. signals contained in root exudates). Plants can alter nutrient availability, causing a

neighbour to alter their root placement, e.g. by segregating its roots to avoid zones of depleted nutrients (Nord, Zhang & Lynch 2011). Conversely, roots may aggregate to take advantage of increased availability of nutrients, such as phosphorus (Li *et al.* 2007b) or nitrogen (Cheng 2009) in the rooting zone of a neighbouring plant. Species identity has been shown to influence root placement. Under intra-specific competition, root segregation has been demonstrated, while inter-specific competition can lead to root aggregation (Bartelheimer, Steinlein & Beyschlag 2006). This may be due to the competitive strength of the neighbour (Schmid, Bauer & Bartelheimer 2015), or due to signals in root exudates (Bais *et al.* 2006). Roots may alter their root traits in response to exudates from non-related individuals, e.g. by increasing root biomass, specific root length and root branching (Semchenko, John & Hutchings 2007; Semchenko, Saar & Lepik 2014). Responses to neighbours in terms of vertical root distribution may differ between functional groups, however, empirical evidence from the field is scarce. As grasses and forbs have been shown to differ in their root traits (Tjoelker *et al.* 2005; Ravenek *et al.* 2016), nutrient foraging ability (Grime & Mackey 2002; Kembel & Cahill 2005), and plasticity (Rose *et al.* 2009), they may alter their vertical root distribution differently in response to their neighbours. Quantifying vertical root distribution with molecular methods (Mommer *et al.* 2008) gives valuable insight into the plasticity in species-specific vertical root distribution in mixtures, and how the diversity in vertical root distribution influences biodiversity effects.

Decomposing the diversity-decomposition relationship

Globally, more than 90 gigatons of terrestrial plant biomass enter the dead organic matter pool annually (Cebrian 1999). The rate that this litter is decomposed has a major effect on carbon and nitrogen cycling (Parton *et al.* 2007; Berg & McClaugherty 2008), and ultimately determines net carbon storage (De Deyn, Cornelissen & Bardgett 2008). Species loss across trophic levels can significantly alter decomposition rates (Gessner *et al.* 2010; Handa *et al.* 2014), with estimated equal or greater effects compared to other global environmental changes: elevated CO₂ or nitrogen deposition (Hooper *et al.* 2012). The species diversity of leaf litter has been shown to alter litter decomposition (Hättenschwiler, Tiunov & Scheu 2005; Handa *et al.* 2014). However, the effect of plant

diversity on root decomposition is less known (Zhang *et al.* 2008). This leaves a meaningful knowledge gap, as roots account for the majority of plant biomass in grasslands (Poorter *et al.* 2012), and are a major carbon input to the soil (Rasse, Rumpel & Dignac 2005). Indeed, soil carbon accumulation and storage were found to increase with plant diversity in long term grassland biodiversity experiments, corresponding with increases in root standing biomass (Fornara & Tilman 2008; Steinbeiss *et al.* 2008a; Adair *et al.* 2009; Cong *et al.* 2014; Lange *et al.* 2015). In the Jena Biodiversity Experiment, root litter decomposition decreased with increasing plant diversity (Chen *et al.* 2017), providing evidence that plant diversity increases carbon storage through multiple pathways. Soil carbon storage is the net result of litter production and decomposition. Therefore, in managed grasslands where aboveground biomass is removed, the importance of root litter production and decomposition is paramount. Further elucidating the mechanisms which underlie the plant diversity-root decomposition relation will inform predictions of how species loss will influence carbon cycling and storage.

Pathways to decomposition

Decomposition rate is determined via two main pathways: the soil environment, and litter quality (the species composition of the litter mixture, or litter mixing effects) (Swift, Heal & Anderson 1979; Aerts 1997; Parton *et al.* 2007). Plant diversity has been shown to influence factors in both of these pathways, leading to changes in decomposition.

Plant diversity has been reported to have a positive (Hector *et al.* 2000; Cong *et al.* 2015b), weak negative (Knops, Wedin & Tilman 2001; Fornara, Tilman & Hobbie 2009; Chen *et al.* 2017), or non-significant effect on decomposition (Scherer-Lorenzen 2008) via the soil environment (comparing decomposition of standard litter). This may signal that plant diversity does not have consistent effects on the underlying factors, and that the factors limiting decomposition differ between study sites. Diverse communities have greater canopy cover (e.g. Spehn *et al.* 2005), which can lead to lower temperatures at ground level (Verheyen *et al.* 2008) and in the top soil (Rosenkranz *et al.* 2012). Especially in spring, this could reduce decomposer activity, and thus decomposition. A more complete use of soil water has been found in diverse communities, but due to increased

evapotranspiration, the growth of these communities was predicted to be negatively affected during drought (Verheyen *et al.* 2008). In contrast, water content of the topsoil has been shown to increase with plant diversity (Caldeira *et al.* 2001; Rosenkranz *et al.* 2012). As soil moisture generally promotes decomposition (Prescott 2010), differential effects of plant diversity on soil water may lead to inconsistent effects on decomposition. Plant diversity can also influence soil biota, increasing decomposer abundance (Eisenhauer *et al.* 2011a) and activity (Balvanera *et al.* 2006), microbial biomass (Eisenhauer *et al.* 2010) and microbial activity (Lange *et al.* 2015), signalling that decomposition would increase with plant diversity. Explicit tests of how plant diversity simultaneously influences soil abiotic and biotic factors, and the relative importance of these factors to decomposition, are needed in order to better predict the effect of plant diversity on decomposition via changes in the soil environment.

Through shifts in litter quality across a diversity gradient (comparison of native root litter decomposing in its home environment), plant diversity has been shown to have negative (Chen *et al.* 2017) or non-significant (Milcu *et al.* 2008; Scherer-Lorenzen 2008; Fornara *et al.* 2009) effects on litter decomposition. Plant diversity can influence litter quality through shifts in species abundance which influence litter trait means or diversity, or via litter mixing effects. For example, plant diversity negatively affected root decomposition due in part to an increase grass presence and the associated increase in root C:N ratio in diverse communities (Chen *et al.* 2017). Litter mixing effects are frequently non-additive, i.e. the decomposition of a mixture cannot generally be predicted from the individual decomposition of the composite species. In their meta-analysis, Gartner and Cardon (2004) showed that leaf litter mixing effects vary from negative non-additive (19% of mixtures), neutral (33% of mixtures), to positive non-additive (47% of mixtures). Wardle *et al.* (1997) tested litter mixing effects of litter mixtures varying in plant diversity and found that litter mixing effects ranged from negative to positive, but did not change with the plant diversity of the litter. Root litter mixing effects are largely unknown. Both studies considering litter mixing effects based on root litter mass loss found positive litter mixing effects (Robinson, Kirkham & Littlewood 1999; de Graaff *et al.* 2011).

Root traits as an underlying mechanism

The outcomes of the plant diversity-decomposition relation could be inconsistent because the functional traits of an individual may have a greater effect on ecosystem processes than its taxonomic identity (Díaz & Cabido 2001; Scherer-Lorenzen 2008). Trait-based approaches are now frequently used to explain ecosystem processes. Previously, aboveground traits were the predominant focus, however, root traits are now being considered with increasing interest due to their importance in nutrient and carbon cycling (Bardgett, Mommer & De Vries 2014). Plant nutrient uptake and growth can be predicted by leaf traits (*the leaf economic spectrum*; Wright et al. 2004; Reich 2014), and root traits (*the root economic spectrum*; Roumet et al. 2016). The root economic spectrum is not ubiquitous to all ecosystems, which may reflect a disconnect between root traits and functioning in certain ecosystems (Weemstra et al. 2016). Traits can also inform outcomes of plant-soil interactions. For instance, leaf traits can explain the composition of soil food webs (Orwin et al. 2010; de Vries et al. 2012b). Plant-soil feedbacks have been explained by traits of leaves (Baxendale et al. 2014) and root (Cortois et al. 2016). Combining leaf and root traits has been shown to predict population biomass (Schroeder-Georgi et al. 2015), and explain community biomass and net biodiversity effects (Roscher et al. 2012). Plant functional traits can explain variation in soil carbon storage across biomes (De Deyn et al. 2008). Litter chemical and physical traits have been shown to be the predominant predictor of leaf and root decomposition through their effects on litter quality (Cornelissen 1996; Silver & Miya 2001; Garnier et al. 2004; Cornwell et al. 2008; Zhang et al. 2008; Freschet, Aerts & Cornelissen 2012a; Smith et al. 2014). Clearly, plant traits above and belowground can influence not only plant nutrient uptake and growth, but complex ecosystem processes such as plant-soil interactions, nutrient and carbon dynamics. Litter traits likely underlie the plant diversity – root decomposition relation. However, it is still unclear which root traits drive this relation, and to what extent it is due to shifts in the relative abundance of species (shifting community traits), or litter mixing effects (interactions between litters of different species). Identification of species-specific relative abundance of roots across the plant diversity gradient allows these effects to be disentangled.

Scope of this thesis

Study sites

This thesis takes place within the Jena Trait Based Experiment (chapters 2-4) and the Jena Experiment (chapter 5), in Jena, Germany, <http://www.the-jena-experiment.de/> (Photo 1). The mission of these experiments is to explore the mechanisms which underlie the relationship between biodiversity and ecosystem functioning. This thesis builds on previous research at the Jena Experiments, by exploring the links and factors underlying the relationships between plant diversity and belowground productivity and decomposition.

The Jena Experiment was established in 2002 to test how plant diversity (1, 2, 4, 8, 16, and 60 species) and functional group richness (mixtures of 1-4 functional groups: grasses, legumes, small herbs and tall herbs) influence ecosystem functioning (Roscher *et al.* 2004). In the Jena Experiment, plant diversity has been shown to increase plant productivity above- (Marquard *et al.* 2009) and belowground (Ravenek *et al.* 2014). Aboveground, this relation has been shown to persist in the face of climate extremes: drought (Vogel, Scherer-Lorenzen & Weigelt 2012) and flooding (Wright *et al.* 2015). The positive net effect of plant diversity on aboveground productivity was shown to be due to an increase in complementarity effects (see above section, *Partitioning the effects of biodiversity*) with diversity (Marquard *et al.* 2009). Belowground, this research question is outstanding, and is addressed in this thesis. Extensive work on the mechanisms underlying the positive diversity effects has been carried out in this experiment. Resource partitioning is commonly hypothesized to underlie complementarity effects, facilitating the diversity-productivity relationship. Resource partitioning indicates that species in mixtures occupy distinct resource niches (Tilman 1982) which should decrease with increasing plant diversity, leading to greater community resource uptake and productivity. Resource uptake has been shown to increase with plant diversity at the Jena Experiment (Roscher *et al.* 2008; Oelmann *et al.* 2011b; a) This was due in part to the presence of legumes, which increased biological nitrogen fixation with plant diversity, facilitating increased nitrogen uptake by neighbouring grasses and herbs (Gubsch *et al.* 2011b). In line with these findings, Gockele *et al.* (*in review*) traced stable isotope analogues for water, potassium, and nitrogen, and found that resource uptake increased

with greater plant diversity. This approach enabled the quantification of species resource niches, and found that species' resource niches did not decrease with plant diversity, indicating that resource partitioning is not the main factor underlying the positive diversity effects on productivity found in Marquard *et al.* (2009) and Ravenek *et al.* (2014). Similarly, Bachmann *et al.* (2015) showed with isotopically labelled soil water that there was no difference in the spatial or temporal uptake (^{18}O of xylem water) of water over a plant diversity gradient. However, increased water uptake from deeper soil layers in diverse communities may depend on environmental conditions, and only increase when photosynthetic activity is high, i.e. during periods of high vapour pressure deficit (Guderle *et al.* 2017).

Plant traits could help explain positive biodiversity effects, as above- and belowground plant traits have been shown to predict resource uptake strategies (Roumet *et al.* 2016), and interactions between plants and soil microbes (Cortois *et al.* 2016). Both factors could underlie biodiversity effects. Above- and belowground trait diversity and dominance (i.e. the trait mean) was shown to explain variation in aboveground net and complementarity effects (Roscher *et al.* 2012), and community biomass production (Roscher *et al.* 2013) in the Jena Experiment. The diversity in belowground traits, e.g. vertical root distribution, may contribute to the explanation of biodiversity effects. At the community level, Ravenek *et al.* (2014) showed that over a 9-year period, roots tended to congregate in the top soil layers, instead of distributing across the soil profile, and community vertical root distribution did not explain increases in aboveground plant productivity. Consideration of vertical root distribution at the species level is needed to determine if diversity in vertical root distributions can explain diversity effects; this is addressed in chapters 2 and 3 of this thesis.

The Jena Trait Based Experiment (Ebeling *et al.*, 2014) commenced in 2011 to explicitly test the effects of temporal and spatial trait diversity on ecosystem functioning by manipulating the functional trait diversity of mixtures as independently as possible from plant species richness. As trait based approaches have been shown to better explain ecosystem functioning than plant species richness per se (Cadotte *et al.* 2009; Reiss *et al.* 2009; Laliberté 2017), manipulating community functional trait diversity allows for a greater mechanistic understanding of how species loss could implicate ecosystem

functioning (Hillebrand & Matthiessen 2009). Thus, the Jena Trait Based Experiment addresses a fundamental question in ecology- does plant functional trait diversity underlie plant diversity-ecosystem functioning relations? Three pools of eight species were chosen from the 60-species pool of the Jena Experiment (Roscher *et al.* 2004) based on six functional traits related to resource acquisition in space (plant height, leaf area, rooting depth, root length density) or time (growth starting date, flower starting date). As the role of legumes in grasslands is already well studied, including in the Jena Experiment, legumes were excluded in the Jena Trait Based Experiment. These six traits were analysed with principal component analysis, which separated the species into two axes according to their resource use along spatial (axis one) and temporal gradients (axis two). Eight species along the first axis, which represented a gradient in traits related to spatial resource acquisition were selected for pool 1. Eight species along the second axis, which represented a gradient in traits related to temporal resource acquisition were selected for pool 2. In each of these pools, four grass and four non-leguminous forb species were selected. Eight species from the extremes of both axes were chosen for pool 3, which included seven non-leguminous forbs, and one grass. In this thesis, only pools 1 and 2 are considered in chapters 2-4, as they are more ecologically relevant to the research questions addressed. Further, comparison of pool 1 and pool 2 allows for differences between spatial and temporal functional trait diversity to be considered. In each pool is composed of 46 plant communities (3.5m * 3.5m), along a gradient of plant species richness (1, 2, 3, 4, or 8 species) and a gradient of functional trait diversity, in space (pool 1) or time (pool 2), from redundant ($FD_{Jena} 1$) to diverse ($FD_{Jena} 4$). Molecular methods (Mommer *et al.*, 2008), with primers developed for pools 1 and 2 of this experiment, allow for the determination of species specific root biomass, facilitating the research questions addressed in chapters 2-4 of this thesis.

The increase in root biomass with plant diversity has implications for carbon cycling, through rhizodeposition and the rate of litter decomposition. At the Jena Experiment, carbon storage was found to increase, while carbon losses were found to decrease with increasing plant diversity, due to higher root biomass (Steinbeiss *et al.* 2008a) and increased rhizodeposition and microbial activity (Lange *et al.* 2015). Early studies at the Jena Experiment found no effect of plant species or functional group diversity on

(chapter 4), we consider how root litter mixing effects affect root decomposition over a diversity gradient, and compare this to effects of plant diversity via changes in litter quality or the soil environment.



Photo 1. The Jena Trait Based Experiment (TBE) in summer 2014 (top left), Sigrid Dassen and Victor Malakhov taking soil cores at the TBE (top right), the data of which appears in chapters 2-4. The Jena Experiment, the study site of chapter 5 (below left), Jan van Walsem and Frans Möller constructing the very useful root sampling carts that they kindly designed and built (below right).

Chapter 1

Research questions

In this thesis plant diversity effects on belowground productivity and decomposition are considered; two interconnected processes that are measures of ecosystem functioning ubiquitous to all ecosystems (Hooper *et al.* 2012). Together, they contribute to the stock of root standing biomass, and to global carbon and nutrient cycling. Figure 1 presents a conceptual diagram of the thesis. The following research questions will be addressed:

1. Do complementarity or selection effects drive the positive diversity-productivity relationship belowground (chapter 2)? Does the diversity in inherent vertical root distribution facilitate complementarity effects (chapter 2)?
2. How do grassland species alter their vertical root distribution when grown in mixtures, and does this relate to increases in relative belowground yield (chapter 3)?
3. How does plant diversity and functional group composition influence root decomposition via changes in the soil environment (chapters 4 and 5), changes in litter quality (chapters 4 and 5), and litter mixing effects (chapter 4)?
4. Can the relations between plant diversity or functional group composition and decomposition be explained by root traits (chapters 4 and 5), soil biota or soil abiotic conditions (chapter 5)?

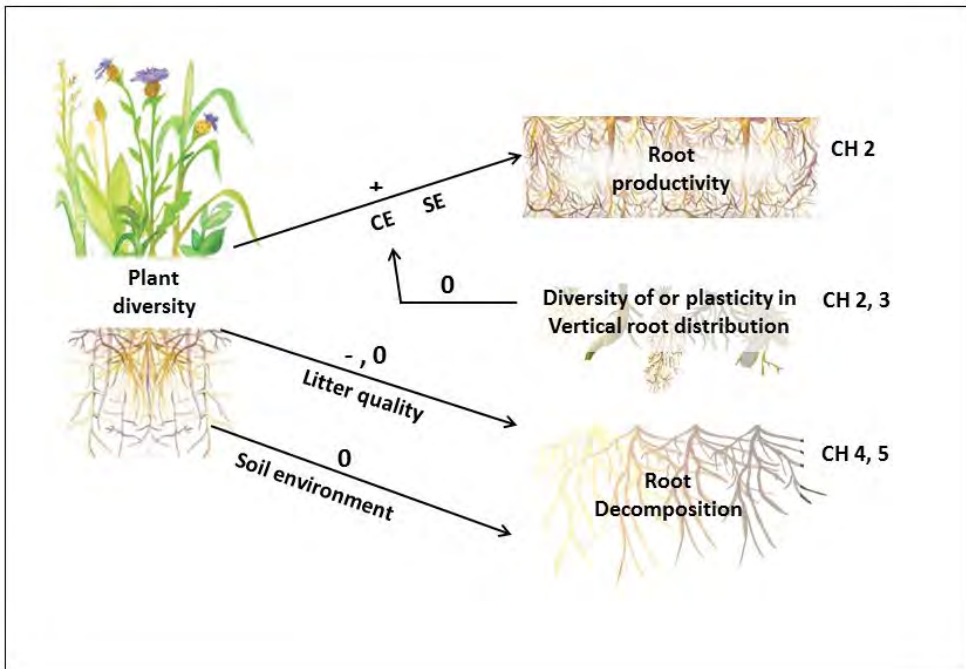


Fig. 1.1. Conceptual framework of this thesis. Plant diversity influences root productivity positively. This positive net effect of diversity can be partitioned into complementarity effects (CE) and selection effects (SE). Positive complementarity effects indicate that on average, species produce more biomass in mixture than expected, based on their biomass production in monoculture. This suggests beneficial interactions in mixtures facilitate higher than expected biomass production. Positive selection effects indicate that species that are productive in monoculture dominate the mixture, which contributes to greater than expected biomass in mixture. The arrow from vertical root distribution to CE + SE indicates that the diversity or plasticity in vertical root distribution could facilitate CE. Plant diversity can influence root decomposition via changes in litter quality or the soil abiotic and/or biotic environment. The effect of plant diversity on root decomposition is due to changes in litter quality, but is strongly driven by changes in functional group composition. There were no effects of plant diversity on decomposition via changes in the soil environment.

Box 1.1. Glossary of terms

Additive partitioning method Analogous to the Price Equation in genetics, this equation separates the net biomass gained in diverse plots, compared to the respective monocultures into complementarity effects (positive interactions between species) and selection effects (the dominance of productive species in mixture), also see Box 1.2 for equation (Loreau & Hector 2001).

Character displacement 'increased differences of size sympatry between closely-related or similar species' (Dayan & Simberloff 2005), i.e. when species that co-exist diverge in traits, but these divergences are not observed when the species are not co-existing.

Community weighted mean the trait values of species in a community, weighted by the relative abundance of the species in the community, analogous to a weighted arithmetic mean.

Competitive ability/strength the ability to acquire and use limiting resources (Westoby *et al.* 2002); the competitive ability of species *a* compared to species *b* can be measured as the growth reduction of species *b*, compared to the growth of species *b* alone (Schmid *et al.* 2015).

Complementarity effect(s) A positive complementarity effect occurs if, on average, species in mixture yield more than expected, based on the weighted average yield of the component species in monoculture (Loreau & Hector 2001)

Complementary interaction(s) an interaction which results in a benefit for both parties.

Diversity-productivity relation the relation between the number of plant species in a community and the community's biomass production

Ecosystem functioning the efficiency by which an ecosystem captures resources, produces biomass, decomposes and recycles biological material (Cardinale *et al.* 2012).

Expected yield the yield expected in a mixture, based on a species yield in monoculture weighted by their relative abundance in mixture.

Functional groups a grouping of plant species based on their phylogeny, in this thesis: grasses (C₃), non-leguminous forbs/herbs and legumes.

Functional trait diversity the value and range of species traits that influence ecosystem functioning (Petchey & Gaston, 2006; Tilman, 2001)

Growth strategy (strategies) ranging from conservative, slow-growing, ruderal, to exploitative, fast-growing, competitive (Grime 1977).

Inherent A trait value that results from genetic controls, with limited influence of a specific biotic or abiotic stimulus (i.e. the trait value in the absence of plasticity). In the context of this thesis, a species' inherent vertical root distributions is its vertical root distributions in monoculture.

Intra-specific competition competitive interactions between plants of the same species

Inter-specific competition competitive interactions between plants of different species

Box 1.1. Glossary of terms (continued)

Litter mixing effect(s) the effect on decomposition of combining litters from different species

Native root litter root litter originating from the same plant community as where it is decomposing, i.e. plot specific litter

Net effect the amount of biomass gained in a mixture of plant species, relative to biomass expected, based on the biomass of the composite species in monoculture, also see Box 1.2 (Loreau & Hector 2001).

Niche complementary hypothesis species differ in their spatial and temporal resource acquisition. Therefore, greater plant diversity leads to greater productivity due to more complete utilization of limiting resources at higher diversity (Tilman 1999).

Niche differentiation when species use the environment differently, in a way that facilitates coexistence (e.g. by acquiring resources from different areas of the soil)

Nutrient use efficiency the amount of aboveground biomass per unit of aboveground nitrogen (van Ruijven & Berendse 2005)

Observed yield the measured yield of a plant community, usually referring to a mixture

Plant-soil feedback(s) interactions between plants and soil organisms which 'feedback' and influence plant performance, plant population and community dynamics (Wardle *et al.* 2004; Bever *et al.* 2010)

Plastic/plasticity the ability of a plant to alter their traits in response to biotic or abiotic stimuli in their environment (Hodge 2004).

Resource complementarity/resource partitioning occurs when plants in more diverse communities increase total nutrient capture due to differences in resource uptake in space, time, or type (Ewel 1986).

Root branching the density of lateral roots growing from a main root

Sampling effect(s) higher biomass in a diverse mixture due to the increased probability of including a highly productive species (Tilman, Lehman & Thomson 1997)

Selection effect(s) the covariance between species monoculture biomass, and their relative yield in mixture (Loreau & Hector 2001)

Specific root length root length per mass, e.g. m g^{-1}

Standard litter a foreign litter added to each plot in order to determine how the soil environment influences decomposition, e.g. cotton, paper, the root or leaf litter of a plant not included in the experiment.

Trait(s) Any physiological, morphological, or chemical characteristic of a plant

Box 1.2. Additive partitioning

Additive partitioning is essentially the Price Equation from genetics (Price, 1970), which was applied in an ecological context by (Loreau & Hector, 2001). Additive partitioning mathematically separates the net effect of biodiversity (ΔY) into complementarity and selection effects using the following equation:

$$\Delta Y = N \cdot \overline{RYM} + N \cdot cov(\Delta RY, M)$$

in which:

$$\Delta Y = Y_0 - Y_E = \sum_i RY_{0,i}M_i - \sum_i RY_{E,i}M_i = \sum_i \Delta RY_i M_i$$

M_i = the yield of species i in monoculture

$Y_{0,i}$ = observed yield of species i in the mixture

$Y_0 = \sum Y_{0,i}$ = total observed yield of the mixture

$RY_{E,i}$ = expected yield of species i in the mixture (relative proportion planted or sown)

$RY_{0,i} = \frac{Y_{0,i}}{M_i}$ = observed relative yield of species i in mixture

$Y_{E,i} = RY_{E,i}M_i$ = expected yield of species i in mixture

$Y_E = \sum Y_{E,i}$ = total expected yield of mixture

$\Delta Y = Y_0 - Y_E$ = deviation from total expected yield in the mixture

$\Delta RY = R_{0,i} - RY_{E,i}$ = deviation from expected yield of species i in the mixture

N = number of species in the mixture

This net effect can be partitioned into a complementarity ($N \cdot \overline{\Delta RY \cdot M}$) and selection effect ($N \cdot cov(\Delta RY, M)$):

$$\Delta Y = \sum_i \Delta RY_i M_i = N \cdot \overline{\Delta RY \cdot M} + N \cdot cov(\Delta RY, M)$$


in which:

$\overline{\Delta RY}$ is the mean deviation from expected yield of all species in mixture

cov indicates covariance

Hence, the net effect of biodiversity (NE) = complementarity effect (CE) + selection effect (SE). NE is the difference between the observed yield in mixture and the expected yield based on the yield of the composite species in monoculture. Positive complementarity effects indicate that species produce more biomass in mixture than expected, based on their biomass production in monoculture. Thus, beneficial interactions in mixtures facilitate higher than expected biomass production. Positive selection effects indicate that species which are productive in monoculture dominate the mixture, hence are responsible for the greater than expected biomass in mixture.





Chapter 2 Molecular identification of species-specific root biomass reveals belowground complementarity effects in a biodiversity experiment

Natalie J. Oram*, Janneke M. Ravenek*, Alexandra Weigelt, Hongmei Chen, Arthur Gessler, Annette Gockele, Hans de Kroon, Jan Willem van der Paauw, Michael Scherer-Lorenzen, Annemiek Smit-Tiekstra, Jasper van Ruijven, Liesje Mommer

* These authors contributed equally

Abstract

It is well established that the positive relationship between plant diversity and aboveground plant productivity is driven by complementarity effects, and not the presence of few productive species. The mechanisms underlying these complementarity effects are assumed to mainly operate belowground. However, experimental evidence for belowground complementarity effects is lacking, because species-specific root biomass could not be easily determined. Here, we provide the first experimental test of belowground complementarity effects in a large biodiversity experiment. Across the gradient of plant species richness in the Jena Trait-Based Experiment, we sampled fine-root standing biomass over depth in 2012 and 2014. A molecular technique (RT-qPCR) was used to quantify species-specific root biomass. The additive partitioning method was used to calculate belowground complementarity and selection effects. In addition, we tested for underlying mechanisms by linking belowground complementarity effects in species mixtures to the functional diversity in species-specific vertical root distributions, as measured in monocultures. Plant species richness was positively related to community root biomass in both years, which was associated with an increase in complementarity effects and a decrease in selection effects. Community root biomass decreased with soil depth, but this pattern was not affected by species richness. The diversity of the vertical root distributions measured in monoculture was not related to belowground complementarity effects in plant mixtures, suggesting that belowground resource complementarity is linked to other functional traits. Alternatively, mechanisms other than resource use complementarity are more important for the positive effects of plant species richness on plant (root) biomass. This study demonstrates for the first time that belowground complementarity effects are an important factor underlying the belowground diversity-productivity relationship

Introduction

Plant diversity is positively related to plant productivity above- and belowground (Reich et al. 2004; van Ruijven & Berendse 2005; Marquard et al. 2009; Mueller et al. 2013; Cong et al. 2014; Ravenek et al. 2014). This positive net effect of plant diversity on productivity can be partitioned into complementarity and selection effects (Loreau & Hector 2001). Complementarity effects refer to the contribution of positive species interactions, e.g. resource partitioning or facilitation, to the net effect of diversity. Selection effects describe the contribution of highly productive species that dominate the mixture (Loreau et al. 2012). Aboveground, the contribution of complementarity effects often is larger than that of selection effects (Cardinale et al. 2007), and complementarity effects have been shown to strengthen with time (Marquard et al. 2009; Van Ruijven and Berendse 2009; Reich et al. 2012).

However, in contrast to aboveground biomass, the increase in root biomass with plant diversity has not yet been partitioned into complementarity and selection effects. This is because roots of grassland plant species in mixtures are impossible to distinguish by eye, which has prevented a quantitative assessment of species-specific root biomass in biodiversity experiments and consequently the calculation of complementarity and selection effects. Recent development of molecular techniques which can determine the relative abundance of species specific root biomass have overcome this limitation (Mommer et al. 2008, 2010, 2011; Hendriks et al. 2015), allowing the assessment of belowground biodiversity effects. Belowground complementarity effects could signal resource partitioning, which is often proposed to underlie the positive diversity-productivity relationship (e.g. van Ruijven and Berendse, 2005). Resource partitioning is based on the assumption that plant species differ in their resource uptake patterns in space and/or time, occupying distinct resource niches (Tokeshi 1999). Therefore, co-occurring plant species are expected to cover the total available niche space more completely, leading to a higher performance of mixtures relative to monocultures (Dimitrakopoulos & Schmid 2004).

It is important to note that complementarity effects are not a direct indication of resource partitioning, as positive complementarity effects could also be caused by facilitation or other positive interactions between plant species (Wright et al. 2017). To test whether

resource partitioning is a factor underlying complementarity effects, it is important to establish links between plant traits related to resource uptake and complementarity effects. A classical belowground example of resource partitioning is vertical differentiation of root distribution among species (Parrish and Bazzaz 1976, Berendse 1982, Levine and HilleRisLambers 2009). Differences in vertical root distribution between species can lead to spatial niche differentiation (Levine and HilleRisLambers 2009; Skinner and Comas 2010; Belter and Cahill 2015), which may allow for a more complete use of resources and consequently, increased productivity (Dimitrakopoulos and Schmid 2004). Mixtures with a greater diversity in vertical root distributions could therefore lead to positive complementarity effects due to a greater volume of soil explored, and higher resource uptake in mixtures, compared to monocultures or mixtures with low diversity in vertical root distributions. Thus, diversity in vertical root distribution may explain complementarity effects better than the number of plant species per se.

Although community root biomass has indeed been shown to increase with plant diversity (Mommer et al. 2010; Mueller et al. 2013; Cong et al. 2014; Ravenek et al. 2014), evidence for differentiation in vertical root distribution in mixtures varies. Vertical root distribution has been shown to be unaffected by plant diversity; plant species roots concentrate in upper soil layers, instead of differentiating over the soil profile (Mommer et al. 2010; Cong et al. 2014; Ravenek et al. 2014). In contrast, in a long-term biodiversity experiment the positive effect of plant diversity on community root biomass was greater in deeper soil layers (Mueller et al. 2013). This could signal that differentiation in vertical root distribution influences the diversity-productivity relationship. Here, we investigate whether the diversity in vertical root distribution can explain variation in belowground biodiversity effects.

We determined the relationship between plant diversity and root biomass in the first and third year of the Jena Trait Based Experiment (Ebeling et al., 2014). We used a molecular technique to determine species-specific root biomass on the root biomass derived from soil coring in each plot (Mommer et al., 2008). With this information we determined belowground biodiversity effects (according to Loreau and Hector, 2001).

We tested if these biodiversity effects could be explained by the diversity in vertical root distributions of the component species. Specifically, we hypothesized:

1. Belowground biomass will increase with plant diversity, i.e. a positive net effect of biodiversity belowground.
2. The positive net effect of biodiversity belowground will be due to positive complementarity effects.
3. Belowground complementarity effects will increase with increasing diversity of vertical root distributions.

Materials and Methods

Experimental design

This study was conducted within the framework of the Jena Trait Based Experiment (TBE). The complete experimental design is described in (Ebeling et al. 2014b). Briefly, the TBE was sown in the spring of 2011 along the river Saale (130 m above sea level) in three spatial blocks to account for variation in soil parameters, parallel to the Jena Experiment (Roscher et al. 2004) (Germany; 50.95 °N 11.62 °E). The soil is a sandy loam (40% sand, 44% silt, 16% clay). Experimental plots (12.25 m²) were mown twice a year (June, September) and weeded three times a year (April, July, October) to maintain target plant community composition.

The TBE consists of three pools of eight species (see Ebeling et al. 2014 for complete details), selected from the original species pool of the Jena Experiment (Roscher et al. 2004). In order to create gradients of spatial and temporal functional trait diversity (FDJena), plant communities were composed of species based on six traits involved in resource acquisition (plant height, leaf area, growth and flowering starting date, rooting depth, and root length density), varying from redundant (FDJena 1) to diverse (FDJena 4). In this study we focus on pool 1 and 2. Species in pool 1 represent a trait axis of spatial resource acquisition: *Avenula pubescens* (Dumort), *Centaurea jacea* L., *Festuca rubra* L., *Knautia arvensis* L., *Leucanthemum vulgare* (Lam), *Phleum pretense* L., *Plantago lanceolata* L. and *Poa pratensis* L. Species chosen for pool 2 represent a temporal trait axis: *Anthoxanthum odoratum* L., *Dactylis glomerata* L., *Geranium pretense* L., *Holcus lanatus* L., *Leucanthemum vulgare* (Lam), *Phleum pretense* L., *Plantago lanceolata* L., and *Ranunculus acris* L. Because Pool 3 is composed of mixtures from the extremes of both trait gradients, i.e. both spatial and temporal trait diversity, we chose to focus on Pools 1 and 2 in order

to disentangle effects of spatial and temporal trait diversity. In total, Pool 1 and 2 include 92 plots (Table S1): 16 monocultures, 32 two-species plots, 24 three-species plots, 18 four-species plots, and 2 eight-species plots.

Root sampling

Community root standing biomass (RSB) of all plots was sampled up to 40 cm depth over the course of 3 weeks in August in 2012 and 2014. Per plot eight root cores (4 cm diameter, 40 cm deep) were taken (Fig. S1) and divided into five depths: 0-5, 5-10, 10-20, 20-30 and 30-40 cm, pooled by depth and stored at 4°C until washing over a 0.5 mm sieve. Washing always took place within 32 hours after sampling, to preserve DNA quality. Roots were separated into coarse (> 2 mm diameter) and fine (< 2 mm diameter), only fine roots are considered in all further analysis. From fine roots, sub-samples of 50 mg were taken for molecular analysis, which were stored at -80 °C. The rest of the fine root biomass was dried at 65 °C for at least 48h and weighed.

Molecular analysis of species proportions in root samples

The relative proportions of species abundance in mixed root samples were estimated using real-time (RT) PCR (Mommer et al. 2008; Hendriks et al. 2015). Root DNA was extracted using DNeasy 96 Plant Mini Kit following the protocol (Qiagen, Venlo, The Netherlands). DNA concentrations were measured using a Qubit Fluorimeter (Thermo Fisher Scientific, Waltham, Massachusetts, USA). In each sample, each species was separately amplified by RT-PCR with species-specific primer pairs (in triplicate). Primer pairs for *A. odoratum*, *F. rubra*, and *L. vulgare* were used as described in Mommer et al. (2008). Primer pairs for *C. jacea*, *D. glomerata*, *G. pratense*, *H. lanatus*, *K. arvensis*, *P. lanceolata*, *P. pratensis*, and *R. acris* were developed using the same protocol as Mommer et al. (2008; Table S2). RT-PCR reactions were performed with HOT FIREPol Eva Green (Solis BioDyne, Tartu, Estonia) qPCR Mix Plus with an addition of 0.94 µM MgCl₂, a primer concentration of 60 nM for *A. odoratum* and *C. jacea* and 120 nM for all other species, and 4 ng genomic DNA for *P. lanceolata* or 1 ng genomic DNA for the other species, in a reaction volume of 20 µl. The qPCR program was as follows: 15 min at 95 °C; then 41 cycles of 20 s at 95 °C, 30 s at 62 °C and 15 s at 72 °C; and finally a melting

curve analysis of 5 sec per cycle, starting at 70 °C and ending at 91 °C with an increment of 0.5°C per cycle. RT-PCR analyses were performed on a CFX96 Touch Real-Time PCR Detection System (Bio-rad Laboratories, Hercules, CA, USA).

To validate the RT-PCR estimates of species-specific root proportions, 16 reference samples were made by manually pooling monoculture roots of different species. Ten so-called 'standard samples' contained equal proportions of all plant species, and the other 6 reference samples contained species-specific proportions between 0-50%. These samples were used to determine the relationship between actual and measured relative abundance for each species. This analysis revealed that monoculture material for two species (*H. lanatus* and *P. pratensis*) contained traces of another species (*P. lanceolata*), probably because the latter established as a weed in these two monocultures. The actual abundance of these three species in the mixed reference samples were consequently corrected for this contamination. Then, the relationship between actual and measured relative abundance was determined again using regression analysis (see Fig. S2). The five standard samples with smallest summed discrepancy between measured and actual presence were used as reference standards on the 96 well RT-PCR plates. A plate thus included 25 samples, 5 standards, 1 positive and 1 negative control, all run in triplicate. We calculated the species specific fine root biomass per layer, per plot by multiplying the total fine root biomass per layer per plot (g m^{-2}) with the relative abundance of each species derived from RT-PCR; as in Mommer et al. (2010).

Calculations

For community root biomass per plot, total root standing biomass (RSB, in g m^{-2}) over the 0-40 cm soil profile was used to allow comparison with other studies. To compare community root biomass among the different soil layers (within plots), root mass density (RMD, mg cm^{-3}) was calculated for each layer.

For each mixed plant community, species-specific root biomass (g m^{-2}) over the 0-40 cm soil profile, determined with the molecular analysis, was used to calculate the net effects (NE), complementarity effects (CE) and selection effects (SE) (Loreau & Hector 2001). NE is the difference between the observed root biomass (g m^{-2}) in mixture and the expected root biomass based on the component species grown in monoculture. The NE is the result

of the sum of CE and SE. Positive SE occur when mixtures are dominated by species with higher than average root biomass in monoculture. Positive CE are observed when on average, species are more productive in the mixture, than is expected based on their productivity in monoculture. Three species occur in both species pools (*L. vulgare*, *P. lanceolata* and *P. pratense*). For these species, the monoculture in the same pool as the mixtures was used in the calculations.

To determine the diversity of vertical root distributions in each mixture, we first assessed the vertical root distribution of each species in monoculture. This distribution was calculated as the parameter β by fitting the following asymptotic equation to proportional root biomass over depth (Jackson et al. 1996):

$$Y = 1 - \beta d$$

Where Y is the cumulative fraction of roots from the surface to depth d , and β describes the decline of proportional root biomass over depth. High values of β (e.g. 0.90) correspond to a greater proportion of roots deeper in the soil, while lower values illustrate a greater proportion of roots near the soil surface (Gale & Grigal 1987; Jackson et al. 1996).

Next, for each mixture of species, the diversity in vertical root distributions of the component species was calculated using the functional diversity index Functional Dispersion (FDis) using the FD package (Laliberté and Legendre 2010) in R (R Core Team 2016). This index measures the mean distance in trait space of individual species to the centroid of all species and is independent of species richness (Laliberté & Legendre 2010). Species-specific trait values (in this case the vertical root distribution of each species, β) were weighted by the relative abundance of species-specific root biomass in mixtures (determined with molecular analysis, above). Higher FDis values indicate that the community is more diverse in terms of the focal trait (β). Here, the trait value used to derive an independent estimate of the diversity in vertical root distribution in mixtures were taken from measurements in monocultures, rather than using trait values from trait databases. This was done to minimize the chance that differences in abiotic conditions affected the trait values. Traits were first scaled to a mean of zero and a standard deviation of ± 1 using the function `scale{base}`.

Statistical analysis

We used the function `lme` (Pinheiro et al. 2016), combined with the function `anova{stats}` (R Development Core Team 2016), to construct models and analyze them with ANOVA with type I (sequential) sums of squares. To account for non-independence between measurements of the same plot in 2012 and 2014 the `lme` model contained the random term `~1|plot`. All data were analyzed using a model that tested the effect of block (as factor: 3 levels), year (as factor: 2 levels, 2012, 2014), species richness (log transformed, continuous: 1, 2, 3, 4, 8), `FDJena` (continuous: 1, 2, 3, 4) and pool (as factor: 2 levels, 1, 2) as explanatory variables.

The model for root mass density (RMD) per layer contained the random term `~1|plot/layer`. In order to incorporate autocorrelation between RMD in sequential depth layers in the same plot (e.g. layer 1 and 2 are more correlated than layer 1 and 5), the correlation structure `corAR1 {nlme}` was used. In this analysis, depth (five levels) was added as a factor to the model described above.

Analysis of NE, CE and SE included the mixtures (SR = 2 - 8). Two outliers were removed, the first due to a possible field measurement error, and the second based on distribution graphs, as it was more than 1.5 times the next highest point. If the number of plant species does not influence belowground productivity (quantified as RSB), the value of NE, CE, and SE will be zero- the observed RSB in mixture is not different than expected based on the RSB of the component species in monoculture. Therefore, we tested if NE, SE, and CE, were significantly different than zero using t-tests for all mixtures combined when 'species richness' was significant, or each diversity level separately, when not.

In the analysis of the vertical distribution of root biomass (β) in monocultures, the effect of species identity could not be determined as there is only one replicate per species. Instead, the effect of functional group (grasses or forbs) was tested in addition to year and pool.

To test the hypothesis that communities with a higher diversity in vertical root distribution (higher `FDis`) would show greater complementarity effects, the additional explanatory variable `FDis` (log transformed, continuous) was included after 'year' in a model identical to the community root biomass model. In this analysis, complementarity effects were standardized by dividing by the average monoculture biomass of all

component species present in the mixture. By using this relative complementarity effects (rCE), potential confounding effects of differences in mean monoculture biomass between mixtures are eliminated (Craven et al., 2016). All statistical analyses were performed in R (R Development Core Team 2016).

Results

Root biomass

Total community root standing biomass (RSB) significantly increased with species richness (Fig. 2.1). This belowground biodiversity effect was independent of year and pool (Table 2.1). RSB significantly increased from 2012 to 2014. In 2012, there was no difference in RSB between Pool 1 and Pool 2, but in 2014, RSB was higher in Pool 1 than in Pool 2 (Fig. 2.1; Table 2.1).

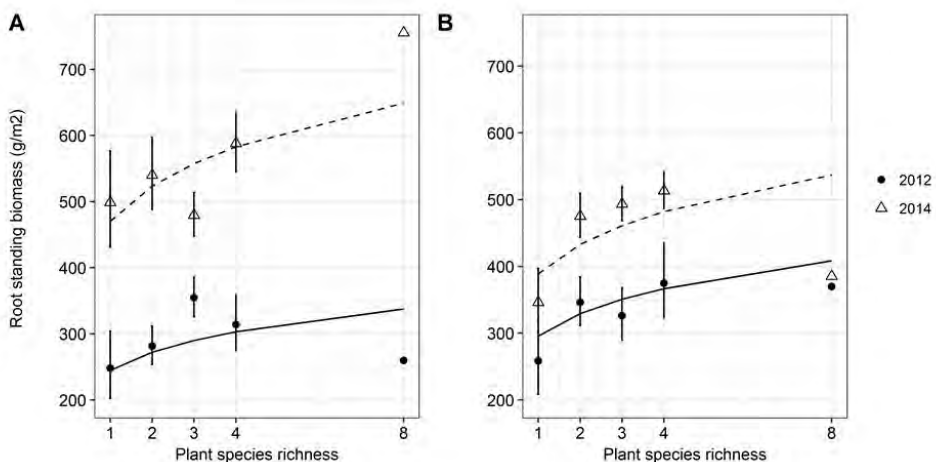


Fig. 2.1. Root standing biomass increased with species richness in both years in pool 1 (A) and pool 2 (B). Root biomass was greater in pool 1 than in pool 2 in 2014. Points represent means, error bars indicate SE.

Biodiversity effects

The positive biodiversity effect on RSB was reflected in a significantly positive net effect (NE) in mixtures in both years (one-sided t-test, $t_{73} = 3.36$, $P < 0.0001$ in 2012 and $t_{74} = 4.95$, $P < 0.001$ in 2014). However, NE did not increase with species richness (Fig. 2.2A). NE was slightly higher in Pool 2 than in Pool 1 (Table 2.1).

Application of the additive partitioning method revealed that the positive biodiversity effect was associated with a positive complementarity effect (CE) and a negative selection effect (SE). CE significantly increased with species richness and selection effects (SE) significantly decreased (Fig. 2.2B, C). This increase with species richness was independent of pool or year. From 2012 to 2014, CE increased and SE decreased. CE and SE did not differ between pools (1).

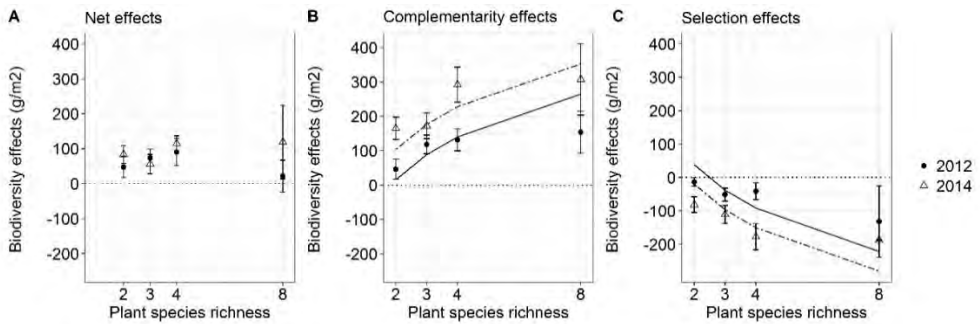


Fig. 2.2. A) Net effects (NE) were significantly greater than zero, but did not increase with species richness and did not differ between years. B) Complementarity effects (CE) increased with species richness, and were greater in 2014 than in 2012. C) Selection effects (SE) decreased with species richness and were more negative in 2014 than in 2012. Relationships with species richness for CE and SE were independent of year and pool. Data show means \pm SE for both pools combined.

Table 2.1. ANOVA (type I) summary of analysis of community root standing biomass (RSB), net effects of biodiversity (NE), complementarity effects (CE), and selection effects (SE). The effect of block (three levels: 1, 2, 3), year (two levels: 2012, 2014), plant species richness (continuous log transformed: 1, 2, 3, 4, 8, logSR), functional trait diversity (continuous: FD_{Jena}, 1, 2, 3, 4), pool (two levels: 1, 2). Additive partitioning was used to calculate NE, CE, and SE (see methods). RSB was log transformed to meet assumptions of normality.

	RSB (g m ⁻²)	NE (g m ⁻²)	CE (g m ⁻²)	SE (g m ⁻²)
block	F _{2,83} =5.74**	F _{2,66} = 8.16***	F _{2,66} =6.55***	F _{2,66} =0.22
year	F _{1,86} =78.94***	F _{1,69} =1.43	F _{1,69} =15.42**	F _{1,69} =18.02***
logSR	F _{1,83} =8.82**	F _{1,66} =1.71	F _{1,66} =9.80**	F _{1,66} =9.35**
FD _{Jena}	F _{1,83} =0.19	F _{1,66} =2.04	F _{1,66} =0.02	F _{1,66} =3.19
pool	F _{1,83} =0.08	F _{1,66} =8.22**	F _{1,66} =2.70	F _{1,66} =0.23
pool: FD _{Jena}	F _{1,83} =0.32	F _{1,66} =0.04	F _{1,66} =0.15	F _{1,66} =0.00
logSR:pool	F _{1,83} =2.59	F _{1,66} =0.12	F _{1,66} =0.00	F _{1,66} =0.001
logSR:FD _{Jena}	F _{1,83} =0.33	F _{1,66} =0.34	F _{1,66} =0.25	F _{1,66} =1.64
year:logSR	F _{1,86} =0.15	F _{1,69} =0.06	F _{1,69} =0.15	F _{1,69} =0.24
year: FD _{Jena}	F _{1,86} =0.01	F _{1,69} =0.31	F _{1,69} =0.18	F _{1,69} =0.60
year:pool	F _{1,86} =5.63*	F _{1,69} =2.65	F _{1,69} =3.61	F _{1,69} =1.79
year:logSR: FD _{Jena}	F _{1,86} =0.09	F _{1,69} =0.09	F _{1,69} =0.18	F _{1,69} =0.02

P < 0.001 ***, P < 0.01 **, P < 0.05 *

Community root biomass over depth

Root biomass (measured as root mass density, RMD) decreased significantly over depth (Fig. 2.3). Like total root biomass, the average root biomass per layer increased with species richness, but the distribution of root biomass over depth was not affected by plant species richness (Fig. 2.3; Table 2.2). The distribution of roots over depth, however, differed between 2012 and 2014. In 2014, root biomass in the upper soil layer (0-5 cm) decreased, but increased in the soil layer directly below (5-10 cm; see Fig. 2.3). Root biomass in the layers below 10 cm did not differ between 2012 and 2014. The distribution

of RMD over depth differed between pools (Table 2.2): root biomass at 10-20 and 20-30 cm was greater in pool 1 than in pool 2.

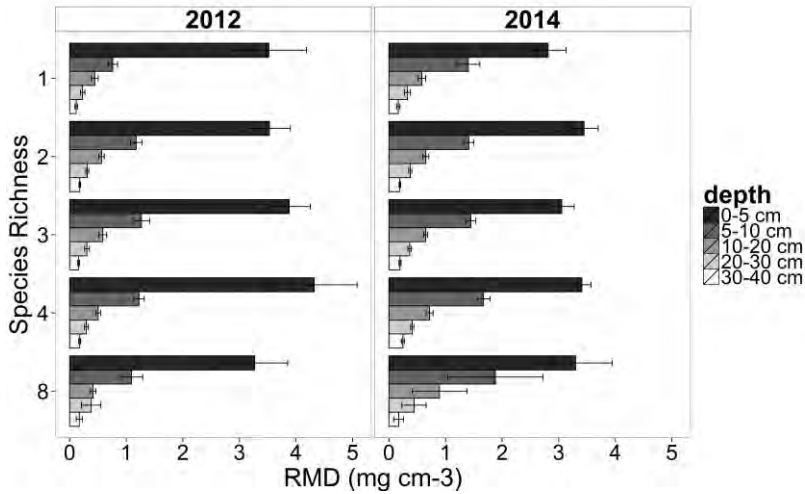


Fig. 2.3. The distribution of root mass density (RMD; mg cm^{-3}) over depth for plant communities along the species richness gradient in 2012 (left) and 2014 (right). Root mass density strongly decreased with depth, but this decrease was not dependent upon species richness. Bars show mean \pm SE; data are presented for both pools combined.

Table 2.2. ANOVA (type I) summary of analysis of root mass density (RMD) over depth layers. The effect of block (three levels: 1, 2, 3), year (two levels: 2012, 2014), layer (five levels: 1, 2, 3, 4, 5), plant species richness (continuous, log transformed: 1, 2, 3, 4, 8; logSR), functional trait diversity (continuous: FD_{Jena} , 1, 2, 3, 4), pool (two levels: 1, 2), and interactions were included in the order specified in the table. RMD was log transformed to meet assumptions of normality.

	RMD (mg cm ⁻³)
block	$F_{2,86} = 5.26^{**}$
year	$F_{1,450} = 44.26^{***}$
layer	$F_{4,356} = 1391.95^{***}$
logSR	$F_{1,86} = 14.06^{***}$
FD_{Jena}	$F_{1,86} = 0.00$
pool	$F_{1,86} = 2.38$
year:logSR	$F_{1,450} = 0.36$
year:layer	$F_{4,450} = 5.02^{***}$
layer:logSR	$F_{4,356} = 1.78$
layer:pool	$F_{4,356} = 3.53^{**}$
year:logSR:layer	$F_{4,450} = 0.43$
$P < 0.001^{***}$, $P < 0.01^{**}$	

Linking complementarity effects to vertical root distributions

In monocultures, individual species showed considerable variation in proportional vertical root distribution (Fig. 2.4). Root distributions ranged from very shallow, with more than 80% of root biomass in the first 5 cm (e.g. *P. pratensis* 2012; $\beta = 0.66$) to deep rooting with less than 40% of root biomass in the upper layer (e.g. *K. arvensis* 2012; $\beta = 0.91$). See Table S3 for a complete list of fitted β and r^2 values. Vertical root distributions (β) were not affected by functional group (grass vs. forb; $F_{1,10} = 0.19$; $P > 0.05$), pool ($F_{1,10} = 0.01$; $P > 0.05$) and year ($F_{1,13} = 2.96$; $P > 0.05$). Due to the differences in β among species in monocultures, mixtures differed considerably in their diversity of root distributions (calculated as FD_{is}) with up to 10-fold differences between plots (Fig. 2.5). However, higher diversity in vertical root distributions did not lead to greater belowground relative complementarity effects (rCE) (Fig. 2.5). Like complementarity effects (CE), rCE

significantly increased with species richness. Mixtures in pool 2 had a greater rCE than in pool 1 (Table 2.3).

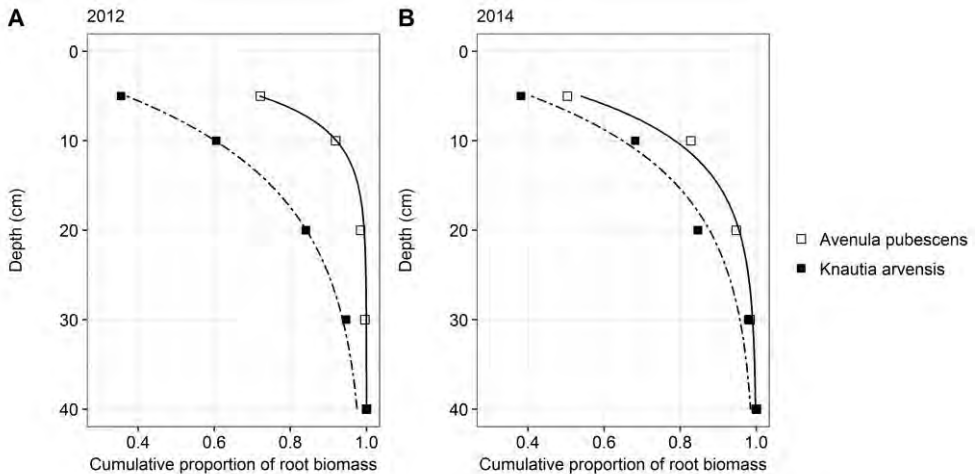


Fig. 2.4. The proportional distribution of cumulative root biomass over depth for a grass, *Avena pubescens*, and a forb, *Knautia arvensis* in 2012 and 2014 growing in monoculture. Species differed considerably in vertical root distribution, with the proportion of roots in the upper soil layer ranging from less than 40 to more than 80%. Points represent the proportion of roots of a species in that layer ($n = 1$). Curves represent the fitted vertical root distribution, β . See Table S3 for β and r^2 values for each species.

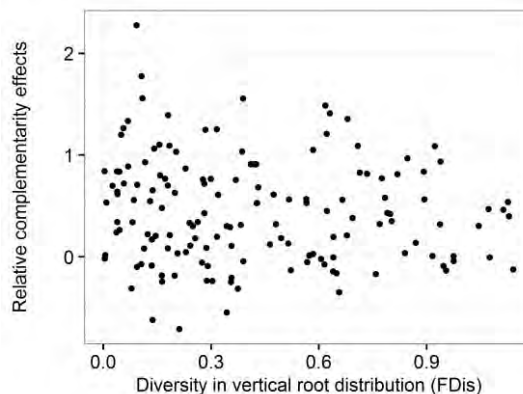


Fig. 2.5. Belowground relative complementarity effects differed strongly between communities, but was not related to the diversity of vertical root distributions of species (FDIs) in those communities. Points show individual plots.

Table 2.3. ANOVA (type I) summary of the analysis of relative complementarity effects (rCE). Effects of block (3 levels: 1, 2, 3), year (2 levels: 2012, 2014), FDis (continuous), logSR (log transformed continuous: 2, 3, 4, 8 species), FDJena (continuous: 1, 2, 3, 4), and pool (2 levels: 1, 2) were tested. FDis is a measure of functional dispersion of vertical root distribution, and was calculated using the root distribution of species when grown in monocultures, and their relative abundance in mixture (see methods). FDJena is a measure of functional trait diversity of six traits, calculated in (Ebeling et al. 2014b).

	rCE
block	$F_{2,70} = 3.68$ *
year	$F_{2,68} = 1.61$
Fdis	$F_{1,68} = 2.26$
logSR	$F_{1,70} = 7.08$ **
FDJena	$F_{1,70} = 0.11$
Pool	$F_{1,70} = 4.09$ *
Fdis: logSR	$F_{1,68} = 0.07$
Fdis: FDJena	$F_{1,68} = 0.42$
Fdis: Pool	$F_{1,68} = 0.21$
Fdis: year	$F_{1,68} = 0.57$

$P < 0.01$ **, $P < 0.05$ *

Discussion

Already one year after the establishment of the Jena Trait Based Experiment (TBE), a positive biodiversity effect on root biomass was observed. Application of the molecular approach enabled the quantification of the relative abundance of species in mixed root samples, which allowed the calculation of complementarity and selection effects. Our results demonstrate that positive belowground complementarity effects contributed significantly to the positive biodiversity effect on root biomass. Individual species showed considerable differences in vertical root distributions, but contrary to our hypothesis, belowground complementarity effects did not increase with increasing diversity of vertical root distributions in mixtures.

The diversity-productivity relationship belowground

As expected, community root standing biomass (RSB) increased over the plant species richness gradient. This belowground biodiversity effect has also been observed in other biodiversity experiments (Ravenek et al., 2014; Mueller et al., 2013). We found no strengthening of the biodiversity effect between the first and third year after the initiation of the experiment, potentially due to the restricted time frame. In the Jena Main Experiment (Roscher et al. 2004), an experiment that started ten years before the TBE experiment at the same site, the biodiversity effect on root biomass increased with time, but only after six years (Ravenek et al. 2014).

In line with our hypothesis, the positive effects of species richness on root biomass were associated with positive complementarity effects (CE) and negative selection effects (SE), a pattern that has also been frequently shown for aboveground biomass (e.g. van Ruijven and Berendse 2005; Roscher et al. 2005; Fargione et al. 2007; Marquard et al. 2009). A meta-analysis of aboveground biodiversity effects showed that CE and SE both contribute, but that CE is most important, particularly in the long term (Cardinale et al. 2007). For root biomass, positive CEs have been found in a mesocosm facility studying a grassland mixture of four species (Mommer et al. 2010) and in a maize/bean/squash polyculture (Zhang et al. 2014), but to our knowledge this is the first experimental evidence for positive belowground CE over a species richness gradient in an experimental grassland.

Diversity in root distributions and complementarity

We hypothesized that belowground complementarity effects would be associated with diversity in rooting distributions, as mixtures composed of species with large differences in their vertical root distributions would occupy more spatial niches and be better able to partition resources (Parrish and Bazzaz 1976, Berendse 1982, Levine and HilleRisLambers 2009). At the community level, the distribution of root biomass over depth did not change with species richness, in line with Ravenek et al. (2014) and Mommer et al. (2010). However, in the monoculture plots, we observed substantial variation among species in their vertical root distribution patterns. This variation in monoculture root distributions translated into a gradient of diversity in root

distributions in mixtures, measured as FDis. However, contrary to our hypothesis, we found no relationship between FDis and belowground complementarity effects. This suggests that either vertical root distribution does not accurately capture spatial resource complementarity, or that other mechanisms are more important.

It is important to note that we used the root distributions of species measured in monocultures to predict the distribution in mixtures. This allowed us to test if an independent measure of species specific root biomass could be used to predict biodiversity effects in mixtures. Using independent trait measures, weighted by the relative abundance of species in mixtures, have been used in trait based approaches, including aboveground at the Jena Experiment (Roscher et al., 2012). This approach does not account for changes in species' vertical root distributions in response to interspecific neighbours. Root distribution has been shown to change in response to nutrient availability (Hodge 2004; Kembel and Cahill 2005; Cahill and McNickle 2011) and neighbouring plants (Semchenko et al. 2014; Belter & Cahill 2015; Mommer, Kirkegaard & van Ruijven 2016). Such adjustments in rooting patterns in mixtures may result in resource complementarity, which would not be detected with our approach. Alternatively, traits other than vertical root distribution may be more important for spatial resource partitioning, and better explain complementarity effects.

Other mechanisms

We cannot rule out that mechanisms other than spatial resource complementarity are more important for the positive plant diversity-productivity relationship (and associated complementarity effects). Several tracer studies found little evidence for spatial difference in nutrient uptake among grassland plants (Mamolos, Elisseou & Veresoglou 1995; Pecháčková et al. 2003; von Felten et al. 2009). It has often been argued that temporal complementarity is important in ecosystems and thus that partitioning of different chemical forms of nutrients may contribute to complementarity (McKane et al. 2002; Ashton et al. 2010). However, experimental evidence for variation in resource acquisition in time, and/or chemical form in grassland communities is limited (Kahmen et al. 2006; von Felten et al, 2009; Bachmann et al 2015). Understanding how these factors

relate to CE and the diversity-productivity relationship in grasslands requires an integrated approach in future research.

Recently, host-specific soil pathogens have been found to be a major determinant of the diversity-productivity relationship by causing negative plant-soil feedback (Bever 1994) and suppressing plant growth at low species richness levels (Schnitzer et al. 2011; Maron et al. 2011; Hendriks et al., 2013). As the additive partitioning method is based on a comparison of performance in mixtures and monocultures, reductions in monocultures due to negative plant-soil feedback can also lead to positive complementarity effects. Identifying the mechanisms underlying the positive biodiversity-biomass relationships in grasslands may require incorporating the plant-microbe interactions that can mediate nutrient uptake, facilitate resource partitioning, and alter competitive dynamics (Bever et al. 2010).

Conclusion and outlook

Aboveground, it is well established that complementarity effects (CE) contribute to the positive biodiversity- productivity relationship. The mechanisms that underlie this relationship are commonly assumed to occur belowground. Here, we provide the first experimental evidence that CE also occur belowground, and contribute to the positive biodiversity effect we observed. We tested if the diversity in vertical root distributions among species, measured in monocultures, could be a mechanism underlying belowground CE. However, we did not find a relationship between vertical root distribution diversity and CE. Future research aimed at elucidating the mechanisms underlying CE would profit from considering several mechanisms (e.g. spatial and temporal resource differentiation, plant-pathogen interactions) simultaneously, and linking these to important functional traits. This may help to enhance our understanding of the importance of biodiversity for productivity in grasslands.

Acknowledgements

We would like to thank our colleagues and student-helpers for washing the roots, the Jena Experiment gardening team for support and maintenance of the field experiment, and the Leipzig Botanical Garden for the use of root washing facilities. We also would

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like to thank Cameron Wagg, Marinka van Puijenbroek and Thijs Fijen for helpful comments on statistics, and Lisette Bakker and Sigrid Dassen for insightful discussion. The Jena Experiment is funded by the Deutsche Forschungsgemeinschaft (DFG FOR 1451). L.M. is supported by NWO-VIDI grant 864.14.006.

Supplementary Information

Fig. S2.1. Sampling design for 2012 and 2014 in each plot of the trait-based experiment. This sampling was part of a longitudinal study on the development of root standing biomass in the trait-based experiment. A root core of 40 mm diameter and 40 cm depth was taken at each sampling point. 'ME' is the Jena Main Experiment, 'Saale' is the Saale river.

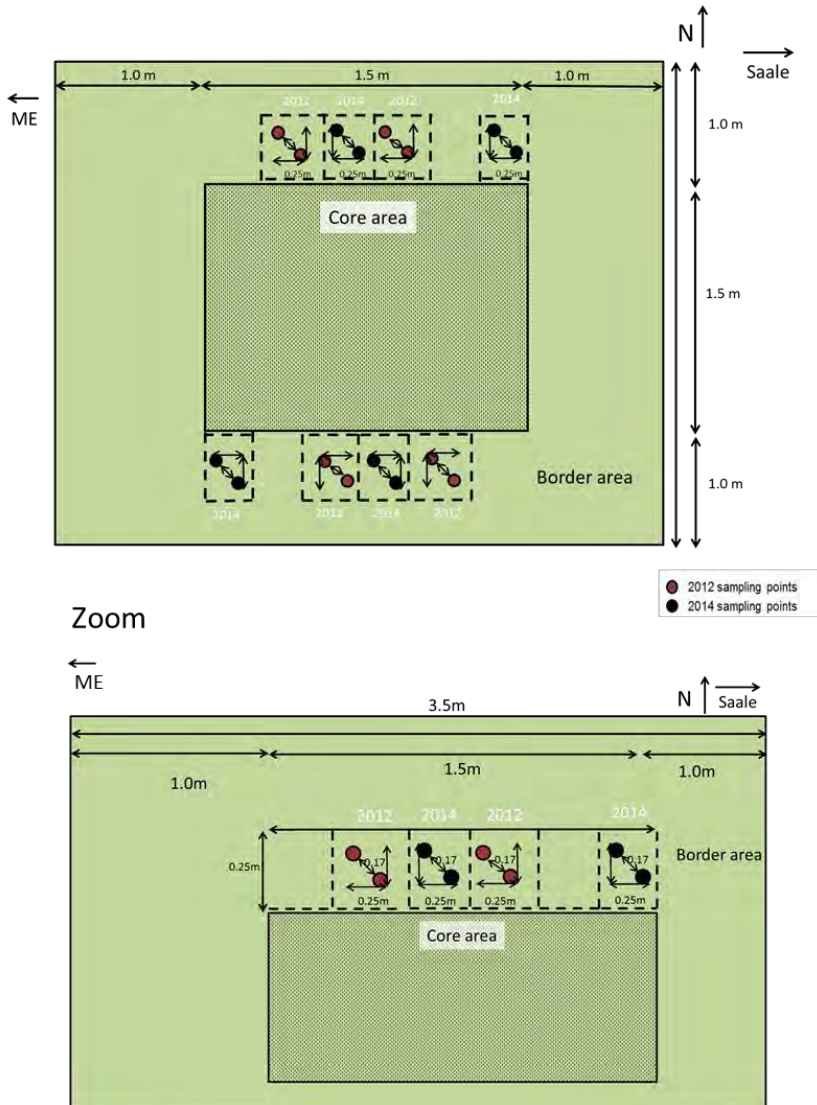


Fig. S2.2. Reference plots of estimated species proportion (y-axis) against actual species proportion (x-axis) in mixed samples, used to check the validity of the RT-PCR analysis for pool 1 in 2012 (A) and 2014 (B), and pool 2 in 2012 (C) and 2014 (D). Each panel represents a different species. Red lines represent linear regressions, based on 56 samples per plot. Black lines represent 95% confidence interval around the regression lines.

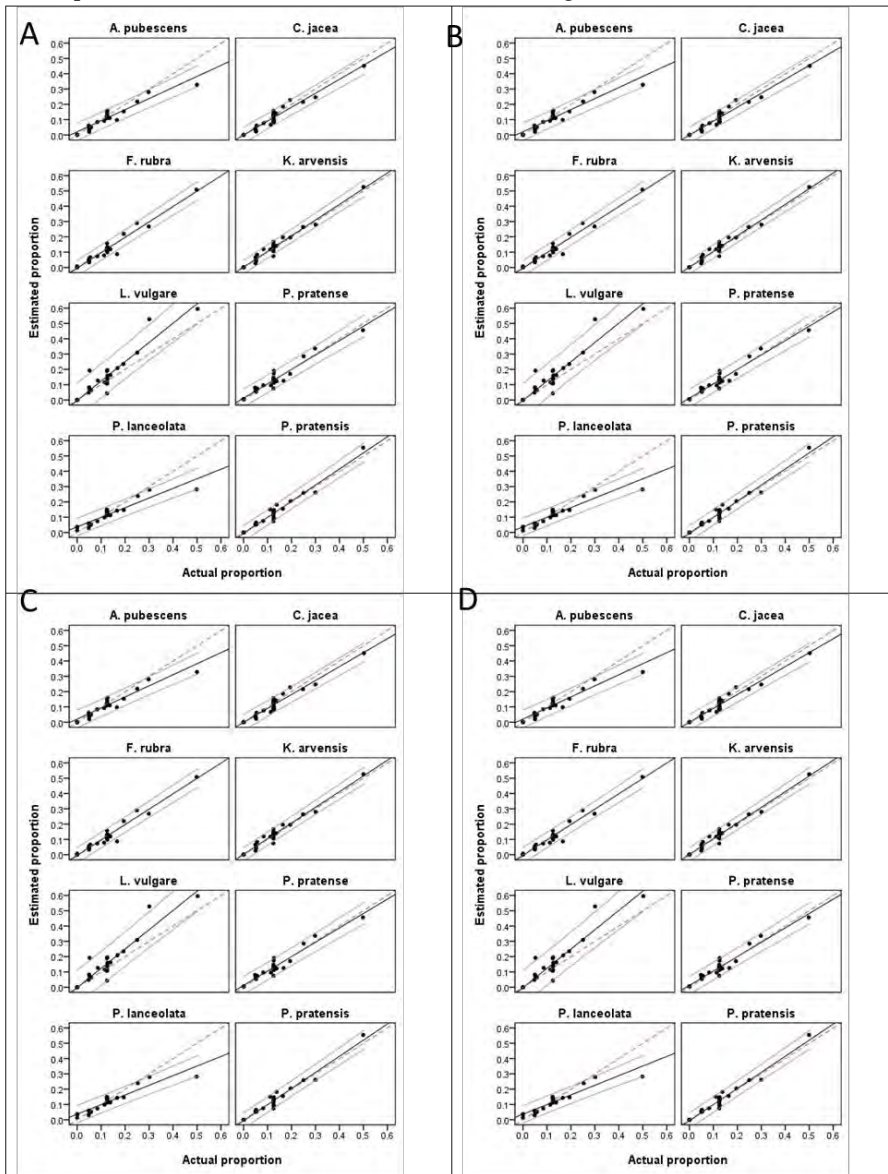


Table S2.1. Adapted from Table 3 in (Ebeling *et al.* 2014b). List of combinations between the design variables plant species richness (PSR) and functional trait diversity (FD_{Jena}) and its respective number of replicates per species pool and for the complete design (species pool 1 and 2), giving a total of 92 plots. FD_{Jena} varies along a gradient of functional trait diversity from redundant (FD_{Jena} 1) to diverse (FD_{Jena} 4).

Plant species richness (PSR)	FD _{Jena} 1	FD _{Jena} 2	FD _{Jena} 3	FD _{Jena} 4	Replicates/ species pool	Total plot number
1	8	-	-	-	8	16
2	4	6	4	2	16	32
3	-	4	4	4	12	24
4	-	3	4	2	9	18
8	-	-	-	1	1	2
Replicates / species pool	12	13	12	9		
Total plot number	24	26	24	18		92

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Table S2.2. Primer sequences used in the RT-PCR method to determine species-specific root proportions of the thirteen species in this study.

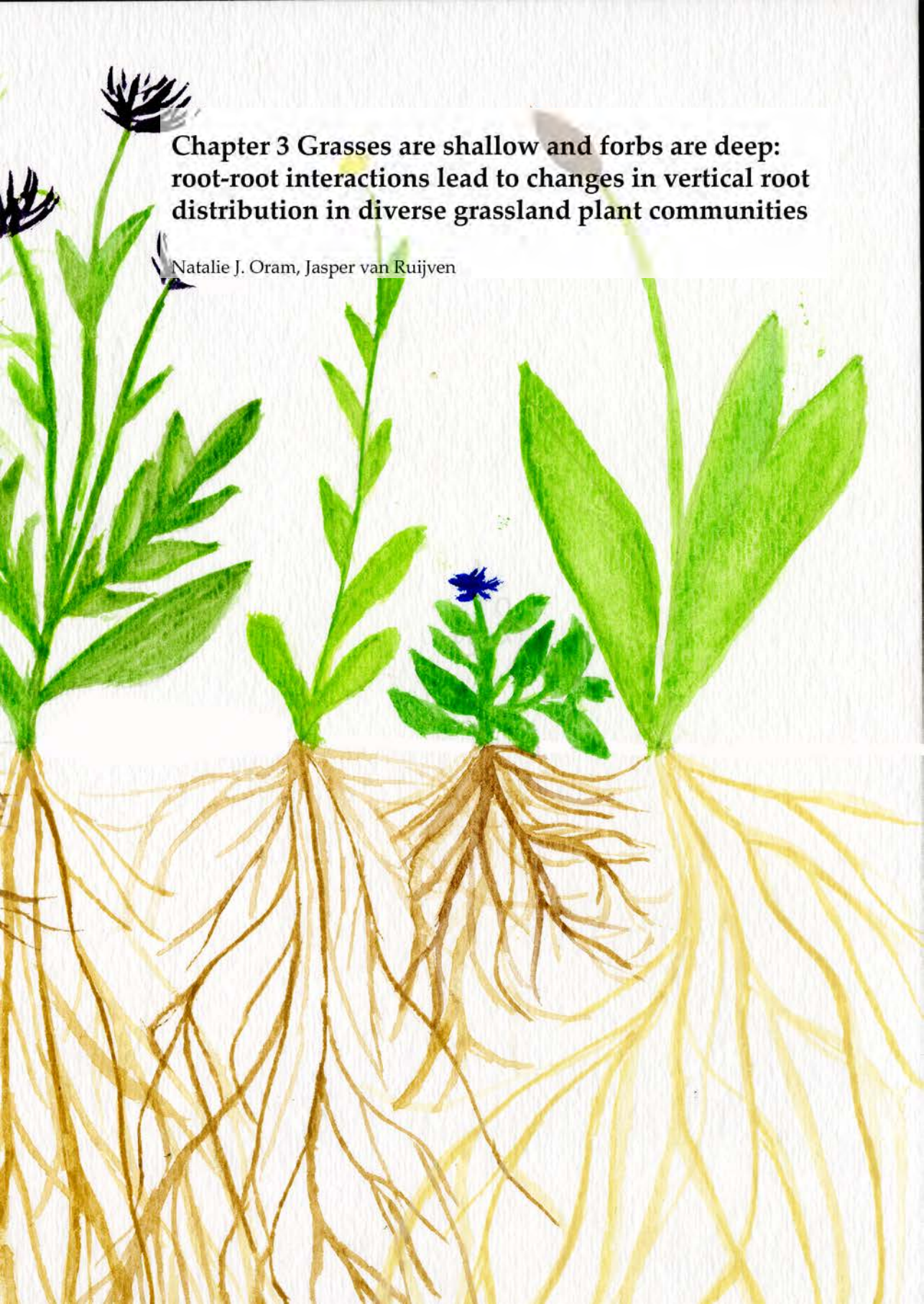
Species	Pool	Forward primers	Reverse primers
<i>Anthoxanthum odoratum</i> *	2	5'-TCATGTACTGTTGTACTGCGAAG-3'	5'-GAATCAAGCTGGACAGTAAATGAC-3'
<i>Avenula pubescens</i>	1	5'-CTGGACGTTCCCATGTTCT-3'	5'-GGTGGTACAGAGGTGGCAGT-3'
<i>Centaurea jacea</i>	1	5'-CTCGCACATCCACGCACAC-3'	5'-TGCAGTGGTTTTTCGTAGGAAGG-3'
<i>Dactylis glomerata</i>	2	5'-CAGGGCATTGAACTGATGATG-3'	5'-AGAACTGGTGTGCGTCTGC-3'
<i>Festuca rubra</i> *	1	5'-ACCGGAGATCGACAGCAAAACAG-3'	5'-TGTCCCTTGGTGGCGTTTTGG-3'
<i>Geranium pratense</i>	2	5'-ACCTTCGGGGAATCGTGTA-3'	5'-TCGACCCAAGTGGTAAGGAG-3'
<i>Holcus lanatus</i>	2	5'-CAAGTTCGGAAGCCGTTAGG-3'	5'-GGACTCCAGTCCAGCGAAGT-3'
<i>Knautia arvensis</i>	1	5'-GACCACAAAAGCAAGGAAGAA-3'	5'-CAAGGCAAGGAATCTCCAAG-3'
<i>Leucanthemum vulgare</i> *	1,2	5'-AAACTCTACAGGCGTTCTTCC-3'	5'-ATTTCACTTCATAGCTCTTCACTG-3'
<i>Phleum pratense</i>	1,2	5'-AGAGAGCAGGACACCGCCTA-3'	5'-GCCCTCTTGATTTTCGCATC-3'
<i>Plantago lanceolata</i>	1,2	5'- GAGAAAGCAGTAGGAAACCACAGTG-3'	5'-GATCGAGATCTCTCACTCAAACCC-3'
<i>Poa pratensis</i>	1	5'-TGCACCCCTTCTGACTCTCA-3'	5'-GTGATAAGCGCGTCACGTTTC-3'
<i>Ranunculus acris</i>	2	5'-CATTGCCACCTCTGCACTTC-3'	5'-TGAAACTTGCAGGTCCGAGA-3'

* Previously published in (Mommer *et al.* 2008) and used in (Mommer *et al.* 2010).

Table S2.3. List of fitted vertical root distributions (β) and corresponding r^2 values for each species in each year and pool. β values were determined by fitting the equation $Y = 1 - \beta^d$ (Jackson et al., 1996) to cumulative root biomass over depth (d).

Species	Pool	Year	β	r^2
<i>Avenula pubescens</i>	1	2012	0.78	0.998
<i>Centaurea jacea</i>	1	2012	0.8	0.855
<i>Festuca rubra</i>	1	2012	0.79	0.816
<i>Knautia arvensis</i>	1	2012	0.91	0.997
<i>Leucanthemum vulgare</i>	1	2012	0.87	0.927
<i>Phleum pratense</i>	1	2012	0.85	0.919
<i>Plantago lanceolata</i>	1	2012	0.84	0.965
<i>Poa pratensis</i>	1	2012	0.66	0.896
<i>Avenula pubescens</i>	1	2014	0.86	0.982
<i>Centaurea jacea</i>	1	2014	0.87	0.984
<i>Festuca rubra</i>	1	2014	0.86	0.975
<i>Knautia arvensis</i>	1	2014	0.9	0.987
<i>Leucanthemum vulgare</i>	1	2014	0.81	0.894
<i>Phleum pratense</i>	1	2014	0.88	0.984
<i>Plantago lanceolata</i>	1	2014	0.84	0.977
<i>Poa pratensis</i>	1	2014	0.81	0.983
<i>Anthoxanthum odoratum</i>	2	2012	0.88	0.999
<i>Dactylis glomerata</i>	2	2012	0.89	0.98
<i>Geranium pratense</i>	2	2012	0.85	0.868
<i>Holcus lanatus</i>	2	2012	0.7	0.651
<i>Leucanthemum vulgare</i>	2	2012	0.67	0.88
<i>Phleum pratense</i>	2	2012	0.83	0.921
<i>Plantago lanceolata</i>	2	2012	0.87	0.988
<i>Ranunculus acris</i>	2	2012	0.8	0.998
<i>Anthoxanthum odoratum</i>	2	2014	0.76	0.972
<i>Dactylis glomerata</i>	2	2014	0.88	0.979
<i>Geranium pratense</i>	2	2014	0.88	0.954
<i>Holcus lanatus</i>	2	2014	0.91	0.989
<i>Leucanthemum vulgare</i>	2	2014	0.87	0.959
<i>Phleum pratense</i>	2	2014	0.86	0.931
<i>Plantago lanceolata</i>	2	2014	0.88	0.991
<i>Ranunculus acris</i>	2	2014	0.77	0.994



A watercolor illustration of four plants with different root systems. From left to right: 1. A plant with a tall, thin stem and several black, spiky flowers. Its roots are shallow and spread out horizontally. 2. A plant with a thin stem and several green leaves. Its roots are shallow and spread out horizontally. 3. A small plant with a short stem and several green leaves, topped with a single blue flower. Its roots are shallow and spread out horizontally. 4. A plant with a short stem and several large, broad green leaves. Its roots are shallow and spread out horizontally. The background is a light, textured white.

**Chapter 3 Grasses are shallow and forbs are deep:
root-root interactions lead to changes in vertical root
distribution in diverse grassland plant communities**

Natalie J. Oram, Jasper van Ruijven

Abstract

Plants are plastic, adjusting their traits in response to abiotic and biotic conditions. Belowground, plants can respond to their neighbours by altering their vertical root distribution: aggregating or segregating their roots. These responses could facilitate belowground niche differentiation, which may contribute the positive plant diversity-productivity relationship observed above and belowground. We determined the change in species' vertical root distribution by quantifying species-specific root biomass and distribution of 7 grasses and 6 non-leguminous forbs over a plant species richness gradient in the Jena Trait Based Experiment using RT-qPCR. We tested if species altered their vertical root distribution in response to the vertical root distribution of their neighbours, and if this change influenced species-specific belowground relative yield (observed root standing biomass of a species in mixture relative to the expected root standing biomass, based on monoculture). We found that species altered their vertical root distribution when grown with inter-specific neighbours, but this was not influenced by plant diversity. Grasses became shallower rooted, irrespective of the vertical root distribution of their neighbours. Forbs became deeper-rooted when grown with deeper-rooted neighbours. Forbs had greater belowground relative yield than expected, based on their belowground yield in monoculture. Grass root biomass in mixtures did not differ from expected. Overall, belowground relative yield was not related to changes in vertical root distribution. Our study provides evidence that species change their vertical root distribution when growing with inter-specific neighbours, and this change differs between plant functional groups. However, as changes in vertical root distribution did not relate with increases in belowground relative yield, other mechanisms may facilitate the observed diversity-productivity relationship in grasslands.

Introduction

Our understanding of how plants respond to neighbours is substantially less advanced below than aboveground (Cahill & McNickle 2011), despite the fact that roots account for the majority of plant biomass (Poorter et al. 2012). Root systems are plastic, changing in response to biotic and abiotic stimulus in their environment (Hodge 2004). There is evidence from pot experiments that plants respond to their neighbours by altering their root length density, root branching, the proportion of fine roots (Nord et al. 2011; Semchenko et al. 2014), and root distribution (Gersani et al. 2001; Hodge 2004; Cahill & McNickle 2011; Nord et al. 2011; Belter & Cahill 2015). These plastic responses can influence resource capture (Hutchings & de Kroon 1994; Grime & Mackey 2002), which has implications for plant community structure and productivity (Belter & Cahill 2015; Abakumova et al. 2016). In the field, above and below-ground productivity has been shown to relate to a deeper rooting depth of the community (Mueller et al. 2013). However, in comparable experiments, no relation was found (Ravenek et al. 2014; this thesis, chapter 2). This discrepancy could be due to changes in vertical root distribution at the species level. How species alter their vertical root distribution in response to neighbours in the field may enlighten how root-root interactions influence species productivity.

Neighbour-induced changes in root distribution can be roughly grouped into two responses: root segregation, in which plants avoid each other (Schenk, Callaway & Mahall 1999; Kesanakurti et al. 2011) or root aggregation, in which plants grow towards each other (Price et al. 2012; Frank et al. 2015). Whether roots aggregate or segregate could depend on the identities of the interacting species, or the distribution of available nutrients in the soil. Plants have been shown to segregate from closely related neighbours (Bartelheimer et al. 2006), and aggregate with distantly related or inter-specific neighbours (Gersani et al. 2001; Falik et al. 2005; Bartelheimer et al. 2006; Belter & Cahill 2015). Information contained in a plant's root exudates may reveal its identity to its neighbours, leading to changes in the neighbour's root traits and distribution. For instance, the grass *Deschampsia caespitosa* increased its root length density, and produced more branched, finer roots in a soil patch treated with exudates originating from an unrelated plant, compared to when it encountered exudates from a related plant

(Semchenko et al. 2014). Soil nutrient availability may also mediate changes in root distribution. It is well established that plants can alter their root distribution in order to proliferate in nutrient-rich patches (e.g. Drew 1975; Zhang & Forde 1998; Hodge 2004; Kembel & Cahill 2005). At a smaller scale, plants can induce changes in nutrient availability in and near the rooting zone, mediating the root-responses of a neighbour. Root segregation has been shown to occur due to plant-mediated resource depletion (Nord et al. 2011). In contrast, roots may aggregate with neighbours in order to take advantage of increased availability of phosphorus (Li et al. 2007b), nitrogen (Cheng 2009), and water (Prieto, Armas & Pugnaire 2012) in the neighbour's rooting zone.

Segregation in vertical root distribution between species has been hypothesized to facilitate belowground niche differentiation and resource partitioning (Parrish & Bazzas 1976; Berendse 1982; Schenk et al. 1999). Recently this hypothesis, in which species-specific differences in vertical root distribution facilitates increased community nutrient acquisition has been put forward again as a potential explanation for the positive effect of plant species richness on plant productivity (Dimitrakopoulos & Schmid 2004; von Felten & Schmid 2008; Mommer et al. 2010; Mueller et al. 2013), which has been observed above- (Hector et al. 1999; van Ruijven & Berendse 2005; Marquard et al. 2009) and belowground (Spehn et al. 2005; Mueller et al. 2013; Ravenek et al. 2014).

Some evidence suggests that vertical root distribution differs between functional groups, i.e. grasses root shallower than forbs (Berendse 1982; Casper & Jackson 1997) or legumes (Mueller et al. 2013) when grown in monocultures. If functional groups differ in their vertical root distribution in mixtures, vertical root segregation at the functional group level could contribute to resource partitioning. Indeed, functional group diversity was found to promote above- and belowground biomass accumulation, independent of plant species richness, due to positive interactions between species in mixtures (Reich et al. 2004). In the same experiment, functional groups differed in their root distribution in monocultures, and presence/absence of specific functional groups had differential effects on the proportion of deep root biomass, which was related to increases in aboveground productivity (Mueller et al. 2013). This could signal vertical root segregation between functional groups. Conversely, no difference in grass and forb vertical root distribution in monocultures has also been observed (chapter 2, this thesis).

Previously, testing if functional groups (or species) differ in vertical root distribution in mixtures was hindered by the methodological constraint of identifying roots of different species. Thus, species specific vertical root distribution in mixtures has remained largely unknown. Molecular identification of species-specific root biomass (Mommer et al. 2008) has alleviated this constraint, facilitating tests of vertical root segregation between species or functional groups. Mommer et al. (2010) found that in mixtures, one grass and forb species segregated, however, another grass and forb species did not alter their vertical root distribution in a four species mix. In the field, species-specific vertical root distribution in mixtures has yet to be elucidated. Previous studies signal that roots respond to their neighbours, and changes in vertical root distribution at the community level can relate to increases in productivity. This justifies a closer look at the species level. Here, we identify species-specific root biomass (g m^{-2}) with a molecular approach (RT-qPCR) (Mommer et al. 2008) and use this information to determine the species-specific vertical root distribution of 7 grasses and 6 non-leguminous forbs across a plant diversity gradient (1 - 8 species) in the Jena Trait Based Experiment (TBE, Ebeling et al. 2014). We test the hypotheses that:

1. In mixtures, grasses and forbs differ in their vertical root distribution
2. Species change their vertical root distribution when grown with inter-specific neighbours and this change differs between functional groups.

We also relate shifts in vertical root distribution to belowground relative yield, and predict that:

3. The change in vertical root distribution facilitates greater belowground relative yield at the species level.

Materials and Methods

Experimental design

Species-specific vertical root distribution was studied in the Jena Trait Based Experiment (TBE), fully described in Ebeling et al. (2014). Briefly, the TBE was sown in spring 2011,

and is situated along the river Saale (130 m above sea level), parallel to the Jena Experiment (Roscher et al. 2004) (Germany; 50.95 °N 11.62 °E). The soil type is sandy loam (40% sand, 44% silt, 16% clay) (Steinbeiss, Temperton & Gleixner 2008b). Plots (12.25 m², n = 138) were assembled into three spatial blocks along the river, following a gradient of soil characteristics. Plots of the species pools (explained below) were distributed evenly over the blocks. Experimental plots were mown twice a year (June, September) and weeded three times (April, July, October) a year to maintain target plant community composition. The Jena TBE consists of three pools of eight species, selected from the original species pool of the Jena Experiment (Roscher et al. 2004). Plant communities in each pool follow a gradient of plant species richness (SR; 1, 2, 3, 4, or 8 species) and functional trait diversity (FDJena). Gradients of FDJena were created by composing mixtures of plant species with similar (redundant) traits or different (diverse) traits. Six traits were considered: plant height, leaf area, growth and flowering starting date, rooting depth, and root length density. Within each pool, plant communities were created to vary from trait redundancy (FDJena 1) to diversity (FDJena 4). Pool 1 contained mixtures that vary over a gradient of spatial trait diversity, differing in traits such as rooting depth. Plant communities in pool 2 vary over a gradient of temporal trait diversity, differing in traits such as flowering start date. Pool 3 considers the trait diversity in both space and time. This study included pools 1 and 2 (n = 92, 46 per pool), which were chosen to test if changes in vertical root distribution depend on the spatial or temporal trait diversity of the community. Each pool contained 4 forbs and 4 grasses: pool 1 included: *Centaurea jacea*, *Knautia arvensis*, *Leucanthemum vulgare*, and *Plantago lanceolata*, and *Avenula pubescens*, *Festuca rubra*, *Phleum pratense*, and *Poa pratensis*. Pool 2 included: *Geranium pratense*, *Leucanthemum vulgare*, *Plantago lanceolata*, and *Ranunculus acris*, and *Anthoxanthum odoratum*, *Holcus lanatus*, *Phleum pratense*, and *Dactylis glomerata*. The 92 plots in pools 1 and 2 generated 16 monocultures, 32 two-species plots, 24 three-species plots, 18 four-species plots, and 2 eight-species plots.

Root sampling

Root standing biomass (RSB, g m⁻²) as well as the species specific relative abundance of root biomass were determined in 2014; see chapter 2, this thesis for complete details.

Briefly, RSB was collected from eight locations per plot from 0-40 cm depth with a 4 cm diameter soil core. The soil core was separated into five depths (0-5, 5-10, 10-20, 20-30, and 30-40 cm), pooled by depth increment in the field, and kept at 4°C until washing over a 0.5 mm sieve. Sub-samples of fine roots (< 2 mm diameter) from each plot and each depth were taken to determine species specific relative abundance using molecular identification (RT-qPCR) (Mommer et al. 2008). Quantifying species specific relative abundance facilitates the determination of species specific vertical root distribution in mixtures, and subsequent calculations. The rest of the root biomass was dried at 65 °C for at least 48 hours and then weighed. As in chapter 2, this thesis, only fine roots are considered in all further analysis.

Calculations

Vertical root distribution is expressed as β , and was calculated by fitting the asymptotic equation to the cumulative proportion of species specific RSB (g m^{-2}) over depth in each plot:

$$Y = 1 - \beta^d$$

Where Y is the cumulative proportion of roots from the surface to depth, d , and β is the index of distribution. Values of β are maximum 1.0, values closer to 1 correspond with a greater proportion of roots in deeper soil layers (Gale & Grigal 1987; Jackson *et al.* 1996), i.e. deep-rooting species have a high β , and shallow-rooting species have a low β . We use β as a parameter for vertical root distribution as it combines all information on root biomass over depth into one value; this value can then be used as a root trait. Using this fitted curve requires root biomass to follow a declining function over depth. The species in our study follow this distribution, which is common for grassland species. Fig.3.1 illustrates this curve fitted to *L. vulgare* growing in monoculture.

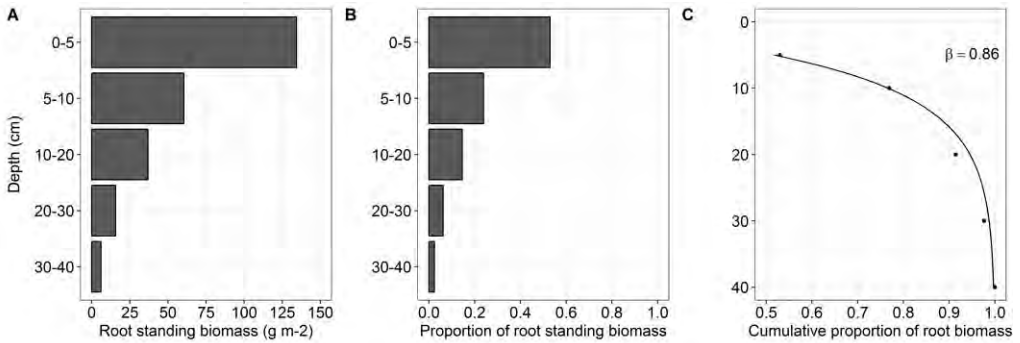


Fig. 3.1. Conceptual diagram illustrating the approach of quantifying vertical root distribution, expressed as β . For each species in each plot, we quantified: (A) root standing biomass (g m^{-2}) over five soil depth (cm) layers, (B) the relative proportion of root biomass over five soil depth (cm) layers, and (C) the fitted curve, $Y = 1 - \beta^d$, where Y is the cumulative proportion of root biomass at depth, d , and β is the parameter describing vertical root distribution. β values are maximum 1.0; larger values indicate that the species allocates more roots to deeper layers, species with shallower roots correspond with smaller β values.

For some species in some mixtures, β could not be fitted accurately. A cutoff point of $r^2 = 0.66$ was set, and β curves with a lower r^2 were excluded from the analysis. This cutoff point was chosen as it is the poorest fit β in monoculture. This conservative criterion is used to prevent inaccurately fitted β curves from influencing the results. After excluding observations below this cutoff, 197/240 observations (16/16 in monocultures, 181/224 in mixtures) remained. The change in vertical root distribution of a species in mixture was calculated as:

$$\Delta\beta = \text{Mono}\beta_i - \text{Mix}\beta_i$$

Where $\text{Mono}\beta_i$ is the vertical root distribution of species i in monoculture, and $\text{Mix}\beta_i$ is the vertical root distribution of species i in mixture. Negative $\Delta\beta$ indicates that the species allocates more roots to deeper layers in mixtures than monocultures, whereas positive values indicate that the species shifts its vertical root distribution to more shallow soil layers.

We determined a species' dissimilarity between its β and β of its neighbours, as measured in monoculture (focal species dissimilarity) using a modified Bray-Curtis calculation (Bray & Curtis 1957):

$$\text{Focal species dissimilarity} = \sum_{i=1}^n \frac{\beta_{\text{neighbor } i} - \beta_{\text{focal}}}{SR - 1}$$

Where β_{neighbor} is the vertical root distribution in monoculture of neighboring species i in that plot and β_{focal} is the vertical root distribution of a focal species in monoculture. SR is the species richness of the plant community. We used the β values measured in monoculture to have an independent measure of vertical root distribution. A positive focal species dissimilarity indicates that the focal species roots shallower than its neighbours, a negative focal species dissimilarity indicates the focal species is deeper rooting than its neighbours. As a measure of species' relative performance in mixtures, we calculated belowground relative yield of each species according to (De Wit 1960):

$$\text{Relative yield species } i = \frac{\text{observed yield}}{\text{expected yield}}$$

Where *observed yield* is the RSB (g m^{-2}) of species i growing in a mixture, and *expected yield* is the RSB of species i in monoculture divided by the species richness in the observed mixture. For the species that occurred in both pools (*P. pratensis*, *L. vulgare*, and *P. lanceolata*), we used the monoculture in the same pool as the mixture plot as a reference.

Statistical analysis

Statistics were performed in R version 3.3.2 (R Development Core Team 2016). We tested our three hypothesis with linear mixed effects models using the function `lme{nlme}` (Pinheiro *et al.* 2016). Significance of these models was determined with a type III (marginal) SS ANOVA, using `anova{stats}`. In all models, the random factor `block/plot` was used to account for spatial variation in soil properties across the field site (`block`; discrete: 1, 2, 3), and multiple observations per experimental (`plot`; discrete: $n = 92$). Assumptions of normality and homogeneity of variance were judged by visual assessment of the model residuals, and the raw data. Homogeneity of variance were further tested with a `bartlett.test{stats}`.

To address our first hypothesis, we tested the effects of pool (discrete: 1, 2), plant species richness ($\log SR$; log transformed, continuous: 2, 3, 4, 8), functional trait diversity (FD_{Jena} ; continuous: 1, 2, 3, 4) and functional group (FG; discrete: grass or forb) on species-specific β in mixtures. All 2-way interactions between explanatory variables were included. A variance structure was specified in our model, using the function `varIdent{nlme}`, as the

variance in β was not homogenous between FG. We also tested if FG and/or pool influenced β in monoculture ($n = 13$: 7 grasses and 6 forbs). We predicted that species would alter their vertical root distribution when grown with interspecific neighbours, and this change would differ between FG (hypothesis 2). We determined if the absolute $\Delta\beta$ over all species in mixtures was significantly greater than zero, using a one-sided t.test, t.test{stats}. We also determined the effects of logSR, FDJena, pool, FG, and focal species dissimilarity (continuous) on $\Delta\beta$. All 2-way interactions were included. To test if $\Delta\beta$ related with belowground relative yield (hypothesis 3), we tested the effects of $\Delta\beta$ (continuous), logSR, FDJena, pool, and FG on species-specific belowground relative yield. All 2-way interactions were included. We used a one-way t.test to determine if belowground relative yield was significantly greater than expected (1.0) for grasses, forbs, and overall.

Finally, we tested the effects of species (discrete, $n = 13$) on β , $\Delta\beta$, and belowground relative yield, with three alternative models which were identical to the ones above, except for the explanatory factor species instead of FG. When 'species' was significant or present in an interaction, a tukey post hoc test was used to determine differences between species using the function `glht{multcomp}` (Hothorn, Bretz & Westfall 2008).

Model simplification was carried out to remove redundant interactions, and find the simplest model which best explains the response variable (Crawley 2007). Models were fit with Maximum Likelihood (ML), and were simplified by sequential removal of the interactions or variables with the highest P value. The function `anova{stats}` was used to compare the fit of nested models based on the Akaike Information Criterion (AIC) of each model. Non-significance ($P > 0.05$) between models signaled that the variation explained by the complex and the simple models was not significantly different, and so the simple model was retained. The final models, presented in the main text, were fitted with REML. Differences between FGs are the focus of our hypothesis, and therefore FG models are discussed in the main text. Species models are presented in the supplementary information. Interactions between factors in the final models were further explored with linear mixed effects models fit with a type III SS ANOVAs as above, to determine the effects of each level of the factor involved in the interaction.

Results

Grasses are shallower rooting than forbs in mixtures

In mixtures, grasses were significantly shallower-rooting than forbs (main effect of functional group, FG, Table 3.1, Fig. 3.2 A). The vertical root distribution parameter, β , of grasses was lower ($\beta = 0.80 \pm 0.012$, mean \pm se) than forbs ($\beta = 0.87 \pm 0.006$, mean \pm se) indicating that grasses allocated a relatively greater proportion of their roots to shallow layers than forbs did. In mixtures, species in pool 1 rooted deeper ($\beta = 0.86 \pm 0.01$, mean \pm se) than in pool 2 ($\beta = 0.82 \pm 0.01$, mean \pm se) (Table 3.1). This effect was due to the difference in the plant species, which differed in their β , between pool 1 and 2. Hence, ‘pool’ was not retained in the model when plant species was included (species model, Table S3.1, Fig. S3.1). Plant species richness (SR) and functional trait diversity (FD_{Jena}) did not significantly affect β in mixtures when either FG or species was included as an explanatory variable (Table 3.1, Table S3.1, respectively). In monocultures, we found no difference in β between grasses and forbs ($F_{1,10} = 0.03$, $P > 0.05$), or between pools ($F_{1,10} = 0.04$, $P > 0.05$).

Table 3.1. ANOVA (type III) summary of species-specific vertical root distribution (β) in mixed plant communities. Explanatory variables include species pool (pool; discrete: 1, 2), plant species richness (logSR; log transformed continuous: 2, 3, 4, 8), functional trait diversity (FD_{Jena}; continuous: 1, 2, 3, 4), and plant functional group (FG; discrete: grass or forb). A random factor (random = ~1 | block/plot) was used to account for spatial variation across the field site and multiple observations per experimental plot. A variance structure (varIdent) was used to account for variance differences in β between grasses and forbs.

Factor	β		
Pool	$F_{1,69} =$	8.43	**
logSR	$F_{1,69} =$	0.18	ns
FD _{Jena}	$F_{1,69} =$	0.04	ns
FG	$F_{1,105} =$	30.72	***
P < 0.001 ***, P < 0.01 **, non-significant (ns)			

Grasses and forbs changed their vertical root distribution from monoculture to mixture

Species altered their vertical root distribution when growing in mixtures, compared to monocultures (absolute $\Delta\beta > 0$, $t_{1,180} = 16.37$, $P < 0.001$). Grasses and forbs differed in how they changed their vertical root distribution when growing with inter-specific neighbours (change in vertical root distribution from monoculture to mixture, $\Delta\beta$, Table 3.2). Grasses placed a greater proportion of their roots in shallow layers in mixtures than in monocultures, resulting in a positive $\Delta\beta$ (Fig. 3.2 B). In contrast, forbs allocated more roots to deeper layers when grown in mixtures compared to monoculture, resulting in a negative $\Delta\beta$ (Fig. 3.2 B). This corresponds with an increase in the proportion of grass root biomass in the top 10 cm of soil from an average of 75% in monocultures to 81% in mixtures. The proportion of forb root biomass in the upper 10 cm was reduced from an average of 77% in monocultures to 69% in mixtures. Species also differed in their $\Delta\beta$, but in general, forbs became deeper, and grasses became shallower (Fig. S3.2, Table S3.2). Pool influenced $\Delta\beta$ in both the FG and species models (Table 3.2, Table S3.2, respectively), as species in pool 1 became marginally deeper, on average ($\Delta\beta = -0.012 \pm 0.007$, mean \pm se), species in pool 2 became marginally shallower ($\Delta\beta = 0.013 \pm 0.009$, mean \pm se).

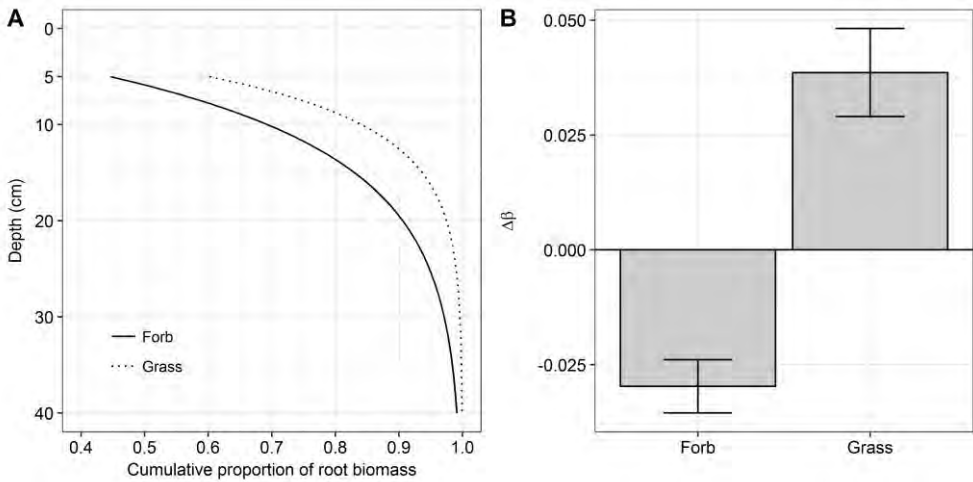


Fig. 3.2. (A) In mixtures, forbs had a deeper mean vertical root distribution (β) than grasses. Curves were derived by fitting the mean cumulative proportion of root biomass for grasses and forbs to the equation $Y = 1 - \beta^d$, where Y is the cumulative proportion of root biomass to depth, d , and β is the root distribution parameter. The β of forbs is illustrated with the solid line, grasses with the dotted line. (B) Forbs and grasses altered their root distribution in mixtures, compared to their respective monocultures ($\Delta\beta$; monoculture β – mixture β). Forbs became deeper-rooting in mixtures (a negative $\Delta\beta$); grasses became shallower rooting in mixtures (a positive $\Delta\beta$). Bars indicate mean \pm SE. See Table 3.2 for statistics.

Table 3.2. ANOVA (type III) summary of the change in species-specific vertical root distribution from monoculture to mixture ($\Delta\beta$). Explanatory variables include species pool (discrete: 1, 2), plant species richness (logSR; log transformed continuous: 2, 3, 4, 8), plant functional group (FG; discrete: grass or forb), functional trait diversity (FDJena; continuous: 1, 2, 3, 4), and focal species dissimilarity (continuous). Focal species dissimilarity is a measure of the difference of a focal species' β from the β of its neighbours. A random factor (random = ~ 1 | block/plot) was used to account for spatial variation across the field site, and multiple observations per experimental plot..

Factor	$\Delta\beta$		
Pool	$F_{1,69} =$	7.24	**
logSR	$F_{1,69} =$	0.10	ns
FG	$F_{1,101} =$	39.32	***
FDJena	$F_{1,69} =$	0.00	ns
Focal species dissimilarity	$F_{1,101} =$	2.00	ns
FG: focal species dissimilarity	$F_{1,101} =$	8.28	**
FDJena: focal species dissimilarity	$F_{1,101} =$	3.75	‡
logSR: focal species dissimilarity	$F_{1,101} =$	5.07	*

$P < 0.001$ ***, $P < 0.01$ **, $P < 0.05$ *, $P < 0.10$ ‡, non-significant (ns)

The relationship between focal species dissimilarity (the difference in β between a focal species and its neighbours) and $\Delta\beta$ differed between grasses and forbs (Table 3.2). Grasses showed no response to their neighbours in terms of β (focal species dissimilarity within grasses, $F_{1,28} = 0.03$, ns). Forbs responded to their neighbours, rooting deeper when growing with deeper-rooting neighbours, evidence for vertical root aggregation (focal species dissimilarity within forbs, $F_{1,37} = 21.93$ $P < 0.001$, Fig.3.3). The relation between $\Delta\beta$ and focal species dissimilarity was influenced by SR (logSR: focal species dissimilarity interaction, Table 3.2, Table S3.2). Focal species dissimilarity was negatively related to $\Delta\beta$ in the 8-SR communities ($F_{1,12} = 5.17$, $P < 0.05$, $r^2 = 0.27$). However, this relation is based on 15 observations within two experimental plots, so likely holds little ecological relevance. Within all other levels of SR, there was no significant relation between focal species dissimilarity and $\Delta\beta$. In the species model only, the relationship between focal species dissimilarity and $\Delta\beta$ differed between levels of FDJena (FDJena: focal species dissimilarity, Table S3.2). A significant relation was only observed in

FDJena 2 ($F_{1,27} = 11.99$, $P < 0.01$) where focal species dissimilarity had a negative effect on $\Delta\beta$ ($r^2 = 0.19$). A negative relation indicates that a focal species roots deeper when in a community with deeper-rooting neighbours.

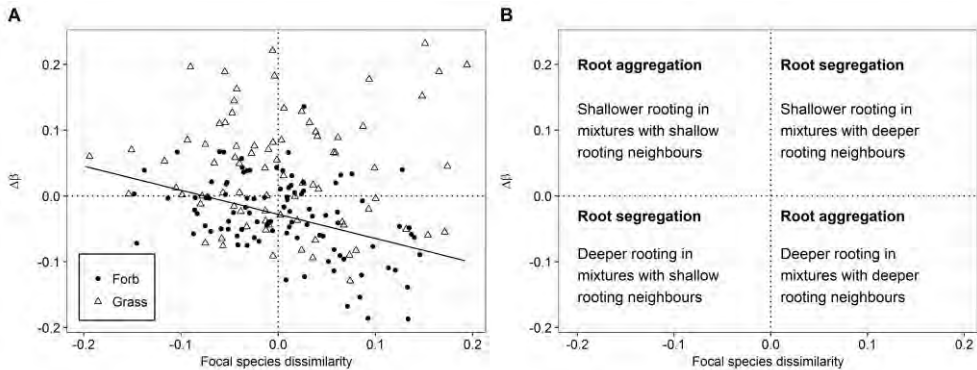


Fig.3.3. (A) The relation between the difference of a focal species' vertical root distribution (β) from the β of its neighbours in a mixture (focal species dissimilarity) and the change in vertical root distribution ($\Delta\beta$; monoculture β – mixture β) differed between forbs and grasses. The $\Delta\beta$ of forbs significantly decreased when grown with deeper-rooting neighbours (black line). The $\Delta\beta$ of grasses did not relate to focal species dissimilarity ($P > 0.05$). The dotted lines highlight no change in $\Delta\beta$ ($y = 0$) and no difference in β between a focal species and the β of its neighbours ($x = 0$). (B) The conceptual figure shows the quadrants which denote the relation between focal species dissimilarity and $\Delta\beta$. A positive $\Delta\beta$ indicates a species that is shallower rooting in mixture than monoculture, a negative $\Delta\beta$ indicates that it is deeper rooting. Positive focal species dissimilarity indicates that the focal species is rooting shallower (lower β) than its neighbours in a mixture; negative values indicates it is rooting deeper (higher β) than its neighbours. See Table 3.2 for statistics.

Belowground relative yield is not affected by the change in vertical root distribution

Overall, belowground relative yield was significantly greater than 1.0 ($t_{180} = 4.94$, $P < 0.001$), indicating over-yielding. A belowground relative yield of 1.0 indicates that the species in mixture produced the amount of root biomass expected based on its monoculture, higher values indicate root biomass production was greater than expected, i.e. over-yielding occurred. We found that the belowground relative yield differed between FG; forbs had a significantly higher belowground relative yield than grasses

(forb belowground relative yield: 2.17 ± 0.22 ; grass belowground relative yield: 1.09 ± 0.12 ; mean \pm SE; Table 3.3). Forbs significantly over-yielded (belowground relative yield > 1.0 , $t_{100} = 5.31$, $P < 0.001$), grasses did not ($t_{79} = 0.71$, ns). The relation between belowground relative yield and $\Delta\beta$ was only significant in FDJena 2 (FDJena: $\Delta\beta$ interaction, Table 3.3), where $\Delta\beta$ had a positive effect on belowground relative yield ($\Delta\beta$ within FDJena 2: $F_{1,27} = 7.64$, $P < 0.05$, Fig. 3.4). Considering the species model, belowground relative yield across an SR gradient differed per species (logSR: species interaction, Table S3.3). The belowground relative yield of two species, the grass *Phleum pratense* and the forb *Ranunculus acris* decreased over the SR gradient (logSR within *P. pratense*: $F_{1,10} = 5.18$, $P < 0.05$, $r^2 = 0.18$; logSR within *R. acris*: $F_{1,10} = 10.34$, $P < 0.01$, $r^2 = 0.46$). The relation between $\Delta\beta$ and belowground relative yield tended to differ between species (species: $\Delta\beta$ interaction, Table S3.3). The belowground relative yield of *Leucanthemum vulgare* increased with increasing $\Delta\beta$ ($r^2 = 0.31$, $\Delta\beta$ within *L. vulgare*: $F_{1,18} = 7.27$, $P < 0.05$), and the belowground relative yield of *R. acris* tended to increase with increasing $\Delta\beta$ ($r^2 = 0.16$, $\Delta\beta$ within *R. acris*: $F_{1,10} = 4.33$, $P = 0.064$), Fig. S3.3. A positive relation between $\Delta\beta$ and belowground relative yield indicates that species which become shallower rooted in mixtures have a higher belowground relative yield.

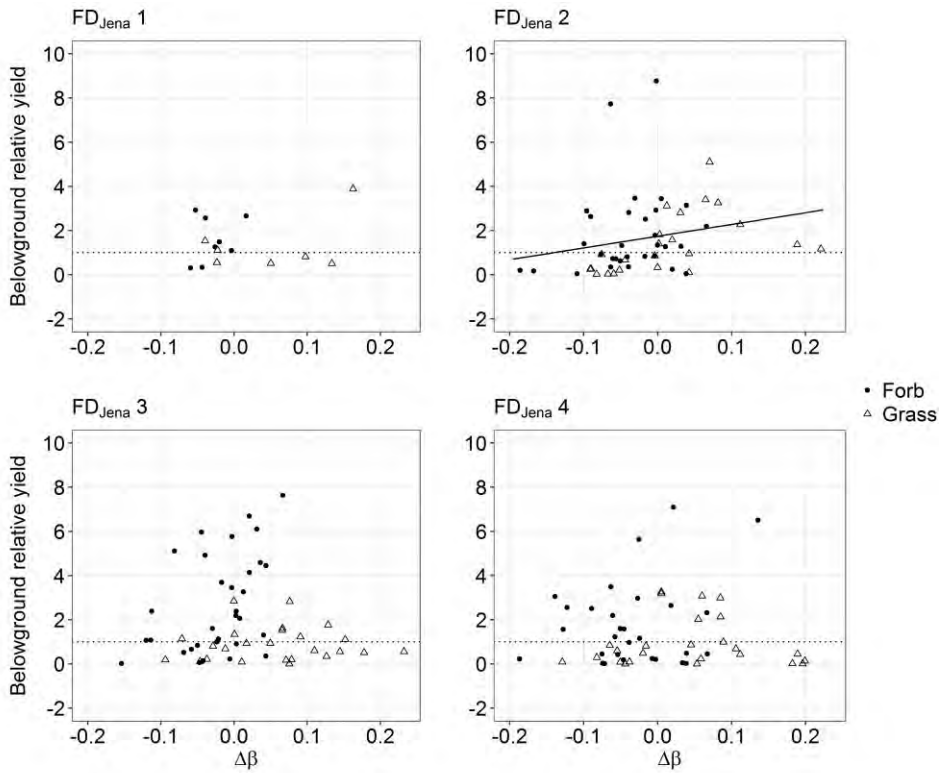


Fig. 3.4. The relation between the change in vertical root distribution from monoculture to mixture ($\Delta\beta$) and species-specific belowground relative yield differed between levels of functional trait diversity (FD_{Jena}). The relation was significant at FD_{Jena} 2, indicated by the black regression line. Positive $\Delta\beta$ indicates species allocated more roots to shallow layers in mixtures, a negative $\Delta\beta$ implies species rooted deeper in mixtures. FD_{Jena} ranges from plant communities with redundant traits (FD_{Jena} 1) to communities with diverse traits (FD_{Jena} 4). Belowground relative yield greater than 1.0 (the dotted line) indicate that the species produces more root biomass in mixture than predicted from root biomass production in monoculture. Black points indicate forbs, open triangles indicate grasses. See Table 3 for statistics.

Table 3.3. ANOVA (type III SS) summary of species-specific belowground relative yield. Explanatory variables include: functional trait diversity (FDJena; continuous: 1, 2, 3, 4), plant functional group (FG; discrete: grass or forb), and the change in vertical root distribution from monoculture to mixture ($\Delta\beta$, continuous). Belowground relative yield is the difference between the observed belowground root standing biomass (RSB, g m⁻²) of a species in mixture, and the expected RSB based on the species' RSB in monoculture. A random factor (random = ~1|block/plot) was used to account for spatial variation over the field site, and multiple observations per experimental plot. Belowground relative yield was log transformed to meet assumptions of normality.

Factor	Belowground relative yield		
FDJena	F _{1,71} =	4.34	*
FG	F _{1,103} =	19.73	***
$\Delta\beta$	F _{1,103} =	8.80	**
FDJena: $\Delta\beta$	F _{1,103} =	4.60	*

P < 0.001 ***, P < 0.01 **, P < 0.05 *

Discussion

Our results provide quantitative evidence for plasticity in vertical root distribution in the field; species change their vertical root distribution when grown in mixtures, compared to monoculture. This change differed between plant functional groups (FG). In mixtures, grasses shifted their vertical root distribution (β) to become shallower, forbs became deeper. This provides empirical evidence for the hypothesis that vertical root distribution differs between FG (Berendse 1982; Casper & Jackson 1997; Schenk & Jackson 2002; Mommer et al. 2010). Grasses did not alter their β in response to the rooting patterns of their neighbours. Forbs responded to deeper-rooting neighbours by allocating more roots to deeper layers, which suggests root aggregation. Contrary to our hypothesis, the change in vertical root distribution from monoculture to mixture ($\Delta\beta$) was not closely related to belowground relative yield (observed root standing biomass relative to expected root standing biomass based on monoculture).

Vertical root distribution differs between functional groups

Species exhibited plasticity in β in response to growing with inter-specific neighbours, which differed between FG but did not change over gradients of plant species richness (SR) or functional trait diversity (FDJena). In line with previous studies (references in Introduction), grasses rooted shallower than forbs in mixtures. The difference in β between grasses and forbs was not observed in the monocultures, similar to Schenk & Jackson (2002), but arose due to a difference in the direction of change in β ; grasses became shallower ($+\Delta\beta$), forbs became deeper ($-\Delta\beta$), in line with previous studies (references in Introduction). Similarly, Mommer et al. (2010) addressed this question by applying a molecular technique (Mommer et al. 2008) in an outdoor mesocosm experiment, and found that grasses tended to root shallower than forbs. In mixtures, they found that the grass *Anthoxanthum odoratum* became shallower rooted, while the forb *Lecantheum vulgare* became deeper rooted. However, the root distribution of *Festuca rubra* and *Plantago lanceolata* was unchanged (Mommer et al. 2010). In our study, there was variation within each functional group (i.e. species differed significantly in their vertical root distribution). However, overall, grasses became shallower, and forbs became deeper in mixtures.

We did not find evidence of vertical root segregation between species in mixtures, in line with von Felten & Schmid (2008), von Felten et al. (2009), and Mommer et al. (2010). Vertical root segregation has been hypothesized to facilitate resource partitioning (Parrish & Bazzas 1976; Berendse 1982; Dimitrakopoulos & Schmid 2004; Levine & HilleRisLambers 2009), as overlap of nutrient depletion zones created by different roots foraging in the same soil volume could reduce resource uptake efficiency. Root segregation has been reported to occur between many plant species (reviewed by: Schenk et al. 1999), as well as in a diverse grassland (Kesanakurti et al. 2011). However, we found that species that had similar β as their neighbours (quantified as focal species dissimilarity), did not change their β to avoid their neighbours. The response of grasses and forbs to their neighbours differed: grasses did not change their β ; forbs became deeper rooted when grown with deeper rooting neighbours, evidence for aggregation. Root aggregation has been shown in a pot experiment with dune grasses (Bartelheimer et al. 2006), and in a natural grassland (Frank et al. 2015). Here, we did not test the

potential mechanisms which may underlie the observed root aggregation. However, based on literature, we venture that this aggregation could be due to increases in resource availability in the rooting zone of neighbours and/or signaling between roots via root exudates.

Resource availability can influence root placement (e.g. Cahill et al. 2010; Nord et al. 2011), growth and branching (reviewed by: Hutchings & John 2003). In the rooting zone, rhizodeposition of labile carbon compounds (i.e. rhizosphere priming) can stimulate nitrogen (N) mineralization near the rooting zone (Cheng 2009). As plant species identity can affect the extent of rhizosphere priming (e.g. Fu & Cheng 2002), plants could benefit from aggregating with a neighbour that can more efficiently stimulate N mineralization. Similarly, the release of organic acids by one plant can mobilize phosphorus (P), and benefit near-rooting neighbours (Li et al. 2007b). Hydraulic redistribution of water from moist to dry soil can occur via roots (Neumann & Cardon 2012), which has been shown to increase the plant's root placement in nutrient-rich patches (Prieto et al. 2012). Placing roots in nutrient patches, and increasing soil moisture in the rooting zone may entice neighbours to aggregate and take advantage of the increased resource availability. Considering the same species as the present study, Ravenek et al. (2016) found that forbs had a greater ability to place roots in nutrient rich patches, compared to grasses. The superior root foraging ability of forbs, compared to grasses, has been found on other studies, e.g. (Grime & Mackey 2002; Kembel & Cahill 2005). Therefore, forbs may be better able than grasses to take advantage of increased resources by selectively placing their roots in their neighbour's rooting zone (hence, aggregating).

Root exudates can influence root-root interactions (Bais et al. 2006; Caffaro et al. 2013). Release of allelo-chemicals and non-toxic signals by a plant can affect the root growth of its neighbour(s) (reviewed by: Schenk 2006). These signals may contain information about the identity of the plant species. A number of studies have shown that the identities of the interacting plants determine root placement patterns. Interacting with inter-specific or unrelated neighbours can lead to proliferation of root biomass (Gersani et al. 2001; Maina, Brown & Gersani 2002; Falik et al. 2003). Semchenko, John & Hutchings (2007) showed that root elongation of *Fragaria vesca* was stimulated by contact with roots of *Glechoma hederacea* compared to intraspecific contact, and that this was not due

to nutrient availability. Root exudates are one mechanism underlying these observations. Exposure to a non-related neighbour's root exudates can lead to increases in root branching and specific root length (Semchenko et al. 2014). Our results show that grasses did adjust their β when grown in mixtures, but unlike forbs, this was not due to the β of their neighbours. Similar to (Semchenko et al. 2014), grasses in the present study may have responded by altering their root traits. Grasses and forbs have been shown to differ in their root traits (Roumet et al. 2008), and may respond differently to signals in their environment.

Changes in vertical root distribution do not alter belowground productivity

Contrary to our hypothesis, $\Delta\beta$ was not closely linked to belowground relative yield. Previously in the Jena Trait Based Experiment (TBE), we found a positive belowground diversity-productivity relationship, which was attributed increasing belowground complementarity effects (this dataset, see chapter 2, this thesis). Positive complementarity effects imply that the positive effect of plant diversity on productivity is due to beneficial interactions between the plant species in mixtures (Loreau & Hector 2001). However, these patterns were not explained by the diversity of inherent (monoculture) β (chapter 2, this thesis), similar to Bakker, Mommer & van Ruijven (2016) who also found no relation between diversity of root traits and productivity or complementarity effects in a grassland biodiversity experiment. This discrepancy could be due to differences in β or root traits between monocultures and mixtures, or because β and the traits studied do not relate to productivity or complementarity effects. Here, we confirm that β does change between mixtures and monocultures. However, similar to Mommer et al. (2010), we found that these shifts do not relate to growth; altering root distribution did not confer a benefit in terms of overyielding.

We did find that overall, forbs had a higher belowground relative yield than expected (based on monoculture yield), which was higher than that of grasses. However, also within FG, belowground relative yield was not related to $\Delta\beta$. Other root traits that represent resource acquisition strategies, such as root length density (RLD, root length/soil volume) and specific root length (SRL, root length/root mass) could increase nutrient uptake and competitive ability (Hodge et al. 1999- RLD; Fort, Cruz & Jouany

2014- SRL; Ravenek et al. 2016- RLD). Heterogeneous nutrient distributions in grassland soils (Fitter 1994) could favour species that are better able to place their roots in nutrient-rich patches (Hodge et al. 1999; Fransen, de Kroon & Berendse 2001; Cahill & McNickle 2011). Forbs may be more plastic than grasses in their root traits (e.g. foraging precision, Grime & Mackey 2002; Ravenek et al. 2016). Plasticity has been shown to affect nutrient uptake (Hodge 2004), interactions with neighbours (Callaway, Pennings & Richards 2003; Fort et al. 2014), and productivity (Padilla et al. 2013). Finally, plant-microbe interactions may have enabled forbs to have a higher belowground relative yield than grasses. In the Jena TBE, forbs belong to five families, and grasses belong to one. Thus, forbs are more likely to grow with neighbours from different families, alleviating negative plant soil feedbacks in mixtures to a greater extent than grasses (e.g. Reynolds et al. 2003).

Conclusion

Our study addresses if and how plant species alter their vertical root distribution in response to neighbours, and if this facilitates a higher belowground relative yield (than expected, based on monoculture yield). In a grassland biodiversity experiment, we provide evidence that grasses and forbs differentially change their vertical root distribution when growing in inter-specific mixtures. Grasses became shallower rooting, irrespective of the vertical root distribution of their neighbours. Forbs became deeper rooting, responding to their neighbours and aggregating with a deeper-rooting neighbours. Contrary to expectations, changes in vertical root distribution did not clearly relate to belowground relative yield at the species level. We found no evidence for vertical root segregation over a plant diversity gradient, and conclude that vertical root segregation over depth may play a minor role in contributing to increasing belowground relative yield over a plant diversity gradient in grasslands.

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Supplementary Information

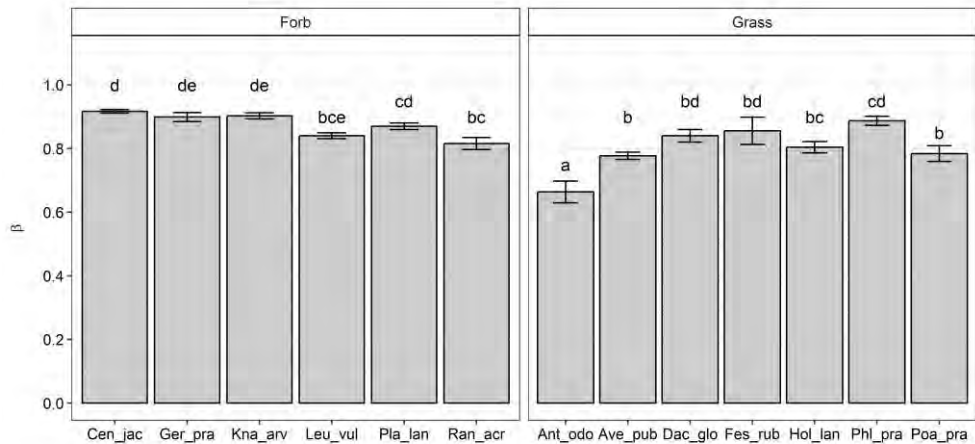


Fig. S3.1. Species-specific vertical root distribution, β , in mixtures. Forbs are indicated in the left panel: Cen_jac (*Centaurea jacea*), Ger_pra (*Geranium pratense*), Kna_arv (*Knautia arvensis*), Leu_vul (*Leucanthemum vulgare*), Pla_lan (*Plantago lanceolata*), and Ran_acr (*Ranunculus acris*). Grasses are indicated in the right panel: Ave_odo (*Anthoxanthum odoratum*), Ave_pub (*Avenula pubescens*), Dac_glo (*Dactylis glomerata*), Fes_rub (*Festuca rubra*), Hol_lan (*Holcus lanatus*), Phl_pra (*Phleum pratense*), and Poa_pra (*Poa pratensis*). Species differed significantly (Table S1) in their β . Deeper rooting species have a higher β , shallower-rooting species have a lower β . Bars indicate mean \pm SE. Letters indicate significant differences between species, indicated by a Tukey HSD post-hoc test.

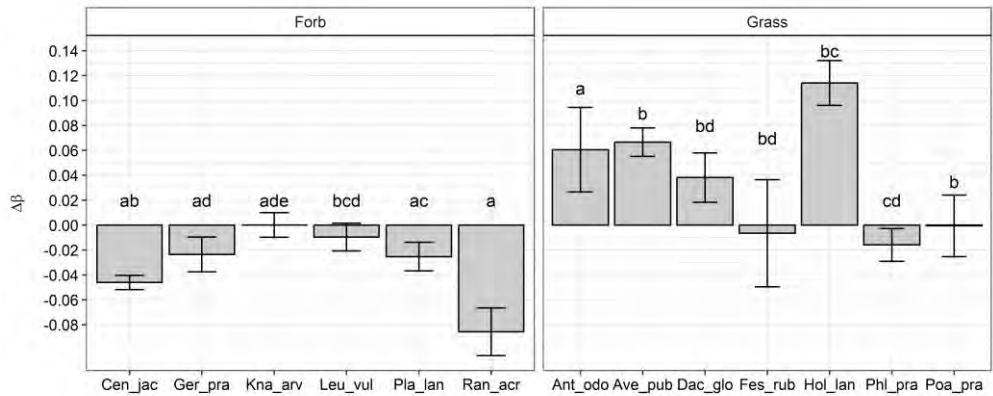


Fig. S3.2: The change in vertical root distribution from monoculture to mixture ($\Delta\beta$). Forbs are indicated in the left panel: Cen_jac (*Centaurea jacea*), Ger_pra (*Geranium pratense*), Kna_arv (*Knautia arvensis*), Leu_vul (*Leucanthemum vulgare*), Pla_lan (*Plantago lanceolata*), and Ran_acr (*Ranunculus acris*). Grasses are indicated in the right panel: Ave_odo (*Anthoxanthum odoratum*), Ave_pub (*Avenula pubescens*), Dac_glo (*Dactylis glomerata*), Fes_rub (*Festuca rubra*), Hol_lan (*Holcus lanatus*), Phl_pra (*Phleum pratense*), and Poa_pra (*Poa pratensis*). Species differed significantly (Table S2) in how they altered their $\Delta\beta$. Bars indicate mean \pm SE. Letters indicate significant differences between species, indicated by a Tukey HSD post-hoc test.

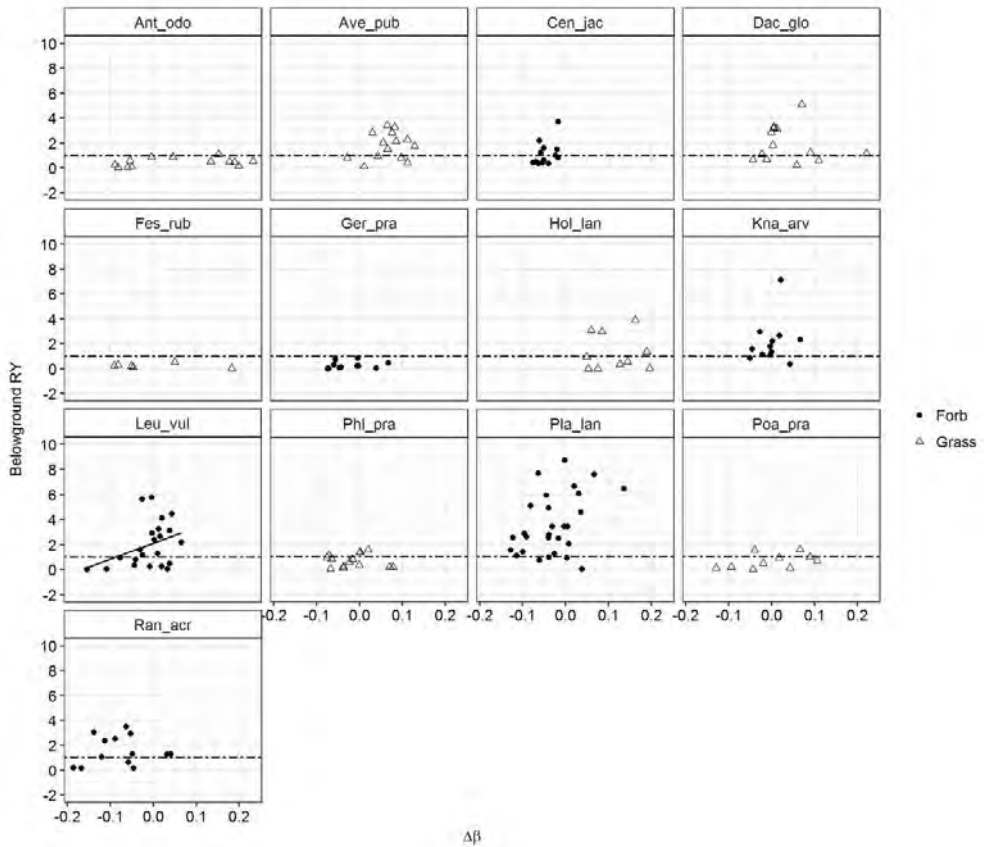


Fig. S3.3. The relation between the change in vertical root distribution from monoculture to mixture ($\Delta\beta$) and species-specific belowground relative yield differed between species. Positive $\Delta\beta$ indicates species allocated more roots to shallow layers in mixtures, a negative $\Delta\beta$ implies species rooted deeper in mixtures. Values above the dotted line ($y = 1$) indicate that belowground relative yield is greater than expected, based on the species' monoculture. Circles and solid lines represent forbs, triangles represent grasses. The regression line for *Leu_vul* indicates a significant ($P < 0.05$) relation. Forb species: *Cen_jac* (*Centaurea jacea*), *Ger_pra* (*Geranium pratense*), *Kna_arv* (*Knautia arvensis*), *Leu_vul* (*Leucanthemum vulgare*), *Pla_lan* (*Plantago lanceolata*), and *Ran_acr* (*Ranunculus acris*). Grass species: *Ave_odo* (*Anthoxanthum odoratum*), *Ave_pub* (*Avenula pubescens*), *Dac_glo* (*Dactylis glomerata*), *Fes_rub* (*Festuca rubra*), *Hol_lan* (*Holcus lanatus*), *Phl_pra* (*Phleum pratense*), and *Poa_pra* (*Poa pratensis*). See Table S3 for statistics.

Table S3.1. ANOVA (type III) results for species-specific vertical root distribution (β) in mixtures, with plant species (species; discrete: $n = 13$, species) as the explanatory variable. A random factor (random = ~ 1 | block/plot) was used to account for spatial variation over the field site, and multiple observations per experimental plot.

Factor	β		
Species	$F_{12,94} =$	15.69	***
P < 0.001 ***, P < 0.01 **, P < 0.05 *, P < 0.10 †, non-significant (ns)			

Table S3.2. ANOVA (type III) summary of the change in vertical root distribution from monoculture to mixture ($\Delta\beta$), with pool (discrete: 1, 2), plant species richness (logSR; log transformed, continuous: 2, 3, 4, 8), plant species (species; discrete: $n = 13$), functional trait diversity (FDJena; continuous: 1, 2, 3, 4), and focal species dissimilarity (continuous) as explanatory variables. Focal species dissimilarity is a measure of the difference of a focal species from its community in terms of β . A random factor (random = ~ 1 | block/plot) was used to account for spatial variation over the field site, and multiple observations per experimental plot.

Factor	$\Delta\beta$		
Pool	$F_{1,69} =$	5.71	*
logSR	$F_{1,69} =$	0.00	ns
Species	$F_{12,91} =$	8.25	***
FDJena	$F_{1,69} =$	0.21	ns
Focal species dissimilarity	$F_{1,91} =$	1.20	ns
logSR: Focal species dissimilarity	$F_{1,91} =$	6.28	*
FDJena: focal species dissimilarity	$F_{1,91} =$	8.45	**
P < 0.001 ***, P < 0.01 **, P < 0.05 *, P < 0.10 †, non-significant (ns)			

Table S3.3. ANOVA (type III) summary of species-specific belowground relative yield with plant species richness (logSR; log transformed, continuous: 2, 3, 4, 8), functional trait diversity (FDJena; continuous: 1, 2, 3, 4), plant species (species; discrete: n = 13), and the change in vertical root distribution from monoculture to mixture ($\Delta\beta$, continuous). Belowground relative yield was log transformed to meet assumptions of normality. A random factor (random = ~1|block/plot) was used to account for spatial variation over the field site and multiple observations per experimental plot.

Factor	Belowground relative yield		
logSR	F _{1,71} =	0.07	ns
Species	F _{12,69} =	0.80	ns
$\Delta\beta$	F _{1,69} =	2.14	ns
logSR: species	F _{12,69} =	1.94	*
Species: $\Delta\beta$	F _{12,69} =	1.76	‡
P < 0.001 ***, P < 0.01 **, P < 0.05 *, P < 0.10 ‡, non-significant (ns)			



Chapter 4 Grass root abundance, not plant species richness, decreases fine root decomposition in an experimental grassland

Natalie J. Oram, Jasper van Ruijven

Abstract

Plant community diversity and composition can influence litter decomposition, the most important process liberating nutrients and governing the soil carbon cycle. As most plant-derived litter in grasslands is belowground, understanding the factors that mediate root litter decomposition are imperative for predicting nutrient and carbon cycling. In the Jena Trait Based Experiment, we used a litterbag experiment to test the effects of plant species richness and functional group composition (grass root abundance) on root decomposition (% mass loss) of plot derived (native) and a standard root litter to determine litter quality and soil environment effects, respectively. Litter mixing effects were determined for native root litter. In addition, we tested if root traits could explain the effects of plant species richness or grass root abundance on root decomposition via changes in litter quality. Plant species richness did not affect root decomposition via changes in litter quality, the soil environment, or litter mixing effects. Litter mixing effects did not differ from zero, indicating that there was no effect of combining litter of multiple species on decomposition. Increasing grass root abundance led to a decrease in root decomposition via changes in litter quality, and a weak increase in root decomposition via the soil environment. Grass root abundance was positively related to litter mixing effects. The negative effect of grass root abundance on root decomposition via reductions in litter quality was captured completely by shifts in specific root length, and partially by root diameter. Root diameter, but not specific root length, explained additional variation in root decomposition, apart from the effects of grass abundance. Our results show plant functional group composition can affect root decomposition in grasslands via shifts in litter quality. Further analyses suggest this effect can be captured by two morphological root traits: specific root length and root diameter.

Introduction

Decomposition of plant litter is an important process providing nitrogen (N) and phosphorus (P) to plants (Hättenschwiler et al. 2005), and regulating net soil carbon storage (De Deyn, Cornelissen & Bardgett 2008). In grasslands, roots account for the majority of plant biomass (Poorter et al. 2012), and have an annual turnover of up to 53% (Gill & Jackson 2000). Therefore, identifying the factors which influence root decomposition is essential to predict carbon and nutrient cycling. Decomposition rate is determined by the abiotic environment, the activity and composition of the decomposer community, and the chemical and structural quality of the litter (Swift et al. 1979; Aerts 1997; Parton et al. 2007; Cornwell et al. 2008; Srivastava et al. 2009). Plant species richness (SR) has been shown to influence all of these factors (Hättenschwiler et al. 2005; Eisenhauer et al. 2011a; Chen et al. 2017), and thus, could alter root litter decomposition via changes in the soil environment, litter quality, or litter mixing effects.

Changes in the soil abiotic and biotic environment are mainly linked to SR of the living plant community. For example, greater SR of the plant community can reduce soil surface temperature (Rosenkranz et al. 2012), increase soil water exploitation (Caldeira et al. 2001; Verheyen et al. 2008), decomposer abundance and activity (Eisenhauer et al. 2011a), microbial biomass (Eisenhauer et al. 2010) and microbial activity (Lange et al. 2015). The rate of litter decomposition responds to changes in these abiotic (Trofymow et al. 2002; Powers et al. 2009) and biotic (Hättenschwiler & Gasser 2005) environmental factors. However, SR-induced environmental effects on decomposition are inconsistent. Increasing SR of the plant community has been reported to increase (Cong et al. 2015b), decrease (Fornara et al. 2009; Chen et al. 2017), or not affect (Scherer-Lorenzen 2008) the decomposition of a standard litter incubated in the soil.

In addition, SR of the litter itself could alter litter decomposition via changes in litter quality, the chemical and structural traits of the litter. Changes in litter quality through SR can happen in two ways. First, through shifts in plant species or functional group dominance across a SR gradient. Shifts in species abundance can have a large influence on decomposition as plant species can vary greatly in their traits (Hättenschwiler & Gasser 2005; Vivanco & Austin 2006), which are the major predictor of decomposition globally (Cornwell et al. 2008). At the Jena Experiment, a long-term field biodiversity

experiment (see Roscher et al. 2004), SR had a negative effect on root decomposition due to an increase in the abundance of grass species, and the associated increase in root litter carbon: nitrogen (C:N) ratio, in more diverse plots (Chen et al. 2017). Similarly, at the Cedar Creek Biodiversity Experiment, Fornara et al. (2009) found that the presence of grass species was negatively related to root decomposition, due to low root litter N concentrations and lignin: N ratio.

The second way SR of the litter can influence decomposition via litter quality is through litter mixing effects, the effects of mixing litters of multiple species on decomposition. In natural environments, plant litter decomposes in multi-species mixtures, rather than individually. Mixtures of multiple species can decompose at a different rate than expected, based on the decomposition of the composite species individually. Interactions between the different litter types may increase or decrease decomposition, leading to non-additive litter mixing effects (Gartner & Cardon 2004; Jonsson & Wardle 2008). Nutrient transfer between litters of different qualities could stimulate decomposition by increasing resource complementarity between decomposers (Gessner et al. 2010), resulting in positive effects of litter diversity on decomposition, i.e. positive litter mixing effects (Wardle, Bonner & Nicholson 1997; Handa et al. 2014). However, empirical support for litter mixing effects is inconsistent. In a review by Gartner & Cardon (2004), mixing leaf litter from multiple species resulted in positive (47% of mixtures), neutral (33% of mixtures) or negative (19% of mixtures) litter mixing effects. For root litter, only three studies have investigated mixing effects, which found positive (Robinson et al. 1999; de Graaff et al. 2011) and non-significant litter mixing effects (Cong et al. 2015b). Litter traits may be better predictors of decomposition than SR per se (Reiss et al. 2009). The composition and diversity of chemical compounds in litter mixtures have been shown to control decomposition processes such as soil respiration and N mineralization to a greater extent than measures of plant diversity (Meier & Bowman 2008). As functional groups differ in their traits, shifts in the dominance of plant functional groups over a SR gradient may better explain decomposition than SR (Lindedam et al. 2009; Fornara et al. 2009). The variation in effects of SR on root decomposition indicates that a more complete understanding of the factors underlying this relation are necessary to

predict how SR influences decomposition, as well as carbon and nutrient cycling in grasslands.

In the present study we addressed how SR and functional group composition (grass abundance) influence fine root decomposition in the Jena Trait Based Experiment (TBE, Ebeling et al. 2014). We tested if SR and/or grass abundance influence root decomposition via changes in soil environment or litter quality by assessing the mass loss of two litter types. First, we tested the effects of SR and grass abundance on the decomposition of 46 different plot-derived (native) root litters, each decomposing in its own plot. These effects may be due to variation in litter quality between plots, but could also be due to differences in the soil environment related to SR or grass abundance of the plant community. To test for the latter, we also determined the effect of SR and grass abundance on the decomposition of a standard litter, an approach used previously by Scherer-Lorenzen (2008), Vogel et al. (2013) and Chen et al., (2017). However, it must be noted that litter quality and the soil environment may also interact to affect litter decomposition (e.g. Aerts, 1997; Zhang et al. 2008). Detecting such interactive effects would require a factorial design, in which each native litter is decomposed in each plot. Unfortunately, this was not feasible due to logistic constraints (i.e. the limited amount of root litter collected and the limited amount of space available within plots to bury litter bags). The potential implications of these interactive effects for our conclusions will be addressed in the discussion.

Grass abundance was based on root biomass determined in Chapter 2 with molecular techniques. This enabled us to determine the relative abundance of grass roots growing in the plot and present in the native root litter, and relate this to changes in decomposition of native root litter and standard root litter. By quantifying species-specific relative abundance of the native root litter (Chapter 2), we were also able to calculate litter mixing effects of the native root litter, and test if SR or grass root abundance altered mass loss via litter mixing effects. Finally, we test if root traits, i.e. root litter quality, can explain the effects of SR and/or grass root abundance on native root litter decomposition. We hypothesize that:

1. SR will alter root decomposition through changes in litter quality, the soil environment, and litter mixing effects.

2. Higher grass abundance will reduce root decomposition via reductions in litter quality.
3. The effects of SR and grass root abundance on root decomposition via litter quality can be explained by changes in root traits.

Materials and Methods

This study was carried out at the Jena Trait-Based Experiment (TBE), described in Ebeling et al. (2014). Briefly, the Jena TBE is located near Jena, Germany (50.95 °N 11.62 °E), on the floodplains of the river Saale (130 m above sea level), parallel to the Jena Experiment (Roscher et al. 2004). The soil type is sandy loam (40% sand, 44% silt, 16% clay) (Steinbeiss et al. 2008b). Plots (12.25 m², n = 138) were assembled into three spatial blocks following a gradient of soil characteristics, were sown in spring 2011. Every year, experimental plots are mown in June and September and weeded in April, July, and October to maintain target plant community composition. The Jena TBE consists of three ‘pools’ of eight species, selected from the original species of the Jena Experiment (Roscher et al. 2004). Each pool of species follows a gradient of plant species richness (SR; 1, 2, 3, 4, and 8 species), and functional trait diversity (FDJena). Due to limitations in time and human resources, we used a subset of plots, pool 1 (n = 46). Since the traits used to calculate FDJena are not predominant factors influencing root litter decomposition, and we found no effect of FDJena, on fine root decomposition, we consider only the SR gradient. Pool 1 includes four grasses - *Avenula pubescens*, *Festuca rubra*, *Phleum pratense*, and *Poa pratensis* - and four non-leguminous forbs- *Centaurea jacea*, *Knautia arvensis*, *Leucanthemum vulgare*, and *Plantago lanceolata* present in monocultures (n=8, one monoculture of each species), and in mixtures of 2 (n=16), 3 (n=12), and 4 (n=9) and 8 (n=1) species.

Our aim was to determine if and how SR and grass root abundance altered root decomposition, and if this was via changes in root litter quality or the soil environment. We used two types of litter to elucidate these pathways. To determine litter quality effects, we placed litterbags containing plot specific (native) fine root litter in each plot, i.e. each plot contained litterbags with roots that originated from that plot. To determine soil environment effects, we placed litterbags containing a standard fine root litter in

every plot (*Lolium perenne*, which is not present in the Jena TBE). Design of the root decomposition experiment

Collection of root material

Root standing biomass of the Jena TBE was harvested in 2014 (chapter 2, this thesis) by taking eight root cores (4 cm diameter, 40 cm deep) per plot, dividing into five depths (0–5, 5–10, 10–20, 20–30 and 30–40 cm), pooling by depth, and storing at 4°C until washing over a 0.5 mm sieve. After washing, a subsample of roots was taken to determine the relative abundance of species-specific root biomass using molecular identification (RT-qPCR) (Mommer et al. 2008). Quantifying the relative abundance of species-specific root biomass in mixtures allows us to determine grass root abundance (the relative abundance of grass roots), and calculate the community weighted mean (CWM) of root traits and litter-mixing effects. The rest of the root biomass was dried at 65 °C for at least 48 hours, weighed, and stored in a dry location until the native root litterbags were prepared. Standard litter comprised of roots of hydroponically grown *L. perenne*, see Chen et al. 2017 for further details.

Preparation of the litterbags

Litterbags (8 · 8 cm) were made with 325 µm nylon mesh (Top Zeven, Haarlem, the Netherlands), and sewn on three sides with polyester thread. As the litterbags were previously used, they were cleaned by soaking in 70% ethanol for 12 hours, rinsed three times with tap water, and dried at 70 °C for 5 hours. Each litterbag was placed inside a paper envelope to collect small root fragments that escaped through the mesh before the litterbags were placed in the soil. To have enough root litter to fill the litterbags, dry roots from the entire plot (i.e. all five layers from 0-40 cm) was mixed homogenously and re-dried overnight at 70 °C, and cooled in a desiccator. Approximately 0.25 g ($\pm 10\%$) of fine roots (< 2 mm) were weighed into each litterbag, which was sealed with a hot press (Impulse Sealer, AIE-200, American International Electric). Three replicated litterbags for each litter type were connected with polyester thread. In the field, litterbags were placed as in (Chen et al. 2017). A vertical cut in the soil 11 cm deep was made with a spade; the litterbag was vertically placed in the hole, so the top of the litterbag was 1 cm below the soil surface. Litterbags were placed at this depth as most the root biomass is in the top

~10 cm of the soil profile (chapter 2, this thesis). Litterbags were 10 cm apart to reduce the potential effects of a neighboring litterbag. Roots that had escaped through the mesh during transport into the envelope were weighed, and this weight was subtracted from the initial weight.

Litterbag collection and processing

After 95 days, litter bags were collected, placed in individual plastic bags, and stored at 4°C until further processing, which took place within a maximum of 7 days. Litterbags that were damaged in the field, e.g. by mice, were excluded from the analysis as most of the root litter fell out during incubation and removal. All damaged litterbags were 'standard' root litter from plots 27 (n=1, SR = 1, *C. jacea*), 42 (n = 1, SR= 2, *C. jacea* and *K. arvis*), and 84 (n = 1, SR = 3, *A. pubescens*, *F. rubra*, and *L. vulgare*). This left two replicate standard litterbags in plot 27, 42, and 84. Adherent soil was carefully removed by rinsing with minimal water to minimise the loss of dissolvable carbon. Fresh roots (identified as white, turgid roots) which had grown into the litterbags were carefully removed using tweezers. After cleaning, litterbags were placed in paper envelopes to reduce the risk of losing roots in the oven, and dried at 70°C. Once dry, all material in the litterbag was removed and weighed. To correct for adherent soil particles, samples were combusted at 550°C for 3 hours in a muffle furnace according to Ball (1964) and Houba, van der Lee & Novozamsky (1997), and the organic matter (OM) fraction of the samples was determined. We did not account for soil organic matter (SOM) which could be present in the soil particles adhered to the root, as SOM is expected to remain constant over the experiment, and including the small fraction of SOM in the OM root fraction was preferable to including mineral contamination. To account for the initial OM and mineral fractions of the roots, standard roots (3 representative samples) and one sample of native roots from each plot that were not placed in the litterbag experiment were also combusted per the same procedure. No initial material remained for the monoculture plot of *P. lanceolata*. Therefore, the mean initial OM fractions of the three other forb monocultures (mean =84.0 %, range = 80.0 - 89.7 %) were used to derive the initial OM fraction in the *P. lanceolata* monoculture. Calculating the mass loss of the *P. lanceolata*

monoculture using the initial OM content of any of the individual forb monocultures did not alter statistical outcomes.

Mass loss was calculated as the % of organic matter decomposed after 95 days:

$$\% \text{ mass loss OM} = \left[\frac{(\text{Initial OM} - \text{Final OM})}{\text{Initial OM}} \right] \cdot 100$$

Where OM is root organic matter (g). The calculated % mass loss of native and standard litter were pooled per plot (i.e. the mass loss % per plot is the mean mass loss % of the replicate litterbags, $n = 3$ when enough material was available).

Litter mixing effects

Litter mixing effects were calculated as follows:

$$\text{Litter mixing effects} = \frac{\text{Observed mass loss}}{\sum_{i=1}^s (w_i \cdot m_i)}$$

Observed mass loss (%) is the mass loss of native root litter of a mixture (SR 2-8) decomposing in its own plot. The denominator is the expected mass loss (%) based on the native root litter mass loss of the composite species decomposing in monoculture; w_i is the relative abundance of species i based on species specific root biomass (g m^{-2}) per plot in the litter mixture, and m_i is the native root litter mass loss (%) of species i in monoculture.

Root litter quality

To assess whether root traits of individual species explain patterns in root decomposition (% mass loss) of plant mixtures, the CMW (Garnier et al. 2004) of each root trait was calculated for each mixture by combining species-specific relative abundance data (chapter 2) with an independent estimates of species-specific root traits. An independent estimate of root traits was used for two reasons: first, root traits cannot be measured in mixtures as physically separating the roots of different species is impossible; second, connecting an independent measure of root traits to root decomposition in the field tests whether standardized trait measures can be connected to an ecosystem process. We considered root traits which have been shown to influence root decomposition. We included three root chemical traits: root nitrogen (root N, %), carbon (root C, %), and root C:N (g g^{-1}). Root litter high in N has been reported to decompose faster than root litter

with low N concentrations (Silver & Miya 2001; Zhang et al. 2008; Prieto, Stokes & Roumet 2016). Root C and root C:N have been found to negatively relate to root litter decomposition (Silver & Miya 2001 and Chen et al. 2017- root C:N ratio; Prieto et al. 2016- root C content). Morphological traits were included, as specific root length (SRL, m g⁻¹) and specific root area (SRA, m² g⁻¹) have been shown to negatively relate to root litter mass loss (Hobbie et al. 2010- SRL; Smith et al. 2014- SRA). Root diameter (RD, mm) has been found to positively relate to root decomposition initially (Hobbie et al. 2010). The influence of root tissue density (RTD, g cm⁻³) was considered as it has been shown to influence grass root lifespan (Ryser 1996), and factors which affect root lifespan may also affect root decomposition. Complete details of the pot experiment can be found in (Schroeder-Georgi et al. 2015). Briefly, plant species were grown in mesocosms (15 cm diameter, 60 cm length) with a mixture of field soil from same location as the Jena TBE and sand in a 5:1 ratio for 12 weeks. Mesocosms were kept outside, and watered equally during dry periods. Root morphological traits were obtained by scanning fresh roots on a flatbed scanner followed by analysis with WinRhizo (Regent Instruments Inc., Canada). Scanned roots were dried for 48 h at 70 °C for calculations. Root N and C were analysed using a EA-IRMS (Delta V, Thermofisher). The CWM of root traits was calculated as follows:

$$CWM = \sum_{i=1}^s w_i \cdot x_i$$

Where w_i is the relative abundance of root biomass (g m⁻²) of species i , and x_i is the species-specific trait value for species i .

Statistical analysis

We took a three-step approach to our statistical analysis. All statistical analyses were performed using R 3.3.0 (R Development Core Team 2016).

1. The effects of SR and grass root abundance on root decomposition

To assess effects on root decomposition, we used linear mixed effects models, `lme{nlme}` (Pinheiro et al. 2016) with SR (continuous: 1, 2, 3, 4, 8), grass root abundance (continuous) and litter type (discrete: native or standard) as explanatory variables, and the interaction between SR or grass root abundance and litter type. A random term, `random =`

~1|block/plot, was included to account for spatial variation across the field site (block: discrete, 1, 2, 3), and standard and native root litter decomposing in the same plot (plot: discrete, n = 46).

Litter type and other explanatory variables significantly interacted, therefore, we tested the effects of SR and grass root abundance on native or standard litter separately, with block as a random factor. The effects of SR and grass root abundance on litter mixing effects were tested in the same way. Litter mixing effects were log transformed to meet assumptions of normality. All models were analysed for significant effects using ANOVA with type I SS (sum of squares) using `anova {stats}`. The Akaike Information Criterion (AIC) is presented from the model summary for the litter quality model, to facilitate comparison between this model and the models which include root traits (below). Marginal R^2 were calculated using the function `r.squaredGLMM {MuMIn}` (Barton 2016). A marginal R^2 describes the variance explained by the fixed factors in a model (Nakagawa & Schielzeth 2013). AIC and marginal R^2 values were derived from models fit by maximum likelihood (ML) to facilitate comparison between models; all other model parameters presented are derived from models fit with restricted maximum likelihood (REML), which reduces bias caused by maximum likelihood by accounting for degrees of freedom lost from estimating fixed effects (Zuur et al. 2009; Nakagawa & Schielzeth 2013).

Overall litter mixing effects were tested with a one-sided t-test, `t.test{stats}`.

2. The effects of root traits on native root decomposition, and grass root abundance on root traits

Linear mixed effects models were used to test the effect of root traits (CWM, continuous) on native root decomposition. Block was included as the random factor. Significant effects were determined with ANOVA type I SS. AIC and marginal R^2 were derived for each model as above. As root traits were correlated (Fig. S1), they were considered in separate models to avoid collinearity.

The effect of grass root abundance (continuous) on root traits was tested, with block as a random factor. The effect of root traits on native root decomposition in monoculture (n = 8) were tested in the same way to exclude the effect of changes in species composition in mixtures. Root C and root N were log transformed.

The difference in root traits and native root decomposition between grasses and forbs in monoculture was tested using a linear model, $\text{lm}\{\text{stats}\}$, with functional group (discrete: grass or forb) as the explanatory variable.

3. Linking grass root abundance to root decomposition via root traits

To determine if root traits could capture the effect of grass root abundance on root decomposition via litter quality, and to test if root traits explained additional variation in root decomposition, we used a linear model, $\text{lm}\{\text{stats}\}$ with a root trait (CWM, continuous) and grass root abundance (continuous) as explanatory variables. We used a type I SS (sequential) ANOVA to determine significance, and alternated the order in which the root trait and grass root abundance appeared in the model. Variables significant in the later position in the model explain unique or more variation in native root decomposition. This analysis tests if root trait(s) capture the litter quality effect of grass root abundance on root decomposition.

Results

Grass root abundance, not plant species richness, influenced decomposition and litter mixing effects

Plant species richness (SR) did not affect native or standard root litter decomposition (Fig. 4.1 A, C; Table 4.2), or litter mixing effects (Fig. 4.1 E, Table 4.2). Overall, we did not find evidence for litter mixing effects; native root decomposition in mixtures was not significantly different than expected, based on the root decomposition of the species in monoculture ($t_{37} = 0.14$, $P > 0.05$).

Grass root abundance significantly affected root decomposition, and its effect was stronger on native than standard root decomposition (grass root abundance: litter type interaction, Table 4.1). Grass root abundance significantly reduced native root decomposition (Fig. 4.1 B, Table 4.2), and increased standard root decomposition (Fig. 4.1 D, Table 4.2). Grass root abundance had a positive effect on root litter mixing effects (Fig. 4.1 F, Table 4.2).

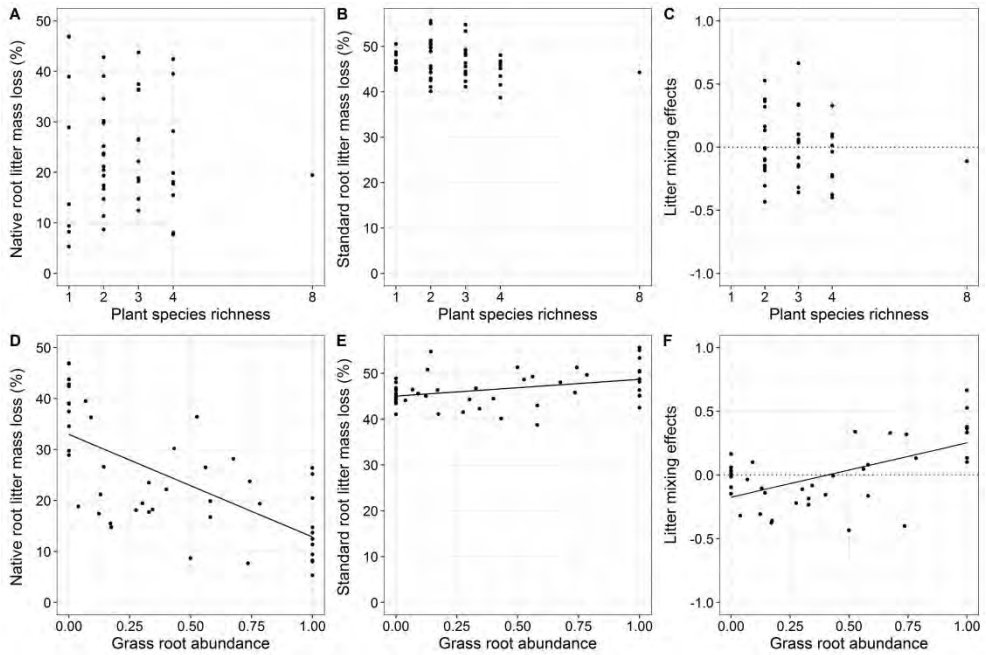


Fig. 4.1. Plant species richness did not affect native root litter mass loss (litter quality effect, fig A) and standard root litter mass loss (soil environment effect, fig B), but grass root abundance did: native root litter mass loss significantly decreased with increasing grass abundance (litter quality effect, fig D) and that of standard root litter mass loss increased (soil environment effect, fig E). Litter mixing effects (the deviation of observed native root decomposition in mixtures from expected based on the decomposition of the composite species in monoculture) were not affected by species richness (fig C), but significantly increased with grass abundance (fig F). Litter mixing effects greater than zero (indicated with a dotted line) indicate that the observed mass loss of the mixture was greater than expected. See Tables 1 and 2 for statistics.

Table 4.1. ANOVA (type I) summary of the effects of plant species richness (SR: continuous 1, 2, 3, 4, 8), grass root abundance (continuous) and litter type (discrete: standard or native) on root decomposition (% root litter mass loss). A random factor (random = 1 | block/plot) was included to account for spatial variation across the field site, and multiple litterbags per plot.

	Root decomposition		
SR	$F_{1,41} =$	1.11	ns
Grass root abundance	$F_{1,41} =$	21.27	***
Litter type	$F_{1,42} =$	275.65	***
SR: litter type	$F_{1,42} =$	0.00	ns
Grass root abundance: litter type	$F_{1,42} =$	47.33	***
P < 0.001 ***, P < 0.01 **, P < 0.05 *, P < 0.10 ‡, non-significant (ns)			

Table 4.2. ANOVA (type I) summary of the effects of plant species richness (SR: continuous 1, 2, 3, 4, 8) and grass root abundance (continuous) on root decomposition (% root litter mass loss) via changes in litter quality (native root decomposition), the soil environment (standard root decomposition), or litter mixing effects. Akaike Information Criterion (AIC) for the litter quality model is presented. Marginal R^2 was calculated for each model. A random factor (random = 1 | block) was included to account for spatial variation across the field site.

	Root decomposition effects (litter quality)			Root decomposition effects (soil environment)			Litter mixing effects		
SR	$F_{1,41} =$	0.38	ns	$F_{1,40} =$	2.55	ns	$F_{1,33} =$	1.29	ns
Grass root abundance	$F_{1,41} =$	39.47	***	$F_{1,40} =$	5.79	*	$F_{1,33} =$	20.10	***
AIC, R^2	334.99, 0.49			252.35, 0.17			-0.63, 0.37		
P < 0.001 ***, P < 0.01 **, P < 0.05 *, P < 0.10 ‡, non-significant (ns)									

Root traits relate to root decomposition and grass root abundance

The community weighted mean (CWM) of all root traits were significantly related to native root litter decomposition (Fig. 4.2, Table 4.3). Native root decomposition increased with increasing root nitrogen (root N, %), root carbon (root C %), root tissue density

(RTD, g cm^{-3}), and root diameter (RD, mm), whereas it decreased with increasing root C:N ratio (g g^{-1}), specific root length (SRL, m g^{-1}), and specific root area (SRA, $\text{m}^2 \text{g}^{-1}$) (Fig. 4.3, Table 4.3). We found that models including RD or SRL best explained root decomposition based on the Akaike Information Criterion (AIC) (Table 3), explaining slightly less variation in root decomposition as the grass root abundance model, based on marginal R^2 (Table 4.2).

All root traits were also significantly related to grass root abundance (Fig. 4.3, Table 4.3). In monoculture, grasses and forbs differed significantly in the root traits RD, SRL, N, and C:N ratio and in native root decomposition (Fig. S4.2, Table S4.1). When grass abundance and a trait were included in a type I SS ANOVA, the significant effect of most traits on native root decomposition disappeared when the effect grass root abundance was considered first; whereas the effect of grass abundance on root decomposition remained significant when included second (Table 4.4). However, there were two exceptions: when SRL was included first the significant effect of grass root abundance disappeared, and vice versa (Table 4.4). RD, on the other hand, remained significant when it was considered after grass root abundance (Table 4.4).

Chapter 4

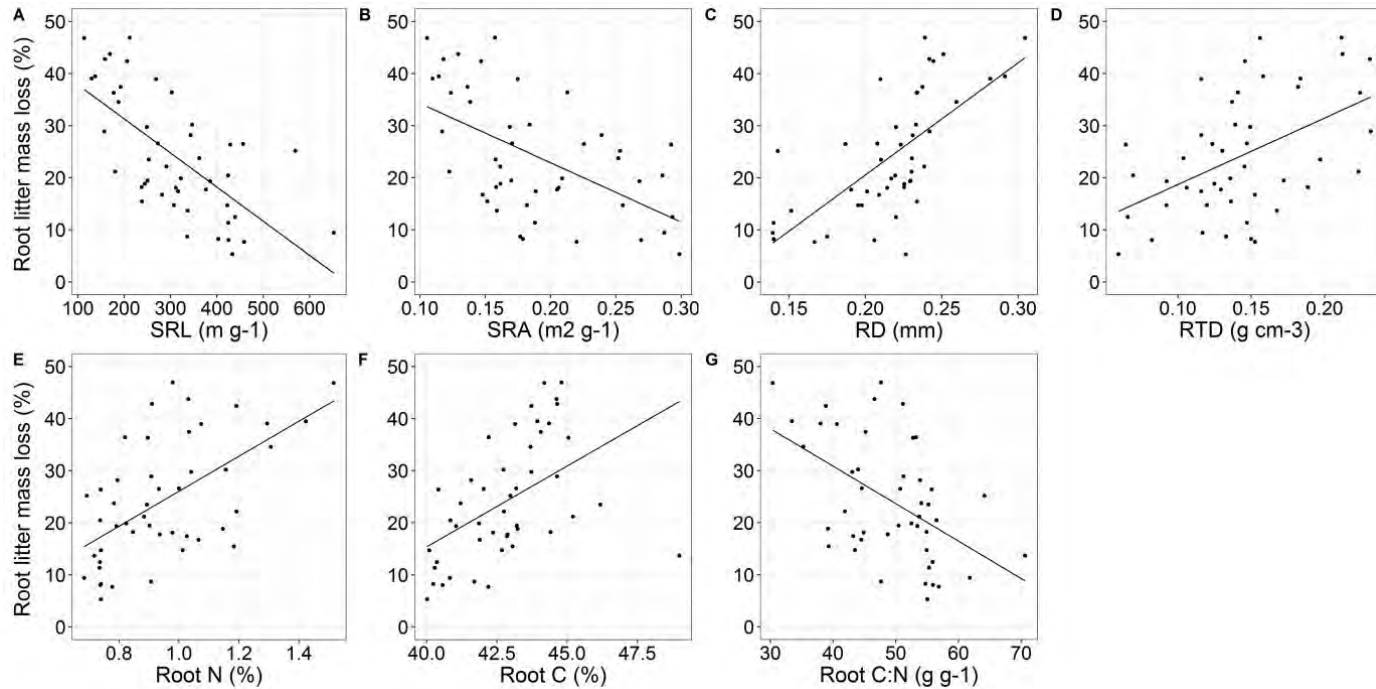


Fig. 4.2. Relationships between community weighted mean root traits and native root decomposition (root litter mass loss, %). Root traits included were: A) specific root length (SRL), B) specific root area (SRA), C) root diameter (RD), D) root tissue density (RTD), E) root nitrogen content (root N) F) root carbon content (root C), and G) root carbon to nitrogen ratio (root C:N). See Table 4.3 for statistics.

Grass root abundance decreases fine root decomposition

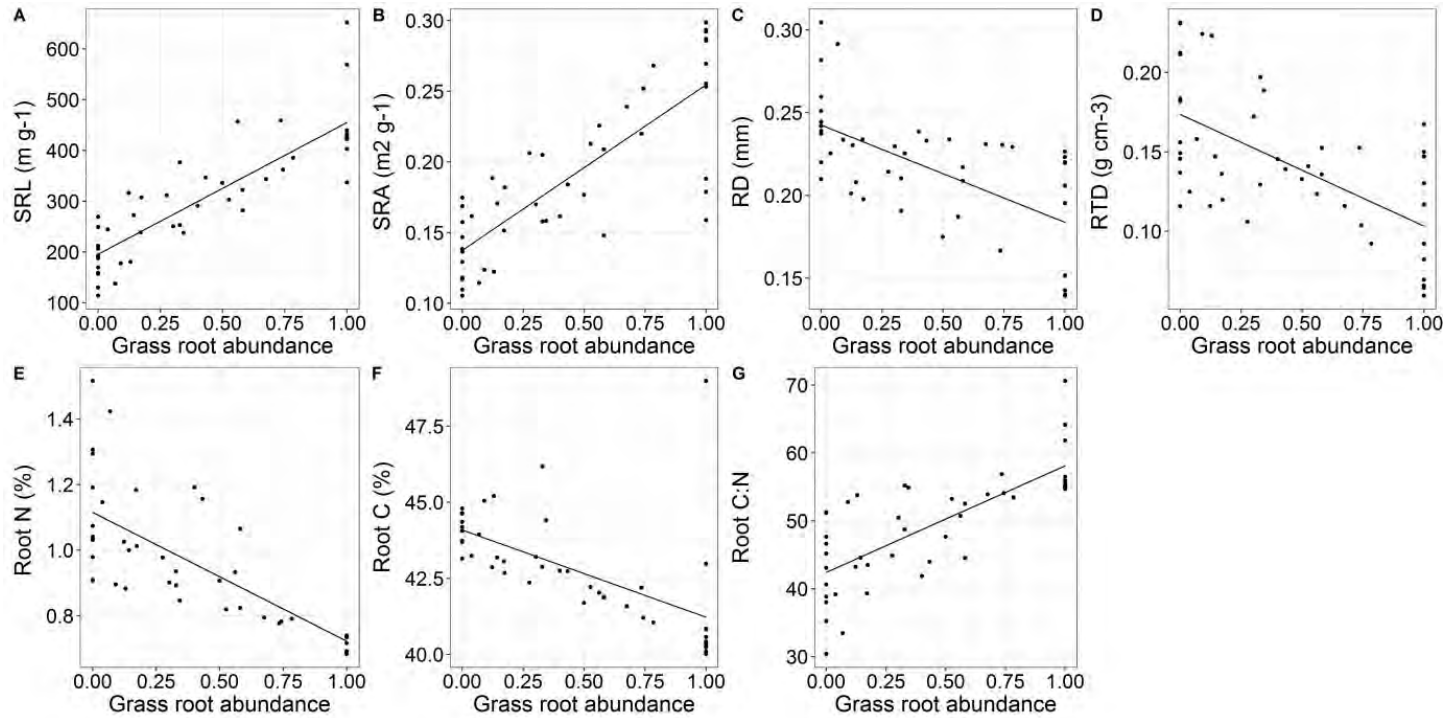


Fig.4.3. Relationships between grass root abundance and community weighted mean root traits. Root traits included were: A) specific root length (SRL), B) specific root area (SRA), C) root diameter (RD), D) root tissue density (RTD), E) root nitrogen content (root N) F) root carbon content (root C), and G) root carbon to nitrogen ratio (root C:N). See Table 4.3 for statistics.

Table 4.3. ANOVA (type I) summary of the effect of root traits (community weighted mean) and root decomposition (% native root litter mass loss), and the relationship between grass root abundance (GRA) and root traits. Root traits included were: root diameter (RD), specific root length (SRL), root nitrogen content (Root N), specific root area (SRA), root carbon to nitrogen ratio (Root C:N), root carbon content (Root C), and root tissue density (RTD). Marginal R^2 and Akaike Information Criterion (AIC) for each model are presented; lower AIC values imply a better-fit model. Δ AIC indicates the difference from the best-fit (lowest AIC) trait model. Marginal R^2 and AIC are based on maximum likelihood models to facilitate comparison. A random factor (random = 1|block) was included to account for spatial variation across the field site. $P < 0.001$ is indicated by ***.

Effect of root traits on root decomposition							Effect of GRA on root traits		
				R^2	AIC	Δ AIC			
RD (mm)	$F_{1,42} =$	37.84	***	0.47	334.26	0	$F_{1,42} =$	28.38	***
SRL ($m\ g^{-1}$)	$F_{1,42} =$	34.56	***	0.45	335.90	1.64	$F_{1,42} =$	124.77	***
Root N (%)	$F_{1,42} =$	22.82	***	0.35	343.47	9.21	$F_{1,42} =$	83.41	***
SRA									
($m^2\ g^{-1}$)	$F_{1,42} =$	18.54	***	0.31	346.40	12.14	$F_{1,42} =$	87.64	***
Root C: N									
($g\ g^{-1}$)	$F_{1,42} =$	14.72	***	0.26	349.37	15.11	$F_{1,42} =$	56.54	***
Root C (%)	$F_{1,42} =$	14.77	***	0.25	349.39	15.13	$F_{1,42} =$	29.60	***
RTD (g									
cm^{-3})	$F_{1,42} =$	13.47	***	0.24	350.37	16.11	$F_{1,42} =$	27.21	***

Table 4.4. ANOVA (type I) summary of the effect of community weighted mean root traits on root decomposition (% native root litter mass loss) when considered before (1st position) or after (2nd position) grass root abundance. Root traits included were: root diameter (RD), specific root length (SRL), root nitrogen content (Root N), specific root area (SRA), root carbon to nitrogen ratio (Root C:N), root carbon content (Root C), and root tissue density (RTD).

	Root decomposition		Root decomposition	
	Root trait (1 st position)	Grass root abundance (2 nd position)	Grass root abundance (1 st position)	Root trait (2 nd position)
RD (mm)	F _{1,43} = 46.69 ***	F _{1,43} = 10.41 **	F _{1,43} = 46.16 ***	F _{1,43} = 10.94 **
SRL (m g ⁻¹)	F _{1,43} = 37.57 ***	F _{1,43} = 3.51 ‡	F _{1,43} = 38.77 ***	F _{1,43} = 2.31 ns
Root N (%)	F _{1,43} = 27.59 ***	F _{1,43} = 10.69 ***	F _{1,43} = 37.48 ***	F _{1,43} = 0.80 ns
SRA (m ² g ⁻¹)	F _{1,43} = 24.31 ***	F _{1,43} = 12.49 ***	F _{1,43} = 36.80 ***	F _{1,43} = 0.00 ns
Root C: N (g g ⁻¹)	F _{1,43} = 20.44 ***	F _{1,43} = 16.37 ***	F _{1,43} = 36.81 ***	F _{1,43} = 0.01 ns
Root C (%)	F _{1,43} = 19.50 ***	F _{1,43} = 18.63 ***	F _{1,43} = 37.41 ***	F _{1,43} = 0.71 ns
RTD (g cm ⁻³)	F _{1,43} = 19.45 ***	F _{1,43} = 18.55 ***	F _{1,43} = 37.35 ***	F _{1,43} = 0.43 ns

P < 0.001 ***, P < 0.01 **, P < 0.05 *, P < 0.10 ‡, non-significant (ns)

Discussion

Our study highlights the importance of functional group composition (grass root abundance) in explaining patterns in root decomposition. Plant species richness (SR) did not affect the decomposition of native or standard root litter, suggesting that SR did not influence the root litter quality or the soil environmental controls of root decomposition. Grass root abundance had a strong negative effect on the decomposition of native root litter, and a marginally positive effect on decomposition of standard litter. As the negative effect of grass root abundance on native root litter was much larger and in the opposite direction compared to the positive effect on the standard litter, this suggests

that grass root abundance reduced native root litter decomposition predominantly via reductions in root litter quality.

However, we cannot rule out that interactions between litter quality and soil environment affected decomposition of native root litter. Limitations in the experimental design do not allow the conclusion that this effect was solely due to changes in litter quality to be made (see below). Our trait analyses revealed that decomposition of native root litter was most closely linked to shifts in specific root length (SRL) and root diameter (RD). SRL captured the effect of grass root abundance on root decomposition completely, but did not explain additional variation in root decomposition. In contrast, RD did not fully capture the effect of grass root abundance on decomposition, but did explain additional variation in decomposition that was not explained by grass root abundance.

Plant species richness does not affect root decomposition

Our study did not find any links between SR and root decomposition, rejecting our first hypothesis. This finding is in line with Scherer-Lorenzen (2008) who found no effect of SR on leaf litter decomposition, but in contrast to Chen et al. (2017) who found a negative effect of SR on root decomposition via decreases in litter quality and changes in the soil environment. Chen et al. (2017) attributed the negative effect of SR on decomposition via litter quality to an increase in grass presence over the SR gradient. Here, we did not find a relationship between SR and grass root abundance, which may be why our findings differed from that of Chen et al. (2017). In general, the effects of SR on decomposition via the soil environment are inconsistent, with positive (Hector et al. 2000), negative (Knops et al. 2001), or non-significant (Milcu et al. 2008) effects. In contrast to our study, Chen et al. (2017) found a weak negative effect of SR on root decomposition via the soil environment in another biodiversity experiment in Jena. This discrepancy may be due to the relation between SR and productivity, which was stronger in the Jena Experiment (Ravenek et al. 2014) considered in Chen et al. (2017) than in the Jena Trait-Based Experiment (TBE), used here (Chapter 2). The weaker diversity-productivity relationship may have resulted in smaller differences in the soil environment over the SR gradient, including temperature and/or moisture and subsequent effects on decomposer community activity.

Grass root abundance affects native and standard root decomposition

Increasing grass root abundance reduced native root decomposition, confirming our second hypothesis. The presence or abundance of grasses has been found to reduce root decomposition via reductions in root litter quality (Fornara et al. 2009; Birouste et al. 2011; Roumet et al. 2016; Chen et al. 2017). Increasing grass root abundance led to a small increase (marginal $R^2 = 0.17$) in root decomposition of the grass roots used as the standard litter via changes in the soil environment, in line with Chen et al. (2017), but in contrast to (Scherer-Lorenzen 2008). This difference could have been caused by the differences in standard root litter used in these studies. Scherer-Lorenzen (2008) used cotton wool as a standard substrate, whereas in Chen et al. (2017) and the present study, roots of *Lolium perenne* were used. Although this species was not present in our experiment, microbial decomposer community in grass-rich plots may be better suited to decomposing grass than forb root litter. Plant roots have been shown to decompose faster in home than away environments (Wang 2016), perhaps due to differences in decomposer communities (Ayres et al. 2009; Freschet, Aerts & Cornelissen 2012b), which were better suited to decomposing grass than forb root litter. Studies which consider the mechanisms underlying the relations between plant community composition (e.g. plant diversity or functional group composition) and the abiotic and biotic soil environment, for example canopy structure (Spehn et al. 2005), complementary water use (Verheyen et al. 2008) or the diversity of soil meso- and macro- fauna (Eisenhauer et al. 2011a), will improve predictions of how SR influences root decomposition via the soil environment.

It must be noted that our results must be interpreted with caution, as changes in native root litter decomposition may also be due to interactions between litter quality and soil environment. These interactive effects can explain variation in litter decomposition (Hättenschwiler et al. 2005). To disentangle the effects of plant community composition via litter quality and decomposition environment, each native litter should be decomposed in each soil environment (e.g. Chen et al. 2017). In the current experiment, this was not possible due to the limited amount of root litter which could be collected, and therefore, it cannot be concluded that the negative effect of grass root abundance on the decomposition of native root litter are caused solely by changes in litter quality. On

the other hand, soil environment effects on standard litter decomposition were small in our experiment (decomposition increased from approximately 45 to 50% across the grass abundance gradient), whereas decomposition of native litter decreased from approximately 35 to 15% along the same gradient. This suggests that the contribution of soil environment effects to native root litter decomposition were relatively small. Similarly, a recent study in which each root litter was decomposed both in its own plot and in a common plot (in which all litters were incubated) showed that the negative effect of plant SR on native root litter decomposition observed when each litter was incubated in its own plot was similar to the effect found in the common plot (Chen et al. 2017). Finally, we found strong relationships between root traits and native root decomposition, which indicates that grass root abundance mainly reduced root decomposition via reductions in root litter quality.

Litter mixing effects are affected by grass root abundance, not plant species richness

Litter mixing effects were not found when considering all mixtures, nor over a SR gradient. As far as we know, only two studies have considered root litter mixing effects based on root litter mass loss (as in the present study), and both found positive effects (Robinson et al. 1999; de Graaff et al. 2011). More studies consider leaf litter-mixing effects, and the effects of combining litters of different species on decomposition vary considerably (Gartner & Cardon 2004). Litter mixing effects may be more closely related to factors related to litter quality than litter SR (Wardle et al. 1997; Jonsson & Wardle 2008). For example, variation in nutrient concentrations could facilitate positive litter mixing effects (Liu et al. 2007) by stimulating decomposition via nutrient transfer between litters (Wardle & Lavelle 1997; Schimel & Hättenschwiler 2007; Handa et al. 2014).

Grass root abundance was positively related to root litter mixing effects, similar to Hector et al. (2000), who found that a synergistic decomposition response was induced when leaf litter from multiple grass species were combined. This is counter intuitive, considering the negative effect of grass root abundance on root decomposition via litter quality found here, and in Chen et al. (2017). It could be that recalcitrant litter can foster a larger diversity of decomposers compared to easily decomposable litter (Lindedam et

al. 2009). Species with poor quality litter may also be more responsive to nutrient transfer in mixtures than richer litter (Handa et al. 2014), leading to the positive relation between grass root abundance and litter mixing effects.

Linking root traits to the effects of grass root abundance on root decomposition via changes in litter quality

RD and SRL best explained native root decomposition: thicker roots (high RD and low SRL) decomposed faster. The morphology of a root system determines its contact with the soil and the decomposer community (Personeni & Loiseau 2005). Therefore, it is expected that fine roots (i.e. a high SRL and low RD) which have a larger external surface, would decompose faster (e.g. Personeni & Loiseau 2005). However, we found the opposite relation, similar to Smith et al. (2014) who found that thicker roots (lower SRA, specific root area) decomposed faster. Roots with a larger diameter may decompose faster due to the presence of larger cortical storage cells near the perimeter of root, which can be easily broken down (Robinson 1990). Hobbie et al. (2010) found that thicker roots (low SRL and high RD) initially decomposed faster than finer roots, but this relation was reversed in the long term. The mass loss of root litter in the present study was between 5 – 49 %; it is therefore possible that in later stages of decomposition, the negative relations between SRL or RD and root decomposition may change.

Root nitrogen (N) was positively related to root decomposition (in line with Vivanco & Austin 2006; Fornara et al. 2009; Aulen, Shipley & Bradley 2012), whereas root C:N ratio was negatively related (in line with Silver & Miya 2001; Chen et al. 2017). Root carbon (C) was positively related to root decomposition, due to the coupling of low root C and decomposition of three grass species. Although total root C content of these species was lower than most other species, these grass species' roots may have a higher proportion of recalcitrant carbon (i.e. lignin), which could lead to low decomposition. However, our results show that root N, C, or C:N ratio explained less variation in root decomposition than SRL or RD, in contrast to Silver & Miya (2001), Prieto et al. (2016), and Roumet et al. (2016). Silver & Miya (2001) found that root decomposition was more closely related to changes in root C:N ratio than root diameter class. Prieto et al. (2016) and Roumet et al. (2016) showed that root decomposition was related to SRL, but was better explained by

chemical traits such as root N, C, and lignin concentrations. This discrepancy may be caused by the wider range of root C:N ratios in Silver and Miya (2001) (~20 – 250) than in our study (~30 – 70), and the narrower range of RD, which was considered in three classes, compared to the continuous variable (0.14 – 0.30, $n = 49$) in our study. The limitation of the decomposer community, which was not considered here, can also influence which root trait explains root decomposition. In a P-limited system, Birouste et al. (2012) found that root P concentration explained variation in root decomposition better than root N or root C:N ratio. Thus, N may not have been the primary nutrient limitation during our experiment, reducing the importance of N or C:N ratio for predicting root decomposition. Here, in contrast to Silver and Miya (2001), Prieto et al. (2016), and Roumet et al. (2016), we connected an independent measure of root traits to native root decomposition in the field. Thus, there is a chance that root N content and C:N ratio differed between the pot and the field experiments. Combining independent trait measures with field-based measures has been shown to explain ecosystem processes, for example, carbon and water fluxes (Everwand et al. 2014) and population biomass (Schroeder-Georgi et al. 2015). Further research on trait plasticity may improve the predictive power of independent trait measures, by selecting functional traits which are more conserved, to reduce the potential discrepancy between species' traits in different environments.

Our results show that SRL captures the effect of grass root abundance on native root decomposition. We cannot rule out that a close correlation between SRL and grass root abundance causes the significant relation between SRL and root decomposition. However, as plant traits are an important predictor of decomposition (Cornwell et al. 2008), it is likely that functional group composition influences root litter quality, and thus root decomposition, via root traits. Compared to SRL, RD was a poorer predictor of the effect of grass root abundance on native root decomposition, but did explain variation in native decomposition that was not explained by grass root abundance. The ideal trait would capture the effect of grass root abundance, and explain additional variation. None of the root traits considered here fulfil that requirement. Other traits that were not considered in the present study may better explain variation in root decomposition, including the effect of grass root abundance on root decomposition found here.

Candidate root traits include those that vary between functional groups, and affect decomposition. For instance: soluble compounds and nutrient concentration (Roumet et al. 2008; Birouste et al. 2011), tensile strength (Pohl et al. 2011), tissue structure (Vogel 2008), lignin concentration (Roumet et al. 2016), and lignin:N (Fornara et al. 2009).

Our findings suggest that functional group composition is important to consider when explaining patterns in decomposition. Functional group composition is relatively easy to quantify, and due to trait differences between grasses and forbs, it can serve as a proxy for a suite of traits which influence decomposition. At the same time, root traits have been reported to vary more within than between functional groups in field experiments; e.g. SRL, RD, root C and root N (Craine et al. 2002b; Roumet et al. 2006) and root lignin:N (Roumet et al. 2016). Further, the traits which best predict decomposition have been shown to differ between graminoid (including grasses) and eudicot (including forbs) functional groups (Roumet et al. 2016). Therefore, it may be necessary to consider if predictors of decomposition differ between functional groups, especially if diverse functional groups are considered. Further research into the factors underlying plant community-induced changes in root decomposition, such as root traits is necessary to gain a mechanistic understanding of how plant community composition (SR or functional group composition) influences root decomposition.

Conclusion

Grass root abundance, not plant species richness, reduced the decomposition of native root litter and had a marginally positive effect on the decomposition of standard root litter and litter mixing effects. Together, these results suggest that plant community effects on decomposition via the soil environment are relatively small, and decomposition of native root litter is predominantly driven by changes in litter quality. However, we cannot rule out that interactions between litter quality and soil environment affected our results. The negative effect of grass root abundance on native root decomposition could be captured by shifts in specific root length (SRL). Root diameter (RD) partially explained the effect of grass root abundance, plus additional variation in root decomposition. Our study demonstrates the importance of functional

group composition (grass root abundance) and two root traits, SRL and RD, for explaining root decomposition in a diverse grassland.

Acknowledgements

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Supplementary Information

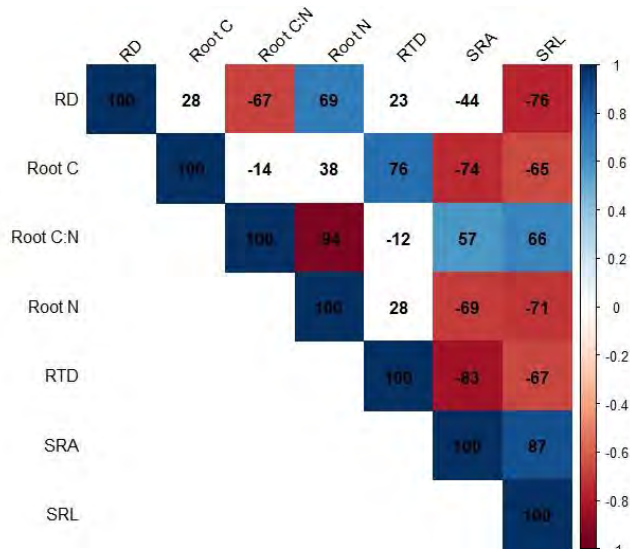


Fig. S4.1. Correlation matrix illustrating the correlations (Pearson's correlation coefficient, %) between the community weighted mean (CWM) root traits. Negative relations are indicated in red, positive relations are blue. The more saturated the colour, the stronger the correlation. Root traits included are: root diameter (RD, mm), root carbon content (root C, %), root carbon to nitrogen ratio (root C:N, g g^{-1}), root nitrogen content (root N, %), root tissue density (RTD, g cm^{-3}), specific root area (SRA, $\text{m}^2 \text{g}^{-1}$), and specific root length (SRL, m g^{-1}).

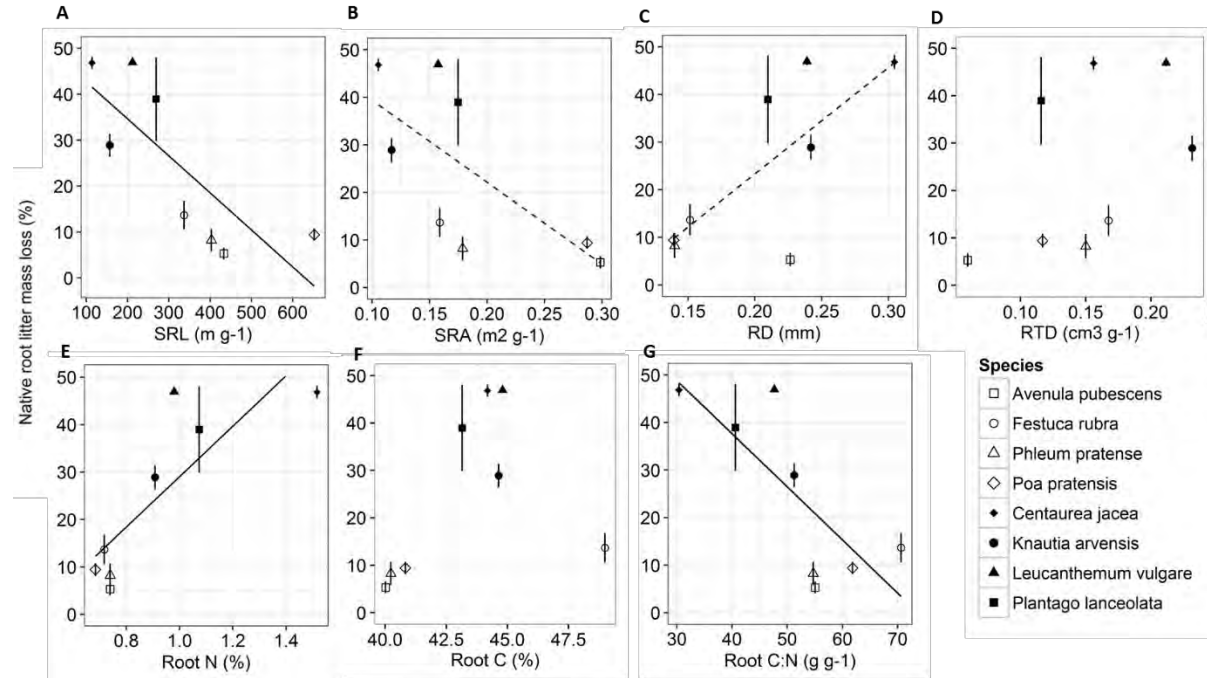
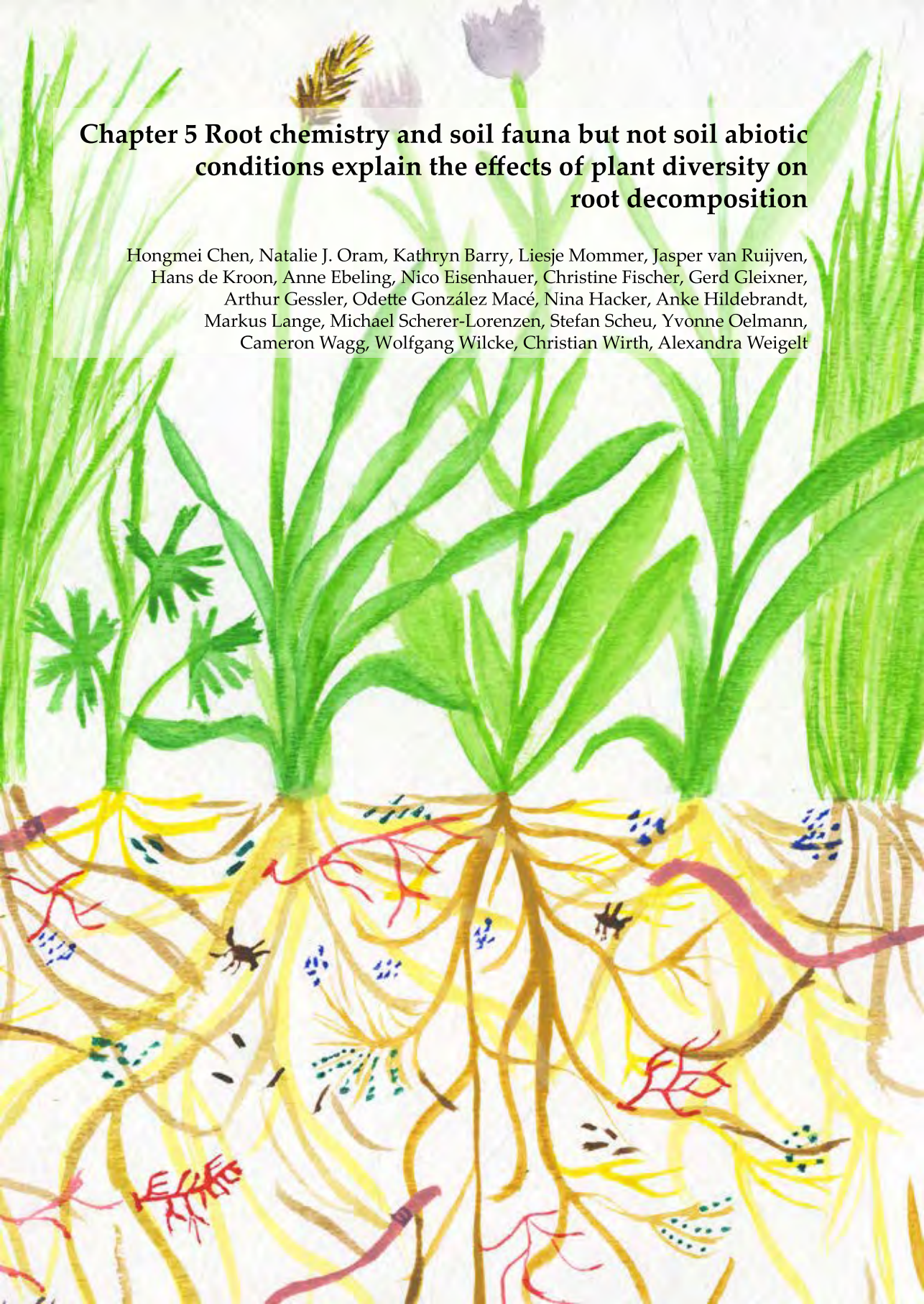


Fig. S4.2. Root decomposition (native root litter mass loss, %) as a function of root traits in monocultures. Grasses are open symbols; forbs are black symbols. Points represent the mean per plot, error bars denote the within-plot standard error of the mean, $n = 3$ when enough material was available. Black lines indicate a significant relationship ($P < 0.05$), dashed lines indicate a tendency ($P < 0.10$). Root traits are: A) specific root length (SRL), B) specific root area (SRA), C) root diameter (RD), D) root tissue density (RTD), E) root nitrogen content (root N), F) root carbon content (root C), and G) root carbon to nitrogen ratio (root C: N). Grasses and forbs differed significantly in their root decomposition, and in the root traits: SRL, RD, root N and root C: N ratio (see Table S4.1 for statistics).

Table S4.1. ANOVA (type I) summary of the differences in root decomposition (% mass loss of native root litter) and root traits between functional groups (FG), and the relations between root traits and root decomposition in monoculture. The Akaike Information Criterion (AIC) for each root trait – root decomposition model is presented; lower AIC values imply a better-fit model. Root traits are: root nitrogen content (root N), specific root length (SRL), root carbon to nitrogen ratio (root C: N ratio), root diameter (RD), specific root area (SRA), root tissue density (RTD), and root carbon content (root C).

	Difference between FG				Root decomposition			
	Forb	Grass	F	P	F		P	AIC
Root decomposition	40.40	9.16	$F_{1,6} = 46.03$	***				
Root N (%)	1.12	0.72	$F_{1,6} = 8.47$	*	$F_{1,4} =$	21.26	**	63.55
SRL ($m\ g^{-1}$)	188.08	456.3	$F_{1,6} = 12.41$	*	$F_{1,4} =$	10.12	*	67.75
Root C: N ratio	42.48	60.55	$F_{1,6} = 9.32$	*	$F_{1,4} =$	8.67	*	68.19
RD (mm)	0.25	0.16	$F_{1,6} = 8.56$	*	$F_{1,4} =$	6.49	‡	69.28
SRA ($m^2\ g^{-1}$)	0.14	0.23	$F_{1,6} = 5.40$	‡	$F_{1,4} =$	5.58	‡	70.4
RTD ($g\ cm^{-3}$)	0.18	0.12	$F_{1,6} = 2.42$	ns	$F_{1,4} =$	1.54	ns	73.36
Root C (%)	44.2	42.52	$F_{1,6} = 0.58$	ns	$F_{1,4} =$	0.89	ns	74.12
P < 0.001 ***, P < 0.01 **, P < 0.05 *, P < 0.10 ‡, non-significant (ns)								





Chapter 5 Root chemistry and soil fauna but not soil abiotic conditions explain the effects of plant diversity on root decomposition

Hongmei Chen, Natalie J. Oram, Kathryn Barry, Liesje Mommer, Jasper van Ruijven, Hans de Kroon, Anne Ebeling, Nico Eisenhauer, Christine Fischer, Gerd Gleixner, Arthur Gessler, Odette González Macé, Nina Hacker, Anke Hildebrandt, Markus Lange, Michael Scherer-Lorenzen, Stefan Scheu, Yvonne Oelmann, Cameron Wagg, Wolfgang Wilcke, Christian Wirth, Alexandra Weigelt

Abstract

Plant diversity influences many ecosystem functions including root decomposition. However, due to the presence of multiple pathways via which plant diversity may affect root decomposition, our mechanistic understanding of their relationship is limited. In a grassland biodiversity experiment, we simultaneously assessed the effect of three pathways, root litter quality, soil biota, and soil abiotic conditions, on the relationship between plant diversity (in terms of species richness, legume presence, and grass presence) and root decomposition using structural equation modeling (SEM). Our final structural equation model explained 70% of the variation in root mass loss. However, the three components of plant diversity included in our model operated via different pathways to alter root mass loss. Plant species richness had a negative effect on root mass loss. This was partially due to increased Oribatida abundance but weakened by enhanced root potassium (K) concentration in more diverse mixtures. Equally, grass presence negatively affected root mass loss. The effect of grasses was mostly mediated via increased root lignin concentration and supported via increased Oribatida abundance and decreased root K concentration. In contrast, legume presence showed a net positive effect on root mass loss via decreased root lignin concentration and increased root Mg concentration which both led to enhanced mass loss. Overall – diversity had a total negative effect on root mass loss when all paths are summed. Furthermore, we found that root chemistry and soil biota but not root morphology or soil abiotic conditions mediated the effect of plant diversity on root mass loss.

Introduction

After over two decades of research, it is widely accepted that plant diversity is essential for maintaining a variety of ecosystem functions (Balvanera *et al.* 2006; Cardinale *et al.* 2011). Yet, the role that plant diversity plays in plant litter decomposition remains elusive (Hättenschwiler *et al.* 2005; Gessner *et al.* 2010). The decomposition of plant litter drives nutrient and carbon (C) cycling in terrestrial ecosystems and therefore is important for primary production and soil C sequestration (Catovsky, Bradford & Hector 2002; Berg & McClaugherty 2008; Lange *et al.* 2015). Making up the majority of the plant standing biomass especially in grasslands (Jackson *et al.* 1996; Poorter *et al.* 2012), roots constitute a substantial portion of plant litter input (Freschet *et al.* 2013). Moreover, root C is better incorporated to the soil than shoot C due to the intimate contact with soil and has a longer residence time (Rasse *et al.* 2005). Thus, root decomposition may be more important than aboveground plant biomass decomposition for C sequestration and stock in the soil (Scheffer & Aerts 2000; Rasse *et al.* 2005; Kramer *et al.* 2010).

In spite of the likely importance of root decomposition for ecosystem functioning, there is little consensus on how plant diversity affects root decomposition (Fornara *et al.* 2009; Liu *et al.* 2009; de Graaff *et al.* 2011; Mommer *et al.* 2015; Chen *et al.* 2017). Several interacting factors may have contributed to the lack of consistency among studies. First, decomposition studies using leaf litter demonstrated that different measures of plant diversity, including plant species richness, functional group richness, and the presence/absence of individual functional groups, may vary in their effects on decomposition (Hector *et al.* 2000; Scherer-Lorenzen 2008). Second, plant species richness and functional group composition can affect root decomposition via three main pathways: (1) root litter quality, (2) soil biota, and (3) soil abiotic conditions (see conceptual model in Fig. 1; (Silver & Miya 2001; Chen *et al.* 2002; Hättenschwiler & Gasser 2005; Solly *et al.* 2014). These pathways are not mutually exclusive and are likely affected differently by different measures of diversity.

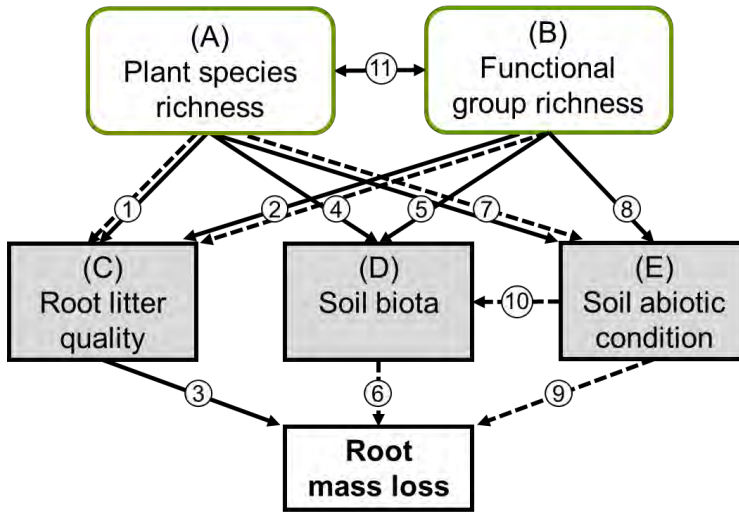


Fig. 5.1 A conceptual framework on the expected causal relationships between plant diversity and root decomposition for *a priori* structural equation model. Different measures of plant diversity, e.g., (A) plant species richness and (B) functional group richness (including presence/absence of individual functional groups) drive root decomposition via three potential pathways: (C) root litter quality comprising root morphological and chemical traits, (D) soil biota comprising basal respiration and mesofauna abundances and (E) soil abiotic conditions such as soil water content, dry bulk density, temperature and nutrient concentrations.

Root litter quality, i.e. root chemical and morphological traits, may determine the rate of root decomposition (Silver & Miya 2001). Roots with low C:N ratios, low lignin, and high nutrient concentrations decompose faster than roots with high C:N ratio, high lignin and low nutrient concentrations (Silver & Miya 2001). Morphological traits such as specific root length (Aulen *et al.* 2012), root diameter (Hobbie *et al.* 2010), and specific root area (Smith *et al.* 2014) are also related to root decomposition (but see (Birouste *et al.* 2011)). Plant species and functional groups show considerable variation in traits related to the root litter quality pathway (Birouste *et al.* 2011; Schroeder-Georgi *et al.* 2015). In addition, traits of individual species might change along a diversity gradient due to resource partitioning, biotic feedback or abiotic facilitation (for example). While there is good evidence for shoot trait plasticity along a diversity gradient (Thein, Roscher & Schulze 2008; Gubsch *et al.* 2011; Roscher *et al.* 2011; Lipowsky *et al.* 2015), we still lack data on

root traits (but see Baxendale *et al.*, 2014). Still, mixed plant communities may produce roots of different quality (Prieto *et al.* 2016; Chen *et al.* 2017), which may in turn lead to non-additive effects on root decomposition rates (Cong *et al.* 2015a; Prieto *et al.* 2017) with increasing plant diversity (Fig. 1, paths 1-3).

Soil biota may mediate the effects of plant diversity on root decomposition in various ways ranging from directly feeding on root litter to indirectly fragmenting litter and interacting with decomposers (Fig. 1, D; Chapin III, Matson & Mooney 2002). Plant diversity may change the community structure of soil decomposers (Salamon *et al.* 2004; Eisenhauer, Reich & Isbell 2012). Both soil microbial biomass and the abundance and diversity of decomposers increase with plant species richness and functional group richness (Fig. 1, paths 4, 5; (Eisenhauer *et al.* 2010, 2011a; Scherber *et al.* 2010; Ebeling *et al.* 2014a). This increase in the abundance and diversity of decomposers may lead to higher litter decomposition rates (Fig. 1, path 6; (Ebeling *et al.* 2014a). In addition, plant species richness and functional group presence/absence alter soil nutrient availability (Tilman, Wedin & Knops 1996; Niklaus *et al.* 2001; Scherer-Lorenzen *et al.* 2003; Oelmann *et al.* 2011) which may in turn influence decomposition via the dietary preferences of decomposer communities (Fig. 1, paths 4-6; (Craine, Morrow & Fierer 2007).

Last, soil abiotic conditions potentially mediate the plant diversity-root decomposition relationships (Fig. 1, E). Soil characteristics such as water content and temperature influence root decomposition (Wildung, Garland & Buschbom 1975; Wang, Liu & Mo 2010; Solly *et al.* 2014) mainly via their effects on the activity of soil microbes and other decomposers (Fig. 1, path 10; (Coleman, Crossley & Hendrix 2004; Butenschoen, Scheu & Eisenhauer 2011). Studies have reported that higher plant species richness could enhance topsoil water content (Rosenkranz *et al.* 2012; Eisenhauer *et al.* 2013; Wright, Schnitzer & Reich 2014) and decrease topsoil temperature (Spehn *et al.* 2000; Rosenkranz *et al.* 2012). As soil water content and temperature interdependently affect root decomposition (Chen *et al.* 2000), it is difficult to predict the net effect of plant diversity-induced changes in water content and temperature on root decomposition. Functional group presence/absence also may alter soil abiotic conditions (Gastine, Scherer-Lorenzen & Leadley 2003).

The three pathways - root litter quality, soil biota, and soil abiotic conditions - are not

mutually exclusive (Chen *et al.* 2017). Rather, the positive effect of one pathway may mask the negative effect of another or vice versa. Despite the putative combined effect of these different pathways, most studies investigated only a single pathway or investigated these pathways separately (Fornara *et al.* 2009; Liu *et al.* 2009; de Graaff *et al.* 2011; Mommer *et al.* 2015). In a previous paper (Chen *et al.* 2017), we tested root litter quality and soil-environmental pathways separately via multiple experiments and found significant negative root litter quality and soil-environmental effects on root decomposition. However, Chen *et al.* (2017) were only able to represent each pathway with one single variable, i.e. root C:N for the root litter quality pathway and soil water content for soil environmental conditions. Thus, we were unable to test the relative importance of the different pathways (due to the use of separate experiments for each pathway) or the individual drivers within pathways (because we could only use a single representative variable for each pathway). Further, we could not separate the biotic and abiotic soil environment effects of plant diversity on decomposition. Previous approaches, including our own, did not examine the effects of diversity on these pathways, the interactions among the pathways, or the way these pathways combine to form the negative diversity-decomposition relationship at the same time. Here, we used SEM to simultaneously test 31 field-measured variables which represent the three pathways through which plant diversity can affect root decomposition. This approach allows us to simultaneously test the relative importance of, and interactions between, the three pathways which underlie the negative plant diversity- root decomposition relationship. We hypothesized that all three pathways significantly affect the relationship between plant diversity and root decomposition as illustrated in Fig. 1.

Materials and methods

The Jena Experiment

This study was conducted in the Jena Experiment (<http://www.the-jena-experiment.de/>), a long-term grassland diversity experiment located on the floodplain of the river Saale, close to Jena, Germany (50° 57' 5" N, 11° 37' 29" E, 130 m a.s.l.). Jena has a mean annual temperature of 9.9°C and mean annual precipitation of 610 mm (1980-2010; (Hoffmann *et al.* 2014). The soil at the field, classified as Eutric Fluvisol, is developed from up to 2 m

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thick loamy fluvial sediments (Roscher *et al.* 2004) and the soil texture (0-30 cm) shifts from loam (40% sand, 44% silt, 16% clay) to silt loam (7% sand, 69% silt, 23%) with increasing distance from the river (Steinbeiss *et al.* 2008a). The study site was an arable field since the 1960s and was fertilized and plowed until the establishment of the Jena Experiment in 2002 (Roscher *et al.* 2004). The full experimental design can be found in (Roscher *et al.* 2004). The present study included 76 experimental plant communities spanning a gradient of plant species richness (1, 2, 4, 8, 16) and functional group richness (1, 2, 3, 4; grasses, legumes, small herbs, and tall herbs). These communities were established by random species sampling from a 60-species pool representing typical Central European mesophilic grassland species and were arranged in a randomized block design to exclude potential confounding effects of soil texture.

Root decomposition experiment

In this study, we used a root decomposition experiment established by (Chen *et al.* 2017) to explore the three potential pathways underlying the effects of plant diversity on root decomposition. In this experiment, plot-specific roots were decomposed in their plots of origin from April to August in 2014 (see below for brief description). This experimental setting included all the potential interactions among the three pathways and thus was suitable for evaluating the three pathways with SEM.

We collected plot-specific roots from each experimental plot in September 2013 by taking two soil samples (size varied from 20×10 cm to 40×15 cm) at 20 cm depth. We based soil sample area on previously measured standing root biomass (Ravenek *et al.* 2014) to ensure sufficient root material for the litter-bag approach. We soaked the soil samples in tap water and washed the soil away over a 630- μ m sieve to collect fine roots (< 2 mm in diameter, see (Chen *et al.* 2017) for details). A subsample of fresh fine roots was preserved in 70% alcohol at 4°C for morphological trait measurement. The remainder was oven-dried at 65°C for 48 h, and then a ~ 1 g subsample was ground with a ball mill (MM 400, Retsch GmbH, Haan, Germany) for chemical analyses. The rest of the oven-dried roots were used to fill litter bags. Litter bags were 8 × 8 cm and made of 325 μ m polyester mesh (Top Zeven B.V., Netherlands). Each litter bag contained 0.25 g of oven-dried roots. Three retrievals were carried out to trace the decomposition process over time. After retrieval,

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litter bags were transported to the lab, stored at 4°C and processed within 2 weeks as follows: 1) soil attached to litter bags was gently flushed away with tap water; 2) litter bags were opened and roots growing in from outside were removed with tweezers. 3) Roots were washed into a 63- μm sieve under tap water and collected; 4) the collected roots were oven-dried at 65°C and weighed. We used the percentage mass loss as a measure of root decomposition.

$$\text{Mass loss (\%)} = (1 - \text{final mass}/\text{initial mass}) \times 100 \quad (\text{Equation 1})$$

Because there was no interaction between time and the drivers of interest (Chen *et al.* 2017), we used mass loss at the final retrieval (120 days) in this study.

Root trait measurements

The preserved fresh roots were washed in a 63- μm sieve under tap water, stained in the neutral red solution overnight at 4°C and scanned with Epson Perfection V700 Photo Scanner (Seiko Epson Corporation, Suwa, Japan) at 600 dpi in greyscale. Then the scanned roots were oven-dried at 65°C for 48 h and weighed. Images were analyzed with WinRHIZO 2009a (Regent Instruments Inc., Ville de Québec, Canada). We extracted average root diameter (mm) from this software and calculated specific root length (total root length divided by root mass; $\text{cm}\cdot\text{g}^{-1}$) and root tissue density (root mass divided by root volume; $\text{g}\cdot\text{cm}^{-3}$).

Subsamples of ground roots were analyzed for total C and N concentrations (%) using an EA-IRMS (Flash 2000, Delta V, Thermo Fisher Scientific Inc., USA). For elemental concentrations, microwave pressure digestion (Speedwave 2, Berghof, Eningen, Germany) was used for sample digestion, where we weighed $0.200 \text{ g} \pm 0.005 \text{ g}$ root powder into 60 ml digestion vials (DAP-60K), added 8 mL of 65% nitric acid and 3 mL of 30% hydrogen peroxide (H_2O_2), waited for 15 min, and digested for 15 min (50 bar, 190°C). Aluminum (Al), barium (Ba), calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), sulfur (S), strontium (Sr), and zinc (Zn) concentrations of digested samples were measured with inductively-coupled plasma atomic emission spectroscopy (Spectro Arcos, Spectro Analytical Instruments GmbH, Kleve, Germany).

To extract and remove the non-cell-wall materials, we added 4 mL of 80% (v/v) aqueous

acetone-ethanol mixture (5:3) to 15 mg of ground roots and incubated them at 70°C for 2.5 h. After centrifuging the samples, we washed the precipitate with distilled water and dried the post-extraction samples (containing no non-cell-wall materials) at 70°C for 48 h. Two replicates were analyzed per plot. Lignin concentrations of the post-extraction samples were measured with a modified acetyl bromide digestion method (Iiyama & Wallis 1988, 1990; Moreira-Vilar *et al.* 2014). We added 5 mL of 25% (v/v) acetyl bromide (99%) in glacial acetic acid (99-100%) to the post-extraction samples (5-15 mg) and incubated them at 70°C for 60 min. Then the samples were cooled on ice for 15 min, adjusted to room temperature for 30 min and centrifuged at 15000 g for 5 min. We diluted 1 mL of supernatant with 8.5 mL glacial acetic acid and 1 mL 2 M NaOH and measured its absorbance at 280 nm with a Jasco V 730 Spectrophotometer (Jasco Inc., Easton, MD, USA). The measurement was repeated three times. Absorbance coefficients (A_s) for each measurement were calculated as:

$$A_s (ml \cdot mg^{-1} \cdot cm^{-1}) = (OD_s - OD_B) \times 52.5/mass \text{ (Equation 2)}$$

where OD_s = optical density of the sample, OD_B = optical density of the blank, and mass = weight of the post-extraction sample. We used the absorbance coefficient to indicate the relative lignin concentration.

Soil biota measurements

Soil samples for analyzing microorganisms were taken in July 2014. In each plot, three soil cores (d = 5 cm, depth = 5 cm) were combined as a composite sample, sieved at 2 mm, and stored at 5°C until further analyses. Basal respiration and soil microbial biomass C (using substrate-induced respiration) were measured using an O₂-microcompensation apparatus (Scheu 1992). O₂ consumption rates without and with D-glucose addition were measured every hour for 24 h to indicate basal respiration and substrate-induced respiration, respectively. The mean of the lowest three readings with D-glucose addition within the first 10 h was taken as the maximum initial respiratory response (MIRR; $\mu\text{L O}_2 \cdot \text{h}^{-1} \cdot \text{g}^{-1}$ soil dry weight). Microbial biomass C ($\mu\text{g C} \cdot \text{g}^{-1}$ soil dry weight) was calculated as $38 \times \text{MIRR}$ (Beck *et al.* 1997).

To measure soil mesofauna abundance, we took one soil core (d = 5 cm, depth = 5 cm) from each plot in July 2014 and extracted the animals by heat (Kempson, Lloyd &

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Ghelardi 1963). Soil invertebrates were collected in diluted glycol and preserved in 70% ethanol. Collembola and Oribatida were identified based on characters described in (Schaefer 2009).

Soil abiotic condition measurements

Soil temperature was measured every 5 seconds at 5 cm and 15 cm depth in the center of each experimental plot with PT100 sensors (home-made by Max-Planck-Institute for Biogeochemistry, Jena, Germany). We averaged soil annual temperature at these two depths to indicate soil annual temperature at 10 cm depth. We used the median of annual mean temperature at 10 cm from 2003 to 2011 in the model.

Volumetric soil water content ($\text{m}^3\cdot\text{m}^{-3}$) was measured with an ML2x Theta Probe (Delta-T Devices, Cambridge, United Kingdom) at 0-6 cm soil depth (the length of the prongs) every two weeks with 5 repetitions in the area surrounding the litter bags. In each plot, we calculated the mean of the 5 measurements for each sampling day and calculated the median soil water content over the decomposition period from April 17th to August 13th, 2014 in the model.

Soil dry bulk density was measured in April 2014 (described in detail in (Steinbeiss *et al.* 2008a). In short, we took three soil cores in each plot ($d = 4.8$ cm, depth = 30 cm) using a split tube sampler (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands), split up each soil sample into 5-cm segments and pooled segments of the same depth from the same plot to one composite sample. Each composite sample was dried at 40°C and weighed. After removal of roots and stones, soil dry bulk density (weight divided by soil volume, $\text{g}\cdot\text{cm}^{-3}$) was calculated. In the model, we included mean soil dry bulk density at 0-5 cm and 5-10 cm depths to indicate soil dry bulk density at 0-10 cm.

We took soil samples for N and P concentrations in October 2014. In each plot, five soil cores ($d = 2$ cm, depth = 15 cm) were combined as one composite sample and then sieved to < 2 mm. We used 1 M KCl to extract soil inorganic N from 5 g of subsamples and measured NH_4 and NO_3 concentrations in the extracts with a continuous flow analyzer (AutoAnalyzer3, SEAL Analytical, Norderstedt, Germany). To calculate NO_3 and NH_4 in $\text{mg}\cdot\text{kg}^{-1}$ dry soil, we determined the soil gravimetric water content by weighing approximately 5 g of soil before and after drying at 105°C for 24 h. After inorganic soil N

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concentration was determined, the remainders of soil samples were air-dried. We measured soil labile P concentrations using the NaHCO₃ extract from the modified Hedley procedure (Hedley *et al.* 1982; Kuo 1996). 0.5 g of air-dried soil was mixed with 20 mL 0.5 M NaHCO₃, shaken for 0.5 h, centrifuged at 2500 rpm, and the supernatant was collected and filtered (MN 619 G ¼, Macherey-Nagel GmbH & Co. KG, Düren, Germany). Inorganic P concentrations in the extracts were measured with a continuous flow analyzer (AutoAnalyzer3, SEAL Analytical, Norderstedt, Germany) with the phosphomolybdate blue method (Murphy & Riley 1962). Inductively-coupled plasma optical emission spectroscopy (DV 5300, Perkin Elmer, Waltham, Massachusetts, USA) was used to determine total P concentrations. Soil N:P ratio was calculated by dividing the sum of NO₃-N and NH₄-N concentrations by total plant labile P concentrations.

Statistical analyses

Prior to statistical analyses, we regressed all dependent variables (including mass loss and all measured variables) against the Cartesian coordinates of the individual plot positions in the field as well as the second order derivatives of these coordinates using a linear model (Niklaus *et al.* 2016). We used the residuals of this model for further analysis, hereafter referred to as specially corrected measures. This analysis controls for differences between plots at the Jena Experiment due to their location (i.e. spatial autocorrelation) and the non-linearity in spatial gradients (see (Niklaus *et al.* 2016) for further details).

We then used a four-step approach to select the most appropriate variables for the SEM following the flowchart in Fig. S1. First, we excluded four out of 76 plots due to missing values, leaving 72 plots for statistical analyses. Second, to select the most important measures of plant diversity affecting root decomposition (Fig 1, A, B) we considered type I sum of squares ANOVA using the function `aov{stats}` (A and B in Fig. 1). We evaluated the effects of different measures of plant diversity, i.e. plant species richness, functional group richness, and the presence/absence of individual functional groups, on root litter mass loss (%). Because of the collinearity between functional group richness and the presence/absence of individual functional groups, we first included plant species richness and functional group richness in ANOVA, then added one of the functional

groups to evaluate the main effect of the specific functional group. This analysis revealed that only plant species richness and the presence/absence of grasses and legumes were significant predictors of root mass loss (Table 5.1). These three factors were included as exogenous variables in structural equation models.

Table 5.1 ANOVA (type I) summary of the effects of plant species richness (log₂-transformed), functional group (FG) richness and the presence/absence of individual FGs (grass, legume, small herb, and tall herb) on the residuals of mass loss of plot-specific roots decomposing in their ‘home’ plots after four months. Arrows indicate positive (↑) or negative (↓) effects; degrees of freedom (df), sum of squares (SS), mean squares (MS).

Source	df	SS	MS	F	P-value	
Species richness	1	10.19	10.19	5.45	0.023	↓
FG richness	1	2.82	2.82	1.51	0.22	
Residuals	69	129.06	1.87			
Main effect of each FG						
Grasses	1	29.82	29.82	20.43	< 0.001	↓
Legumes	1	15.29	15.29	9.14	0.004	↑
Small herbs	1	0.97	0.97	0.51	0.48	
Tall herbs	1	0.66	0.66	0.35	0.57	

Third, to satisfy the multi-normality assumption of SEM, we ran a linear model for each variable against all significant predictors and a linear model for mass loss against each variable, and tested the residuals of these models for normality with the function `shapiro.test{stats}`. Variables were transformed when the residuals of the model were not normally distributed, or when the variable was not linearly related to root litter mass loss or plant species richness (Kline 2005), see Table S5.1 for all variables in the original dataset and transformation. Transformed variables were used in further analyses. Fourth, to minimize collinearity within pathways for SEM, we calculated the Pearson correlation coefficient for all variables within each pathway (i.e. root litter quality, soil biota and soil abiotic conditions; `rcorr{Hmisc}`(Harrel *et al.* 2016) and grouped correlated

variables into subgroups based on a Pearson's r of 0.6 or greater (Dormann *et al.* 2013). We grouped root litter quality variables into four groups (Table S5.2): (1) Al, Ba, C, Fe, Mg, Mn, and Zn; (2) Ca, N, Na, P, S, Sr, and lignin; (3) K; (4) specific root length, root diameter, and root tissue density. From each subgroup, we chose one representative variable based first, on their importance in decomposition literature (Kline 2005) and then on the Akaike Information Criterion AIC of linear regressions between mass loss and the variables. That is, if two or more variables were equally important in the literature (e.g., lignin and N concentrations), We selected the variable present in the linear regression with the lowest AIC (Table S5.4). According to this process, we chose the following variables as representatives for correlated variables from their corresponding groups: Mg from group (1), lignin from group (2), and root diameter from group (4). Among soil biotic variables, only basal respiration and microbial biomass C were highly correlated (Table S5.3). We chose basal respiration as a representative variable because it is the most direct measurement of microbial activity (Joergensen & Emmerling 2006). For the soil abiotic variables (Table S5.3), soil total labile P and inorganic P concentrations formed one group while soil NO_3 , NH_4 , total inorganic N concentrations, and soil N:P ratio formed another group. We chose soil total labile P concentration in the former group based on AICs (Table S5.4) and chose soil N:P ratio in the latter group because a ratio is likely to be more important than absolute concentrations for soil microorganisms (Sinsabaugh, Hill & Follstad Shah 2009). Because this process provided potential biases, we also recreated the variable selection process using a principal component analysis (PCA) to assess whether these groups were consistent across methods (see supplemental methods and discussion for PCA results which were largely similar). Because SEM cannot handle large scale differences in variance among variables, we multiplied or divided variables by 10 to reduce scale difference (Kline 2005). The usage of all variables is summarized in Table S5.4.

We constructed an *a priori* structural equation model following the conceptual framework (Fig. 5.1). We included root Mg, lignin, K, and root diameter for the root litter quality pathway (Fig. 5.1, C), soil basal respiration, abundances of Collembola and Oribatida for the soil biota pathway (Fig. 5.1, D), and soil total labile P, N:P ratio, temperature, bulk density, and water content for the soil abiotic condition pathway (Fig.

5.1, E). We allowed covariance between plant species richness and the presence of grasses and legumes to account for the frequency change of the functional group presence/absence along the plant species richness gradient due to the experimental design (Fig. 5.1, path 11). Adequate model fit is indicated by insignificant χ^2 -test (P -value > 0.05 ; (Grace 2006) and low root mean square error of approximation (RMSEA, preferably < 0.05 ; (Kline 2005). The *a priori* structural equation model was not adequate and we re-specified the model. Starting from the *a priori* structural equation model, we added reasonable covariance or paths among mediating variables when the modification indices implied missing relationships in the model (Grace 2006) and sequentially removed non-significant paths based on their P -value. χ^2 and the AIC of the model were inspected to identify the most parsimonious structural equation model. We considered two models to be significantly different if their AIC values differ by more than 10 (Burnham & Anderson 2007). After we had the most parsimonious structural equation model, we also tested if community aboveground biomass (Wagg, personal communication) and root standing biomass (Oram et al., unpublished) improved the explanatory power of the diversity drivers on the mediating variables of soil biota or soil abiotic conditions. However, biomass was not significant in any of the tested models and is thus not presented in the final results. All statistical analyses were conducted in R version 3.3.1 (R Core Team 2016). Structural equation modeling was done using {lavaan} version 0.5-20 (Rosseel 2012).

Results

The final structural equation model explained 70% of the variation in root mass loss ($\chi^2_{13} = 14.93$, P -value = 0.31, RMSEA = 0.045 with 90% confidence interval = 0.000, 0.130). The effects of plant species richness and presence of grasses and legumes on root mass loss were mediated by root lignin, K, and Mg concentrations, and Oribatida abundance in soil. Root lignin concentration and Oribatida abundance reduced root mass loss, while root K and Mg concentration increased root mass loss (Fig. 5.2).

To determine the total effects of each plant diversity measure on root mass loss, we summed all the direct and indirect paths connecting plant species richness, legume presence, or grass presence with root mass loss (Kline 2005). Based on this approach,

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plant species richness had an overall negative effect on root mass loss (sum of standardized path coefficients = -0.24), which was partially explained by changes in root K concentration and Oribatida abundance (Fig. 5.2). Higher plant species richness increased the abundance of Oribatida and thereby negatively affected root mass loss. This negative effect of plant species richness was diminished by a concurrent positive effect on K concentration in roots which increased root mass loss. Plant species richness did not affect root lignin or Mg concentrations. The standardized path coefficient of the root K pathway was 0.13, which was higher in magnitude than the Oribatida pathway (standardized path coefficient = -0.04) but opposite in direction. In addition to the indirect pathways, there was a direct negative pathway left from plant species richness to root mass loss (Fig. 5.2).

Grass presence had an overall negative effect on root mass loss (sum of standardized path coefficients = -0.32), which was partially explained by changes in root lignin and K concentrations, and Oribatida abundance (Fig. 5.2). Plant communities with grasses produced roots with higher lignin concentration and lower K concentrations and increased the abundance of Oribatida. The sum of standardized path coefficients of the root chemical pathways was -0.55 and higher than the Oribatida pathway (standardized path coefficient of -0.06). In addition, there was a direct positive path from the presence of grasses to root mass loss (Fig. 5.2).

Legume presence had an overall positive effect on root mass loss (standardized path coefficient 0.36). This positive effect of the presence of legumes was explained by changes in root lignin and Mg concentrations, i.e. there was no indirect path via root K concentration and Oribatida abundance, nor a remaining direct path from legume presence to root mass loss. Plant communities with legumes produced roots with lower lignin concentrations and higher Mg concentrations than those without legumes, which led to higher root mass loss (Fig. 5.2).

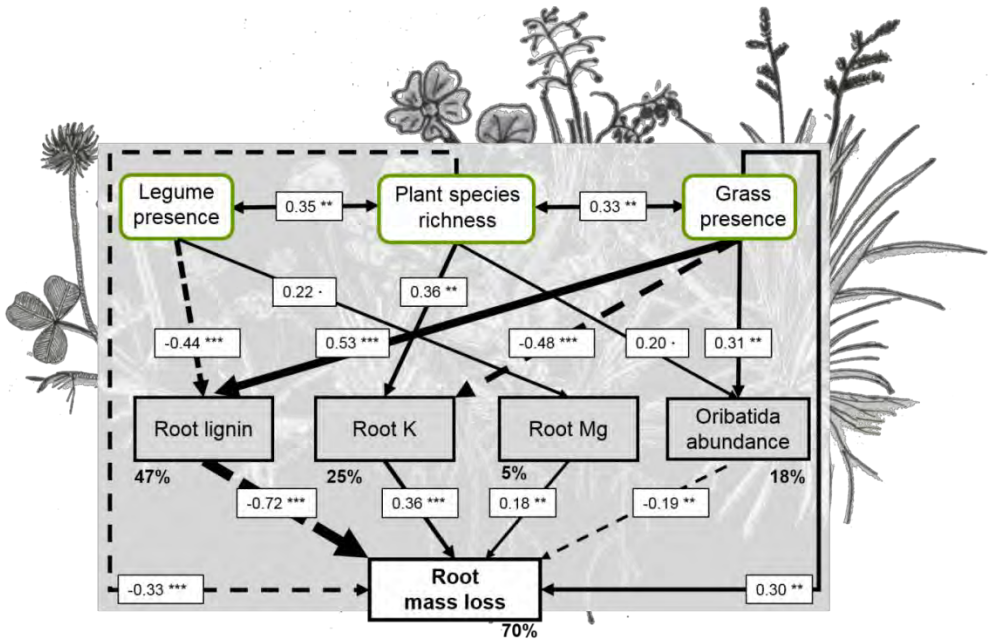


Fig. 5.2. The final structural equation model with direct and indirect pathways mediating effects of plant species richness and presence/absence of grasses and legumes on root mass loss (residuals after spatial auto-correlation being removed). Numbers on arrows give standardized path coefficients with their significance indicated as *** P -value < 0.001, ** P -value < 0.01, * P -value < 0.05, and P -value < 0.1. Solid arrows represent positive relationships, dashed arrows indicate negative relationships. Arrow width reflects path coefficient magnitude. Numbers below the variables indicate the percentage variation explained in corresponding variables (R^2).

Discussion

By applying SEM to a root decomposition experiment within a large-scale biodiversity experiment, we partitioned the effects of plant diversity on root decomposition into three pathways: root litter quality, soil biota, and soil abiotic conditions. Our results suggest that the effects of plant species richness and the presence/absence of grasses and legumes on root decomposition are primarily mediated by root chemical traits, including lignin, K and Mg concentrations, and to a lesser extent by the abundance of Oribatida. Notably, the soil abiotic conditions and root morphologies that we considered did not explain the

effects of plant species richness or functional group composition on root decomposition.

Root chemistry: lignin, K, and Mg drive root decomposition

Root chemical traits including lignin, K and Mg concentrations were important mediators of the plant diversity-root decomposition relationship. However, the role of root chemical traits differed per measure of plant diversity. Root lignin concentration mediated the effects of legume and grass presence on root decomposition, but was unrelated to plant species richness. The lack of a plant species richness effect on root lignin concentration signals that although abiotic and biotic stressors, which could be influenced by plant species richness, may alter the biosynthesis of lignin in plants (Moura *et al.* 2010), variation in root lignin concentration is mainly determined by phylogenetic differences between functional groups. Lignin was negatively related to mass loss, consistent with most literature considering the effect of lignin on decomposition (Cornwell *et al.* 2008; Aulen *et al.* 2012). This is likely due to its resistance to microbial degradation (Swift *et al.* 1979), or its role in structural protection of labile carbon compounds (Austin & Ballaré 2010). Many root decomposition studies have shown that lignin:N ratio is a good predictor for root decomposition (Silver & Miya 2001; Solly *et al.* 2014). We found similar results, yet lignin alone explained more variation in root mass loss than lignin:N ratio (58.2% vs. 52.9%, r^2).

We found that plant species richness and grass presence were positively correlated with root K and root K in turn was positively correlated with root decomposition. The positive relationship between root K and plant species richness may be due to enhanced root production in mixture relative to monoculture (Ravenek *et al.*, 2014; this thesis chapter 2). Young roots have significantly higher K concentration than older roots (Sturner & Elser 2002; Kramer *et al.* 2010; Abrahamson & Caswell 2017). Young roots are likely to be found in higher proportion in mixtures if root production increases in mixtures (Ma & Chen 2016). The positive effect of plant species richness on root K concentration may also be related to increased soil organic C (Lange *et al.* 2015) and increased topsoil water content. Increased soil organic carbon provides more cation exchange sites which prevent K from being leached and serve as a stock of exchangeable K (Peverill, Sparrow & Reuter 1999). Increased soil water content stimulates K diffusion in the soil and the

resupply of K to the soil solution, and thus the uptake of K by plants (Kuchenbuch, Claassen & Jungk 1986). However, this explanation is unlikely in our experiment as we did not observe a significant correlation between root K concentration and topsoil water content ($t_{70} = 1.81$, P -value = 0.074).

The positive effect of root K concentration on root decomposition agrees with previous findings using leaf litter (Cornelissen & Thompson 1997; Makkonen *et al.* 2012; Yue *et al.* 2016). However, the mechanistic role of K in root decomposition is not well studied. One potential explanation for the positive relationship between K and root decomposition is that K is a surrogate for other root traits that we did not measure. Studies showed that concentrations of K and total water-soluble compounds were highly correlated in leaf litter (Makkonen *et al.* 2012; Schreeg, Mack & Turner 2013). Most water-soluble compounds are readily available for microbial decomposers (Berg & McLaugherty 2008) and contribute largely to the mass loss in the early stage of decomposition (Li, Han & Zhang 2007a).

We found that legume presence significantly increased root Mg, and Mg increased mass loss. This may be for several reasons. Legumes are able to acquire more Mg than grasses when grown together (Meerts 1997). Mg content in legumes is typically about 20% higher than in grasses (Whitehead 2000). Higher concentrations of Mg in legumes could promote root growth, and alter root morphology (Marschner 1995; Lambers, Stuart Chapin III & Pons 2008). Further, the allocation of Mg throughout the plant may differ between legumes and grasses, and has shown to be higher in the roots than shoots of dicots, whereas the Mg distribution in monocots is more uniform (Whitehead 2000). Litter Mg concentration has been shown to be an important driver of leaf decomposition rate globally (Makkonen *et al.* 2012), and is positively related to root decomposition during the initial stages of decomposition (Berg 1984).

Soil biota: Oribatida as the main biotic mediator in root decomposition

Among the soil biotic measurements we examined, only Oribatida had a significant negative effect on root decomposition. This diversity effect on Oribatida abundance was caused by both grass species presence and plant species richness confirming similar findings in earlier years in the Jena Experiment (Eisenhauer *et al.* 2011a) as well as effects

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of understory diversity in a forest ecosystem (Eisenhauer *et al.* 2011b). Leaf litter diversity is known to increase Oribatida diversity and abundance – this link is also likely present for root litter diversity (Hansen & Coleman 1998). However, the explicit link to grasses may be because grasses provide low-quality litter input which is more readily decomposed by fungi, a primary food source of Oribatida (Siepel & Ruiters-Dijkman 1993; Hättenschwiler *et al.* 2005). The link between high Oribatida abundance and low root decomposition may reflect the dominance of the fungal energy channel (fungi and fungivores, (Cardon & Whitbeck 2011) rather than the direct reduction of root decomposition by Oribatida feeding on fungi. Although Oribatida preferentially feed on fungi (Siepel & Ruiters-Dijkman 1993; Schneider *et al.* 2004) this is unlikely to result in reduced litter decomposition. Rather, decomposition processes typically are stimulated by microarthropods grazing on fungi (Joo, Yim & Nakane 2006; A'Bear, Jones & Boddy 2014). Alternatively, Oribatida abundance may reflect the abundance of fungi with fungi being less efficient in decomposing roots than bacteria as the fungal energy channel is assumed to respond to resource input more slowly than the bacterial energy channel (Wardle 2002; Moore, McCann & De Ruiters 2005).

Root morphology and soil abiotic conditions do not mediate the plant diversity-decomposition relationship

Contrary to our hypothesis, root morphology did not influence the relationship between plant diversity and root decomposition in our study. None of the root morphological traits that we measured (specific root length, average root diameter, and root tissue density) were significantly correlated with root mass loss or were affected by plant species richness and functional group presence/absence (Table S4). (Birouste *et al.* 2011) also found that root morphology did not account for root decomposition while (Roumet *et al.* 2016) and (Prieto *et al.* 2016) found support for a minor role of root morphology. (Roumet *et al.* 2016) showed that the root morphological traits that best predicted decomposition differed between graminoids and eudicots; root morphology explained variation in the decomposition of graminoid but not eudicot roots. Overall, specific root length explained significant variation in root decomposition. The discrepancy between our study and theirs may be the wider range of morphology trait values in their study,

which considered 74 species, compared to ours. (Prieto *et al.* 2016) studied a broad range of plant communities from agricultural crops to natural forests. Our study included only grassland ecosystems and the range of root morphology was narrow. Also, the mixing of species from different functional groups may blur potential effects of root morphology on root decomposition.

In addition, we found no evidence that soil abiotic conditions are stable mediators of the relationship between plant diversity and root decomposition. Soil moisture, temperature, dry bulk density, and soil labile P were all significantly affected by plant species richness and/or the presence/absence of grasses and legumes, but were not significantly correlated with root decomposition in the majority of our models (Table S4). Notably, when we used PCA axes as mediators in our analyses (Fig. S3) soil temperature was sometimes included, though not in the best model (Table S6). Furthermore, soil temperature appeared in no other model. Our results with regards to abiotic conditions contradict previous studies which showed that soil moisture, temperature, and soil nutrient availability are generally important for the decomposition of leaf and root litter (Bontti *et al.* 2009; Solly *et al.* 2014).

However, our experimental approach was constrained in some ways. First, the spatial scale in our study is smaller than those explicitly testing e.g. the effects of abiotic conditions on decomposition (Solly *et al.* 2014). Thus the variation induced by plant diversity on this smaller spatial scale may not be sufficient to show the effect of soil abiotic conditions. Second, our three pathways (soil abiotic conditions, soil biota, and root morphology) were varied as indirect effects of our diversity gradient. Biodiversity is known to have a profound effect on nutrient availability, root biomass and length density, soil biotic communities, and decomposition. However, the indirect variation of root morphology and soil abiotic conditions due to diversity may not create sufficient variation to demonstrate the effects of soil abiotic conditions and root morphology on root decomposition. Third, we present data for only the first four months of decomposition, and thus focus on early-stage decomposition effects. As decomposition proceeds, the variables that we found to be important may remain so, or their effects may change in extent or direction (Berg & McClaugherty 2008). For example, during leaf decomposition high N concentration increased the rate of mass loss initially, but was

negatively related to mass loss during later stages of decomposition (Berg & McClaugherty 2008). Other factors, including root morphology and soil abiotic conditions may become important at later stages of decomposition across a diversity gradient. Finally, our selection process allowed us to include only one variable from each major group of mediators. We believe that using individual variables enhances clarity, and when other variables from the groups were tested as the representative variable, conclusions remained the same. Moreover, the groups and their effects remain largely consistent using PCA.

Conclusions

In this study, we simultaneously examined the three pathways - root litter quality, soil biota, and soil abiotic conditions - that are hypothesized to mediate the relationship between plant diversity and root decomposition. Our results provide evidence that plant diversity affects early-stage root decomposition via changes in root chemical traits and soil biota rather than via changes in root morphological traits or soil abiotic conditions. We also show, for the first time, that plant species richness and functional group presence affect early-stage root decomposition via different pathways: both plant species richness and grass presence operate via root chemistry and soil biota pathways, while legume presence operates only via the root chemistry pathway. Our study confirms that root lignin, a commonly studied root chemical trait, is a good predictor of root decomposition in the context of biodiversity. We also highlight the importance of less commonly used the predictors root K and Mg concentrations and Oribatida abundance for root decomposition across biodiversity gradients. These findings provide a significant advance in our understanding of the pathways via which plant diversity affects root decomposition.

Acknowledgements

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Supplementary Information

Material and Methods

Statistical analyses

Due to the collinearity among root chemical traits, in addition to grouping based on correlation matrix, we performed a principal component analysis (PCA) on the transformed root chemical traits using `rda{vegan}` (Oksanen *et al.* 2017). We then used the PCA axes in two ways: 1) to replace individual root chemical traits in SEM; and 2) to select individual representatives from groups for the SEM and assess the generality of the groups that we selected based on the correlation matrix. All transformed data were normalized using `scale{base}` before PCA. We used the first three PCs for root chemical traits in the *a priori* structural equation model. The *a priori* structural equation model was not adequate and we re-specified the model as we did for SEM with individual variables.

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Table S5.1: Descriptive summary of variables (n=72, raw data) included in the analyses. Abbreviations: SR, species richness; SD, standard deviation; SRL, specific root length; RD, average root diameter; RTD, root tissue density; BR, basal respiration; C_{mic}, microbial biomass C; P_{tot}, soil total labile P; Pi, soil inorganic labile P; N_{tot}, soil total inorganic N; SWC, soil water content. Oribatida and Collembola are given as number of individuals per sample. The right column gives the data transformation used in the analyses. All transformation were performed on variable residuals after spatial auto-correlation being removed.

Pathway	Variable	Unit	SR=1 (n=13)		SR=2 (n=15)		SR=4 (n=15)		SR=8 (n=16)		SR=16 (n=13)		Transformation
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Root litter	Lignin	ml mg ⁻¹ cm ⁻¹	2.7	1.1	2.6	1.3	2.7	1	2.9	1	2.7	0.8	square root
quality	Al	mg g ⁻¹	8.6	3.2	8.8	3.6	8.9	3.1	9.7	3.4	8.3	2.5	square root
	Ba	mg g ⁻¹	0.1	0	0.1	0	0.1	0.1	0.1	0.1	0.1	0	reciprocal
	C	%	39.2	2.3	38.6	2.2	38.3	2.2	37.3	2.7	39.3	2.3	-
	Ca	mg g ⁻¹	16.1	3.9	15.5	3.6	15.3	3.4	16.4	3.6	15.2	2.9	reciprocal
	Fe	mg g ⁻¹	6.5	2.3	6.4	2.6	6.9	2.5	7.8	2.9	6.5	2	-
	K	mg g ⁻¹	6.3	1	7.5	1.9	7.2	2.4	8	2.2	7	1.8	square root
	Mg	mg g ⁻¹	4.5	1.1	4.7	1	4.4	0.9	4.8	0.6	4	0.5	-
	Mn	mg g ⁻¹	0.3	0.1	0.3	0.1	0.3	0.1	0.3	0.1	0.3	0.1	-
	N	%	1.94	0.56	1.72	0.52	1.61	0.59	1.51	0.56	1.45	0.37	square root
	Na	mg g ⁻¹	4	1.8	3.8	2.4	3	2.3	3.2	1.9	2.6	1	square root
	P	mg g ⁻¹	2.8	0.5	3	0.8	2.7	0.8	2.5	0.7	2.5	0.4	-
	S	mg g ⁻¹	3	0.7	2.9	0.7	2.7	0.9	2.8	0.8	2.7	0.6	-
	Sr	mg g ⁻¹	0.1	0	0.1	0	0.1	0	0.1	0	0.1	0	-

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Table S5.2: Pearson correlation matrix among root chemical and morphological characters (transformed residuals). Significant correlation (P -value < 0.05) are marked in bold. Abbreviations: SRL, specific root length; RD, root average diameter; RTD, root tissue density. We grouped root chemical and morphological traits using a Pearson's r of > 0.6. We then selected a representative variable based on biological relevance. When two traits within a group were of equal biological relevance, we ran linear models with mass loss and chose the variable that provided a better fit to mass loss (based on AIC).

	Lignin	C	Al	Ba	Ca	Fe	K	Mg	Mn	N	Na	P	S	Sr	Zn	SRL	RD
C	-0.33	-															
Al	0.38	0.81	-														
Ba	-0.02	0.52	0.59	-													
Ca	-0.03	0.33	0.49	0.52	-												
Fe	0.38	0.82	0.95	0.66	0.53	-											
K	-0.31	0.01	0.12	0.39	0.05	0.05	-										
Mg	-0.29	0.57	0.65	0.55	0.59	0.60	0.15	-									
Mn	0.28	0.55	0.73	0.58	0.51	0.82	0.06	0.43	-								
N	-0.65	0.23	0.05	0.12	0.24	0.06	0.07	0.35	0.03	-							
Na	-0.55	0.32	0.22	0.17	0.05	0.28	0.05	0.34	0.21	0.62	-						
P	-0.64	0.42	0.40	0.00	0.00	0.39	0.45	0.19	0.29	0.51	0.74	-					

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S	-0.67	0.37	0.28	0.01	0.18	0.28	0.22	0.30	0.20	0.76	0.87	0.81					
Sr	-0.52	0.09	0.01	0.44	0.69	0.07	0.33	0.47	0.18	0.56	0.49	0.59	0.67				
Zn	-0.12	0.29	0.48	0.50	0.53	0.41	0.04	0.60	0.28	0.39	0.21	0.20	0.28	0.46			
SRL	0.23	0.23	0.41	0.16	0.22	0.35	0.20	0.25	0.27	0.15	0.04	0.21	0.00	0.02	0.34		
RD	-0.23	0.13	0.25	0.04	0.19	0.18	0.26	0.11	0.18	0.00	0.00	0.28	0.11	0.05	0.19	0.82	
RT																	0.2
D	-0.21	0.22	0.33	0.18	0.23	0.32	0.05	0.28	0.25	0.05	0.09	0.01	0.11	0.09	0.33	0.60	0

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Table S5.3. Pearson correlation matrix among soil biotic community and abiotic conditions (transformed residuals). Significant correlation (P -value < 0.05) are marked in bold. Abbreviations: P_tot, soil total labile P; Pi, soil inorganic labile P; N_tot, soil total inorganic nitrogen; SWC, soil water content; BR, basal respiration; C_{mic}, microbial biomass C.

	N:P	P_tot	Pi	NO ₃	NH ₄	N_tot	SWC	Temperature	Bulk density	BR	C _{mic}	Oribatida
P_tot	-0.59											
Pi	-0.50	0.84										
NO ₃	0.57	-0.22	-0.17									
NH ₄	0.59	-0.02	-0.04	0.13								
N_tot	-0.75	0.12	0.07	-0.65	-0.74							
SWC	0.10	0.03	-0.04	0.21	-0.04	-0.12						
Temperature	0.25	0.08	0.17	0.14	0.41	-0.39	-0.12					
Dry bulk density	0.28	-0.12	-0.03	0.14	0.33	-0.28	-0.34	0.41				
BR	-0.04	0.19	0.10	-0.12	0.09	-0.03	0.37	-0.09	-0.23			
C _{mic}	0.01	-0.03	0.00	-0.18	0.04	0.13	0.36	-0.12	-0.19	0.63		
Oribatida	-0.27	0.11	0.08	-0.09	-0.20	0.21	0.01	0.06	-0.28	0.13	0.17	
Collembola	-0.16	-0.14	-0.22	-0.05	-0.31	0.20	0.22	-0.15	-0.32	0.13	0.09	0.18

Root chemistry and soil fauna explain the plant diversity effect on decomposition

Table S5.4. Summary of linear models with individual variables against plant species richness (SR) and presence/absence of grasses and legumes and linear models with mass loss against individual variables. All linear models were performed with transformed residuals after spatial auto-correlation being removed. Abbreviations: SRL, specific root length; RD, average root diameter; RTD, root tissue density; BR, basal respiration; C_{mic}, microbial biomass C; P_{tot}, soil total labile P; Pi, soil inorganic labile P; N_{tot}, soil total inorganic nitrogen; SWC, soil water content. Significant estimates (*P*-value < 0.05) are marked in bold. Variables included in the *a-priori* structural equation model are in bold.

Pathway	Variable	SR		Grass		Legume		<i>R</i> ²	<i>P</i> -value	Mass loss			
		<i>Estimate</i>	<i>P</i> -value	<i>Estimate</i>	<i>P</i> -value	<i>Estimate</i>	<i>P</i> -value			<i>Estimate</i>	<i>P</i> -value	<i>r</i> ²	AIC
Root	Lignin	0.019	0.349	0.278	<0.001	-0.267	<0.001	0.49	<0.001	-3.751	<0.001	0.58	197.5
litter quality	Al	-0.002	0.976	0.313	0.047	-0.131	0.405	0.08	0.138	-0.569	0.033	0.06	254.6
	Ba	0.013	0.235	-0.025	0.375	0.019	0.500	0.05	0.365	0.900	0.561	0.00	258.9
	C	-0.112	0.598	-1.259	0.024	0.465	0.401	0.11	0.052	0.097	0.200	0.02	257.6
	Ca	0.004	0.595	-0.015	0.489	0.022	0.314	0.03	0.504	-1.240	0.539	0.01	258.9
	Fe	0.120	0.562	1.140	0.036	-0.333	0.536	0.09	0.082	-0.176	0.022	0.07	253.8
	K	0.136	<0.001	-0.456	<0.001	-0.118	0.248	0.27	<0.001	1.211	<0.001	0.15	247.4
	Mg	-0.039	0.605	-0.207	0.293	0.361	0.070	0.07	0.151	0.697	<0.001	0.15	247.6
	Mn	0.004	0.596	0.035	0.087	-0.003	0.875	0.06	0.220	-3.936	0.057	0.05	255.5
	N	-0.056	<0.001	-0.123	<0.001	0.217	<0.001	0.55	<0.001	3.530	<0.001	0.22	241.0
	Na	-0.088	0.019	-0.346	<0.001	0.395	<0.001	0.39	<0.001	1.634	<0.001	0.30	234.0
	P	-0.060	0.201	-0.661	<0.001	0.335	0.008	0.42	<0.001	1.490	<0.001	0.42	219.8
	S	-0.095	0.050	-0.757	<0.001	0.699	<0.001	0.57	<0.001	1.089	<0.001	0.31	232.3
	Sr	0.000	0.895	-0.011	<0.001	0.008	0.004	0.34	<0.001	45.301	<0.001	0.15	248.0

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Pathway	Variable	SR	Grass		Legume		R^2	P -value	Mass loss			AIC	
		<i>Estimate</i>	<i>P</i> -value	<i>Estimate</i>	<i>P</i> -value	<i>Estimate</i>	<i>Estimate</i>	<i>P</i> -value	r^2	<i>Estimate</i>	<i>P</i> -value		r^2
	Zn	-0.020	0.049	-0.020	0.439	0.032	0.220	0.09	0.083	2.788	0.078	0.04	256.0
	SRL	-790.032	0.145	957.918	0.491	687.864	0.623	0.03	0.533	0.000	0.537	0.01	258.9
	RD	0.007	0.470	-0.041	0.107	-0.019	0.462	0.04	0.418	1.921	0.258	0.02	257.9
	RTD	0.003	0.333	0.008	0.230	0.005	0.507	0.07	0.201	5.407	0.378	0.01	258.5
Soil	BR	0.224	<0.001	-0.091	0.528	-0.190	0.193	0.20	0.002	-0.294	0.280	0.02	258.1
biota	C_{mic}	48.633	0.002	-74.589	0.064	-20.068	0.616	0.14	0.014	0.000	0.845	0.00	259.2
	Oribatida	0.130	0.048	0.412	0.016	-0.178	0.293	0.19	0.002	-0.637	0.006	0.10	251.3
	Collembola	0.028	0.639	0.216	0.163	0.211	0.175	0.08	0.147	-0.314	0.251	0.02	257.9
Soil	N:P	-0.006	0.057	-0.007	0.393	0.008	0.346	0.09	0.096	6.841	0.182	0.03	257.4
abiotic	P_tot	0.243	0.047	0.051	0.870	-1.018	0.002	0.16	0.009	-0.144	0.263	0.02	258.0
condition	Pi	0.951	0.283	-0.195	0.932	-7.822	0.001	0.15	0.011	-0.014	0.415	0.01	258.6
	N_{tot}	0.069	0.141	-0.010	0.933	0.080	0.511	0.06	0.262	-0.186	0.599	0.00	259.0
	NO_3	0.004	0.920	-0.111	0.296	-0.088	0.410	0.03	0.613	0.595	0.144	0.03	257.1
	NH_4	-0.067	0.304	0.050	0.766	-0.064	0.706	0.03	0.628	0.021	0.936	0.00	259.3
	Dry bulk	-0.020	<0.001	-0.007	0.570	-0.022	0.091	0.32	<0.001	3.299	0.250	0.02	257.9
	SWC	0.022	0.021	-0.029	0.228	0.009	0.712	0.10	0.062	0.900	0.609	0.00	259.0
	Temperature	-0.074	0.011	0.178	0.017	-0.348	<0.001	0.42	<0.001	-0.334	0.465	0.01	258.7

Table S5.5: Loadings of root chemical traits on the first three principal components (PCs). The first three PCs captured 77% of information in the data. In general, these groups are similar to those from the correlation matrix and are represented by single variables in the SEM included in the main text. PC1 generally represents our metal cation group which we represented in the main text with Mg. PC2 generally represents the group that we represented with lignin in the main text. PC3 is represented by K in the main text.

Variable	PC1	PC2	PC3
Lignin	0.67	1.00	0.14
Al	1.38	-0.15	0.24
Ba	1.00	-0.61	-0.60
C	-1.26	-0.05	0.07
Ca	0.83	-0.79	0.05
Fe	1.41	-0.16	0.09
K	-0.09	-0.48	-1.32
Mg	0.82	-0.99	0.11
Mn	1.18	-0.20	0.07
N	-0.30	-1.12	0.57
Na	-0.63	-1.05	0.44
P	-0.76	-1.06	-0.30
S	-0.64	-1.23	0.18
Sr	0.04	-1.28	-0.21
Zn	0.63	-0.88	0.22

Table S5.6 Structural equation model comparison. Abbreviations: CFI = comparative fit index; RMSEA = root mean square error of approximation; SR = species richness. We wanted to make sure that the structural equation model that we presented in the main text was both stable and generalizable. In the structural equation model using the PCA axes rather than the individual variables as chosen by the correlation matrix – the results were largely similar with the exception of soil temperature. To assess the degree to which soil temperature may have been excluded due to our variable selection process and the stability of soil temperature effect, we ran the SEM using PCA axes through several iterations. When temperature was included in the model – a direct path from species richness to mass loss was no longer necessary (fit improved when the direct path was removed; third vs. second column below, Fig S3 B vs. A). However, allowing for a direct path from species richness to mass loss and removing soil temperature significantly improved the fit of our model (in terms of % explained variation and BIC; forth vs. second and third columns below, Fig S3 C vs. A and B). We then compared this model (using the PCA axes, PCA-SEM (SR)) to the model using individual variables (first column below, Fig. 2). The model using individual variables represented very similar principal components of root chemical traits but explained significantly more variance and had a lower BIC. Thus, we included the model using individual variables rather than the one with the PCA axes in the main text.

		Fig. 2 (main text)	PCA-SEM (temperature+ SR)	PCA-SEM (temperature)	PCA-SEM (SR)
% of explained variation in mass loss	70.3	56.3	58.2	59.0	
χ^2	<i>df</i>	13	14	15	10
	<i>statistics</i>	14.93	10.94	13.63	5.06
	<i>P-value</i>	0.312	0.691	0.554	0.887
CFI		0.990	1.000	1.000	1.000
BIC		1045.6	1273.5	1271.9	1233.4
RMSEA		0.045	0.000	0.000	0.000
	Lower 90% CI	0.000	0.000	0.000	0.000
	Upper 90% CI	0.130	0.090	0.102	0.060

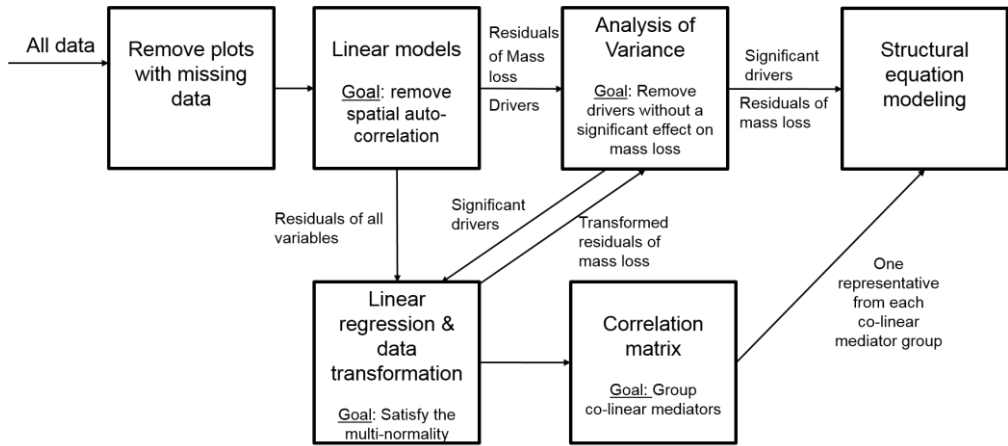


Fig. S5.1 A flowchart for statistical analyses used to select variables to be included in structural equation modeling. Mediators are the variables measured for each pathway

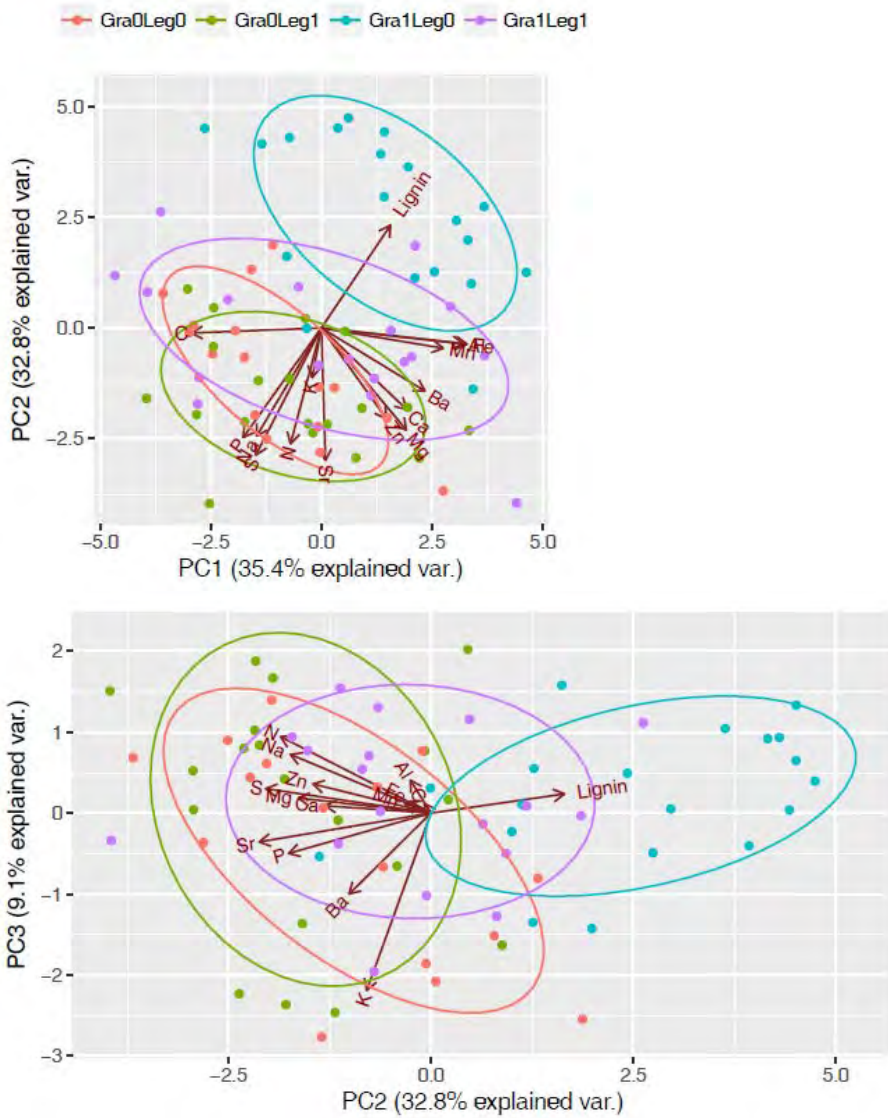


Fig. S5.2. PCA covariance biplot for root chemical traits: A) the first two PCs, B) the second and the third PCs. Gra0Leg0 indicates no grasses or legumes, Gra0Leg1 indicates legumes are present, Gra1Leg0 indicates grasses are present, Gra1Leg1 indicates that both grasses and legumes are present.

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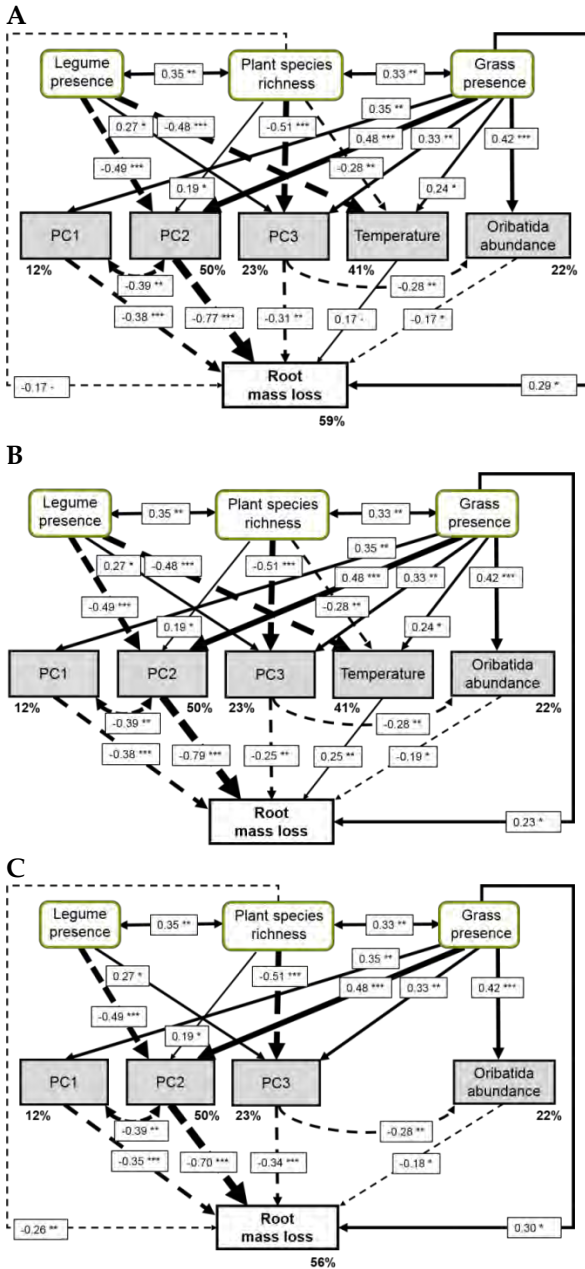


Fig. S5.3 (description on following page)

Fig. S5.3 Structural equation models using PCA components for root chemical traits. A) most parsimonious model including direct link from species richness to mass loss plus soil temperature (PCA-SEM (SR+temperature)), B) most parsimonious model plus soil temperature but without direct link from species richness to mass loss (PCA-SEM (temperature)), and C) most parsimonious model including direct link from species richness to mass loss (PCA-SEM (SR))

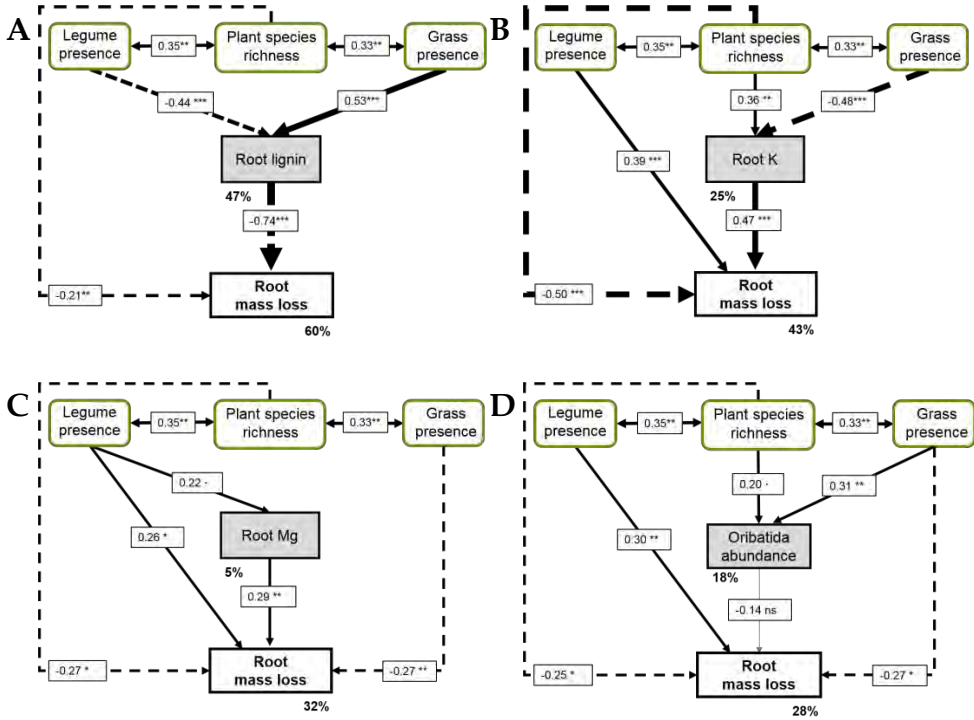


Fig. S5.4 Structural equation models with only one mediator (which was included in the final structural equation model): A) lignin, B) K, C) Mg, and D) Oribatida abundance. From these figures – we concluded that the inclusion of more than one mediator significantly improved the variance explained by our model



Chapter 6 General Discussion



Plant diversity and ecosystem functioning

Plant diversity plays a pivotal role in ecosystem functioning (Hooper et al. 2012; Cardinale et al. 2012). As species loss is accelerating (Steffen et al. 2015), it is now more important than ever to determine how plant diversity affects how ecosystems produce biomass, recycle nutrients, and store carbon. In all ecosystems, the rates of biomass production and decomposition drive key ecosystem functions such as carbon storage and nutrient cycling (Hooper et al. 2005; Cardinale et al. 2011). It is well established that plant diversity promotes plant community productivity aboveground (Hector et al. 1999; Tilman et al. 2001; van Ruijven & Berendse 2009; Marquard et al. 2009) and belowground (Reich et al. 2004; Mueller et al. 2013; Cong et al. 2014; Ravenek et al. 2014). The mechanisms which underlie this relation are predicted to operate belowground (van Ruijven & Berendse 2005; de Kroon et al. 2012). With increasing diversity, greater belowground productivity contributes to greater soil carbon storage (Fornara & Tilman 2008; Steinbeiss et al. 2008a; Cong et al. 2014; Lange et al. 2015), which also depends on diversity-mediated changes in root decomposition. The relation between plant diversity and decomposition is less clear (reviewed by Cardinale et al., 2011), as decomposition may be influenced more strongly by the dominant functional traits in a plant community than by species richness (Hooper et al. 2005). This thesis takes a belowground perspective, and explores the roles of root traits and root-root interactions in mediating the relations between plant diversity and belowground productivity and decomposition. As part of the Jena Experiments, this thesis contributes research on belowground plant diversity-ecosystem functioning relation, complementing research in this long-term project on the aboveground diversity-productivity relation and its underlying factors, and on diversity-related drivers of carbon cycling.

The belowground plant diversity-productivity relationship

The effect of plant diversity on productivity can be partitioned into complementarity and selection effects, which gives insight into why diverse communities are more productive (Loreau & Hector 2001). Increasing productivity with plant diversity could be due to positive interactions between species (complementarity effects) or dominance of productive species (selection effects) in mixtures. A key finding of this thesis was that in

the Jena Trait Based Experiment the positive belowground diversity-productivity relation was due to an increase in belowground complementarity effects (chapter 2), aligning with findings aboveground at the Jena Experiment (Marquard et al. 2009), and at other biodiversity experiments (Loreau & Hector 2001; HilleRisLambers et al. 2004; van Ruijven & Berendse 2005; Fargione et al. 2007). This is the first study to partition the effect of biodiversity on productivity belowground in a field biodiversity experiment, and could indicate that complementarity effects also underlie the positive diversity-productivity relationship belowground in the nearby Jena Experiment (Ravenek et al. 2014) and in other biodiversity experiments (Mueller et al. 2013; Cong et al. 2014). Further, our results in chapter 2 shows that belowground complementarity effects also underlie positive belowground biodiversity effects in a biodiversity experiment without legumes, similar to findings aboveground (van Ruijven & Berendse 2005). Due to their nitrogen fertilization effects, legumes have a large effect on plant community interactions and ecosystem functioning. These effects are well studied at the Jena Experiment (e.g. Temperton et al. 2007; Gubsch et al. 2011b; Roscher et al. 2011). Evidence that positive biodiversity and complementarity effects persist belowground in the absence of legumes is a contribution to biodiversity research.

Resource partitioning is proposed to underlie the positive diversity-productivity relationship (niche complementarity hypothesis, Tilman 1999) and positive complementarity effects (van Ruijven & Berendse 2005; von Felten & Schmid 2008). At the Jena Experiment, tests of resource partitioning have been carried out which have found that resource uptake is higher in mixtures compared to monocultures (Gockele et al.; Oelmann et al. 2011b; a). Oelmann et al. (2011) showed that phosphorus uptake increased over the plant diversity gradient, which did not depend on the presence or absence of specific functional groups. A later study in the same experiment showed that the positive relation between diversity and plant phosphorus uptake could be attributed to increased phosphatase activity with plant diversity, which can increase phosphorus mobilization from organically bound phosphorus to an inorganic, bioavailable form (Hacker et al. 2015). Nitrogen uptake was also found to increase with plant diversity, which was driven by the presence of legumes (Oelmann et al. 2011a). In the Jena Trait

Based Experiment, we tested if resource partitioning was likely to underlie belowground complementarity effects (chapter 2) with a belowground trait based approach. Differences in traits related to resource acquisition, e.g. functional trait diversity, could explain complementarity effects. Functional trait diversity could enhance resource partitioning by differentiating the location, timing, or form of nutrient uptake between plants (McKane et al. 2002). As a community containing more species is predicted to be more diverse in its traits, diversity in functional traits may explain the increase in community productivity with diversity. Previous research, mainly focused on aboveground plant traits, has shown that trait diversity can explain aboveground productivity in grasslands (Petchey, Hector & Gaston 2004; Mokany, Ash & Roxburgh 2008; Cadotte et al. 2009; Schumacher & Roscher 2009), including the Jena Experiment (Roscher et al. 2012). Diversity of vertical root distributions has been predicted to facilitate resource partitioning due to spatial segregation of resource niches (Parrish & Bazzas 1976; Mamolos et al. 1995; Dimitrakopoulos & Schmid 2004; Mommer et al. 2010). Therefore, the expectation is that a high diversity of vertical root distributions leads to increased complementarity effects and greater productivity. However, in the Jena Trait Based Experiment, we did not find a relation between the diversity of species-specific vertical root distributions and complementarity effects (chapter 2). This signals that vertical root segregation at the scale considered does not facilitate complementarity effects, via resource partitioning or other mechanisms, in the Jena Trait Based Experiment. This is in line with results of community level root biomass in the nearby Jena Experiment, where vertical root distribution of the whole plant community did not change over the plant diversity gradient (Ravenek et al. 2014). The factors which underlie positive diversity effects likely differ between environments. In dry environments, the depth or diversity in vertical root distributions has been shown to contribute to the positive diversity-productivity relation (depth- Mueller et al. 2013; diversity-Zhang et al. 2014). Deep rooting species can access soil with higher moisture content than the top soil, benefiting their growth. Shallow rooting species may also benefit due to reduced competition in the top soil, and hydraulic redistribution of water from deep to shallow layers via roots (Prieto et al. 2012). Vertical root segregation may be less advantageous in environments that are not typically water limited, such as the Jena Experiments.

Instead, resource partitioning over soil depth may occur at a finer scale, or result from the diversity in other root traits which influence nutrient uptake, for instance specific root length (Fort, Cruz & Jouany 2014). However, resource partitioning over soil depth was not a major factor underlying greater nutrient uptake in diverse communities in the Jena Experiment (Gockele et al. in review). Resource partitioning could be facilitated by differences in species' preferred chemical form of nitrogen (Weigelt, Bol & Bardgett 2005) or due to changes in species' preferred chemical form of nitrogen in response to growing in different communities (Ashton et al. 2010; Gubsch et al. 2011b). In an Ecotron experiment derived from the Jena Experiment, more diverse plant communities (16 versus 4 species) were found to shift their water uptake to deeper layers to a greater extent during dry (high transpiration demand) periods, although no difference in vertical root distribution was observed (Guderle et al. 2017). This may signal that resource partitioning is dynamic and species may shift their uptake patterns depending on their demands, which may not be reflected by changes in vertical root distribution.

Alternatively, plant-soil feedbacks may drive the positive diversity-productivity relation to a greater extent than resource partitioning. Accumulation of pathogens at low diversity has been shown to underlie the plant diversity-productivity relation (Schnitzer et al. 2011; Maron et al. 2011). Higher plant diversity can dilute the negative effects of pathogens, by increasing the diversity of species specific pathogens, and reducing the abundance of any specific pathogen (Hendriks et al. 2013). Mutualistic soil organisms, e.g. mycorrhizal fungi, have been proposed to mediate facilitation between plants (van der Heijden & Horton 2009; Wagg et al. 2011b; Wright et al. 2017). More diverse arbuscular mycorrhizal fungi communities could extend total niche space and improve individual species performance (Wagg et al. 2011a). Mycorrhizal fungi and nitrogen fixing bacteria are responsible for 5-20% of nitrogen and up to 75% of phosphorus acquired by plants in grasslands; and, along with pathogens, should be considered important drivers of the diversity-productivity relation (van der Heijden, Bardgett & van Straalen 2008). Both negative and positive plant soil feedbacks could simultaneously contribute to diversity effects: at low diversity, interactions with pathogens limit plant growth (negative feedback) while at higher plant diversity interactions with beneficial soil organisms, e.g. mycorrhizal fungi and plant growth promoting rhizobacteria, can

increase productivity (reviewed by: Eisenhauer 2012). At the Jena Experiment, plant diversity was found to have a positive effect on the abundance of anti-fungal producing bacteria. Increase in the abundance of these bacteria was shown to lead to the suppression of the fungal pathogen *Rhizoctonia solani* in an assay experiment (Latz et al. 2012). This indicates that plant community resistance against fungal pathogens may increase with plant diversity. However, soil suppressiveness may differ in more complex environments with multiple pathogens. Considering the entire microbial community of the bulk soil, Dassen et al. (2017) found that plant diversity had a marginally positive effect on fungal richness, but did not affect other microbial groups (bacteria, protists, or archaea). However, as plants have been shown to select their rhizosphere community (Zak et al. 2003; Chung et al. 2007) and root endophyte community (Berg et al. 2005; Gottel et al. 2011), effects of plant diversity may be clearer in these communities. Changes in the root endophyte community composition with plant diversity in the Jena Experiments have not yet been reported. In the Jena Trait Based Experiment, the effect of plant diversity on the composition of the fungal root endophyte communities is being studied (Dassen et al., in prep). An increase in the relative abundance in beneficial root endophytes, and subsequent decrease in pathogens could indicate that plant-endophyte interactions play a role in the positive belowground diversity-productivity relation in the Jena Trait Based Experiment (chapter 2).

In summary, the positive belowground diversity-productivity relation can be attributed to complementarity effects. The lack of relation between the functional diversity in vertical root distribution and complementarity effects suggest that vertical root segregation is not an important factor underlying the positive plant diversity-productivity relation in the vegetation types and under the environmental conditions investigated here.

Root-root interactions and root trait plasticity

Root trait plasticity has been empirically shown (reviewed by: Hodge 2004), and evidence is accumulating that root trait plasticity is influenced by neighbouring species (e.g. Callaway 2002; Schenk 2006; Semchenko, John & Hutchings 2007; Semchenko, Saar & Lepik 2014; Nord, Zhang & Lynch 2011). Trait plasticity can implicate niche

differentiation (Chesson 2000; Gubsch et al. 2011a; Lipowsky et al. 2015), species competition (Fort et al. 2014), and community composition (Callaway et al. 2003), and ecosystem functions including productivity and decomposition. Therefore, root trait plasticity is not only important to characterise, but also to incorporate into the framework of diversity-ecosystem functioning relations. It is largely unknown how plants alter their root traits in response to neighbours in the field, because the roots of different species cannot be visually identified in mixtures. With molecular methods, the relative abundance of species-specific root biomass can be determined, and used to quantify vertical root distribution (chapters 2, 3). Research in chapter 3 showed that species altered their vertical root distribution when grown with interspecific neighbours compared to when grown in monoculture. Furthermore, the direction of this change depended on functional group: grasses became shallower rooted, forbs became deeper rooted when growing with interspecific neighbours. This finding provides the first quantitative evidence in a field biodiversity experiment that functional groups shift a root trait in response to growing with interspecific neighbours. In the nearby Jena Experiment, the role of aboveground trait plasticity in niche differentiation across the plant diversity gradient has been studied in grasses (Gubsch et al. 2011a) and non-leguminous forbs (Lipowsky et al. 2015). Both grasses and forbs responded to increased light competition when growing in diverse communities, altering traits related to light acquisition, i.e. increasing shoot height and specific leaf area (Gubsch et al. 2011a; Lipowsky et al. 2015). The presence of legumes had a large effect on shifts in aboveground functional traits of grasses and forbs, due to nitrogen fertilization effects (Gubsch et al. 2011a; Lipowsky et al. 2015). As the Jena Trait Based Experiment does not contain legumes, there is no nitrogen fertilization effect, and plasticity in response to neighbours would be due to local resource uptake or signals in neighbour's root exudates. Neighbour-induced shifts in functional traits in response to neighbours has two main implications relevant to trait-based approaches in diversity-ecosystem functioning research.

First, root trait plasticity, intraspecific rather than interspecific trait variation, could facilitate belowground biodiversity effects (chapter 2). Plasticity in aboveground traits related to resource use has been found to facilitate functional trait diversity between

species (Grassein, Till-Bottraud & Lavorel 2010). Functional trait diversity has been shown to explain aboveground biodiversity effects (Flynn et al. 2011) and complementarity effects (Roscher et al. 2012). Plasticity in vertical root distribution could facilitate resource partitioning (Mueller et al. 2013; Hernandez & Picon-Cochard 2016), as plasticity could lead to increased nutrient uptake (Hodge et al. 1999). However, we found that belowground relative yield was unrelated to shifts in vertical root distribution. Species altered their vertical root distribution in response to their neighbours, but not to the benefit of increased belowground productivity. In The Jena Experiment, an increase in specific leaf area was associated with increased photosynthetic capacity in grasses (Gubsch et al. 2011a) and forbs (Lipowsky et al. 2015), which increased with plant diversity. Therefore, changes in plant traits over the plant diversity gradient affected aboveground resource uptake. Belowground resource (nutrient) uptake was not considered in our study, however, meaningful increases in nutrient uptake would have translated into increases in biomass. The discrepancy between our results and those from the Jena Experiment could be due to the disconnect between root traits and root function (Mommer & Weemstra 2012; Bardgett et al. 2014), i.e. the resource economic spectrum (described further below in the Outlook section). The second implication is that independent measures of species-specific root traits, e.g. measured in monoculture (chapter 2), pot experiments (chapter 4) or taken from a database, may not represent root trait values in mixtures. For trait based approaches to be useful in explaining ecosystem processes or community dynamics, a key assumption that traits vary more between than within species must be fulfilled (McGill et al. 2006). Trait-based approaches using independently measured trait means have varied in their effectiveness of explaining biodiversity effects (Roscher et al. 2012; Bakker, Mommer & van Ruijven 2016; this thesis, chapter 2) This may be due to intraspecific trait variation, which can be substantial (Hulshof & Swenson 2010; Jung et al. 2010; Albert et al. 2012), but is often neglected in trait based approaches (De Bello et al. 2011). Incorporating intraspecific variation into trait-based approaches has been shown to improve predictions of community trait response to environmental gradients (Jung et al. 2010; Albert et al. 2012; Kichenin et al. 2013; reviewed by Berg & Ellers 2010; Violle et al. 2012) and drought (Jung et al. 2014). Recently, intraspecific variation in specific root length has

been shown to explain variation in aboveground complementarity effects over a species richness gradient in forests (Bu et al. 2017). Although variation is generally greater between than within species, the relative importance of inter- or intraspecific trait variation to the community's trait response to a change in the environment can differ between traits. For instance, intraspecific variation has been found to be greater than interspecific variation for specific leaf area (Messier, McGill & Lechowicz 2010; Auger & Shipley 2013; Kichenin et al. 2013), leaf dry matter content (Messier et al. 2010), and leaf nitrogen (Auger & Shipley 2013). Plasticity in root traits has been demonstrated (Hodge 2004), although comparisons of intraspecific and interspecific variation in root traits are limited. Hajek, Hertel & Leuschner (2013) found that the intraspecific variation in root diameter, specific root area, specific root length, root tip abundance, and root nitrogen was as greater than interspecific variation for three of the eight demes of aspen trees considered. The root nitrogen and phosphorus concentration of woody species has been shown to be more plastic than morphological traits in response to differences in soil fertility (Kramer-Walter & Laughlin 2017). Databases, such as the Fine-Root Ecology Database (FRED, Iversen et al. 2017), which bring together root trait data from the same species grown in different conditions could reveal differences in plasticity between different traits.

Intraspecific variation has been incorporated into measures of functional diversity by including measured traits from multiple individuals within a species, e.g. individual-level functional diversity (Cianciaruso et al. 2009), or by considering trait means per species and intraspecific variability (by replicating the mean by the number of individuals) as input variables (Schleuter, D., Daufresne, M., Massol, F., and Argillier 2010). Functional diversity can also be partitioned into within and between species variation, which allows for comparison of the contribution of intra- or interspecific variation to the functional diversity of a community (De Bello et al. 2011). These approaches require traits to be measured on all individuals (iFD, Cianciaruso et al. 2009) or a random selection of individuals (FD, De Bello et al. 2011). These criteria are difficult to meet when measuring root traits, especially in the field. A new simulation approach developed by Ross et al. (2017) facilitates the incorporation of intraspecific trait variation when data at the individual level (e.g. root traits of a plant individual, rather than a plant

species) are scarce or difficult to measure. This approach could be used to combine independent measures of root traits, while incorporating intraspecific trait variation. For instance, in a pot experiment, root traits could be measured on individual plants exposed to environmental factors like those encountered in the field when growing with neighbours (differences in resource availability, exposure to neighbour exudates, etc.). This trait variation could then be combined with relative abundance data from the field in a simulation approach (e.g. Ross et al. 2017) to incorporate trait plasticity into measures of functional diversity. The diversity indices could then be used to predict ecosystem functions such as community productivity.

We further found that functional groups respond differently to their neighbours: grasses became shallower in mixtures, irrespective of the vertical root distribution of their neighbours; forbs became deeper when growing with deeper rooting neighbours (chapter 3). Trait plasticity has been shown to differ between species with contrasting resource strategies; exploitative species alter their traits more than conservative species (Grassein et al. 2010). . Differences in growth strategy may have influenced the target species' response to its neighbours, and the direction of change. More research is required before generalities of how species or functional groups respond to neighbours can be made. Results in chapter 3 provide evidence that forbs aggregate their roots with neighbouring species. This was shown by relating the change in vertical root distribution from monoculture to mixture to the vertical root distribution of neighbouring species. When forbs were grown with deeper-rooting species, they altered their root distribution in mixtures to become deeper than in monoculture. Root aggregation has been shown in grasslands (Price et al. 2012; Frank et al. 2015) and pot experiments (Gersani et al. 2001; Falik et al. 2003; Bartelheimer et al. 2006; Semchenko et al. 2007). Mechanisms underlying why roots segregate or aggregate were not tested in this thesis. However, root response to neighbours has been shown to depend on the local availability of nutrients (Li et al. 2007b; Cheng 2009; Hodge 2009; Nord et al. 2011; Schmid et al. 2015) or water (Neumann & Cardon 2012), or due to neighbour identity (Falik et al. 2003; Gruntman & Novoplansky 2004; Bartelheimer et al. 2006; Semchenko et al. 2007).

The increase in belowground performance of forbs, but not grasses, in mixtures (chapter 3) could be due to differences in plant-microbe interactions. The size and direction of

feedbacks between the plant and the soil microbial community (i.e. plant-soil feedbacks) have been shown to differ between plant functional groups (Meisner et al. 2014; Cortois et al. 2016). Graminoids (including grasses) have a predominantly negative plant-soil feedback, i.e. a build-up of species-specific pathogens reduces the species' performance (Kulmatiski et al. 2008). Forbs may benefit more from positive plant-soil feedbacks, e.g. due to association with mycorrhizal fungi (Cortois et al. 2016). A more negative plant-soil feedback in grass species could mean that grasses would over-yield due to the 'release' from pathogens in mixtures. However, as grasses are from the same family (Poaceae) in the Jena Trait Based Experiment, they are more likely to grow near a family member in a mixture than forbs, which come from five families. Therefore, forbs may experience a greater release from family-specific pathogens in mixtures. Summarizing, forbs root significantly deeper than grasses in mixture due to plasticity in vertical root distribution. This plasticity was not related to species specific belowground overyielding, and therefore is not a mechanism underlying complementarity effects.

Decomposing the diversity-decomposition relationship

The production of biomass over a diversity gradient is commonly studied as a measure of ecosystem functioning. Less known is the relation between plant diversity and carbon cycling, which could underlie a plant community's capacity to sequester carbon (Milcu et al. 2014). Decomposition of root litter plays a key role in grassland carbon cycling, as most plant biomass is root biomass in grasslands (Poorter et al. 2012), and aboveground biomass is frequently removed for animal feed. Plant community composition can alter litter decomposition via changes in litter quality or changes in the soil environment. We tested the effects of plant diversity and functional group composition on root decomposition in two biodiversity experiments- the Jena Trait Based Experiment (chapter 4) and the Jena Experiment (chapter 5). Results in chapters 4 and 5 consistently show that changes in litter quality, rather than changes in the soil abiotic environment, had a greater effect on root decomposition. Plant diversity did not have a consistent effect on root decomposition in this thesis (chapters 4, 5). Plant diversity reduced decomposition in the Jena Experiment, mainly due to a decrease in litter quality associated with the increase in grass presence and decrease in legume presence (chapter

5). Plant diversity was unrelated to functional group composition in the Jena Trait Based Experiment, and subsequently, did not affect root decomposition (chapter 4). In both experiments, functional group composition, i.e. grass root presence (chapter 5) or abundance (chapter 4), consistently reduced root decomposition by reducing root litter quality.

Our results indicate that root litter traits are an important factor driving decomposition, in line with previous research on root (Silver & Miya 2001) and leaf decomposition (Cornwell et al. 2008). Thus, measures of plant community composition which are related to root traits can predict root decomposition. The negative relation between plant diversity and decomposition in the Jena Experiment, along with the increase in root biomass over the diversity gradient in the same year (Oram et al., unpublished), are in line with the findings of Steinbeiss et al. (2008) and Lange et al. (2015) which show that carbon storage increases with plant diversity and of Milcu et al. (2014) who found that more diverse communities have higher ecosystem carbon uptake rates. The increase in carbon storage in diverse plots was attributed early on to higher root biomass in diverse plots (Steinbeiss et al. 2008a). Lange et al. (2015) found that greater carbon storage in diverse plant communities was related with increases in rhizosphere carbon deposits and microbial activity. Increased microbial activity increased the sequestration of carbon inputs from the roots. Greater root biomass in diverse plots (Oram et al., unpublished) likely contributed to the increase in rhizosphere carbon deposits (i.e. through root exudation and rhizodeposition), and carbon storage found by Lange et al. (2015). Carbon cycling in the Jena Trait Based Experiment is currently being studied with carbon isotope tracing methods, and results are forthcoming (Chen et al., in prep).

Carbon and nutrient cycling are intrinsically connected, and thus, nutrient cycling is influenced by root carbon inputs via rhizodeposition or the decomposition of dead roots (root litter). Feedbacks between these carbon inputs and the microbial community also play a role in nutrient cycling. Inputs of low quality litter (high concentration of recalcitrant carbon compounds and low concentration of nutrients) can foster a soil microbial community with a higher fungal than bacterial biomass (de Vries et al. 2012b). Plant diversity was found to have a marginally positive effect on soil fungal richness in the Jena Experiment (Dassen et al. 2017). A study conducted at the Jena Experiment in

2007 found that the ratio of fungal to bacterial biomass increased with plant functional group richness. The fungal: bacterial ratio increased over part of the plant diversity gradient (from 1 to 8 species) but this relation was not maintained at high species richness (16 and 60 species) (Lange et al. 2014). With increasing time from establishment of the Jena Experiment, this relation may have strengthened. Increases in the biomass and abundance of soil fungi have been found to increase nitrogen retention, and decrease nitrogen loss in species-rich grasslands (de Vries et al. 2012a). Nitrogen uptake has been shown to increase with plant diversity (Oelmann et al. 2011a), a relation which increased in strength over time (Meyer et al. 2016). This could be due to increased nitrogen retention, i.e. reductions in nitrogen lost through leaching, as well as a greater volume of soil explored in diverse plots, increased uptake efficiency. In the Jena Trait Based Experiment, where there are no legumes, nitrogen cycling, and effects on ecosystem functioning related to nitrogen fertilization will differ from the Jena Experiment. This may lead to an increase in the fungal: bacterial biomass ratio, compared to in the Jena Experiment. We found that nitrogen was not the most important factor in determining root decomposition in the Jena Trait Based Experiment, indicating that nitrogen does not limit decomposition (chapter 4). This could be due to a greater abundance of saprotrophic fungi, which play an important role in decomposition and nutrient cycling (Deacon et al. 2006), especially in low-nutrient environments.

At the Jena Experiment, carbon and phosphorus cycling has been shown to become more closely coupled with increasing plant diversity. Microbial carbon use efficiency (microbial biomass produced per unit carbon substrate) was shown to increase with plant diversity, increasing phosphatase activity (Hacker et al. 2015). This aligns with findings that microbial biomass (Eisenhauer et al. 2011a) and plant phosphorus uptake (Oelmann et al. 2011b) increase with plant diversity. This is also supported by results in this thesis. Reduced decomposition of root litter in diverse plots (chapter 5) may cause phosphorus limitation, causing plant roots and soil microbes to increase exudation of phosphatase enzymes (Olander & Vitousek 2000). In diverse communities, greater root biomass (Ravenek et al. 2014) provides a greater surface area and labile carbon compounds for microbes, which could have caused the increased microbial carbon use efficiency (Hacker et al. 2015).

The traits which best characterized the plant community composition effects on root decomposition differed between experiments (chapters 4 and 5). Grass presence reduced root decomposition by contributing root litter with high lignin and low potassium concentrations in the Jena Experiment (chapter 5), and through an increase in specific root length (root length per root mass, $m\ g^{-1}$) in the Jena Trait Based Experiment (chapter 4). Differences in the root traits underlying the plant community-root decomposition relationship in chapters 4 and 5 are in part due to differences in traits tested and trait ranges. Legume presence in the Jena Experiment, but not in the Jena Trait Based Experiment, led to a larger range of root trait values in the Jena Experiment. Further, root traits were measured on the root litter in the Jena Experiment; in the Jena Trait Based Experiment, the community weighted mean of root traits was calculated using an independent measure of root traits weighted by species-specific relative abundances of the root litter (chapter 4). The community weighted mean describes changes in traits resulting from changes in species composition, but does not incorporate intraspecific trait variation/trait plasticity which could result from growing with interspecific neighbours (see discussion in the previous section).

The negative effect of plant diversity on root decomposition in the Jena Experiment was associated with an increase in Oribatida (mite) abundance (chapter 5). The increase in Oribatida abundance may be facilitated by poorer litter quality in diverse plots, which promotes accumulation of fungi (de Vries et al. 2012b), the preferred food source of Oribatida (Siepel & Ruiter-Dijkman 1993). The role of fungi in litter decomposition is expected to increase with more recalcitrant litter, as fungi are capable of degrading lignin, whereas bacteria are not (de Boer et al. 2005; van der Heijden et al. 2008). Thus, Oribatida mites could signal that fungal decomposition is predominant in communities with poor quality litter (diverse or grass rich communities, chapters 4, 5). This result indicates that feedbacks between the plant community, litter quality, and the soil biotic community affect decomposition. Plant diversity could alter root decomposition by altering soil microbial community composition, via root exudate diversity (Steinauer, Chatzinotas & Eisenhauer 2016), and increased belowground productivity (Zak et al. 2003). Greater plant productivity, and thus, more plant-derived organic matter inputs, can favour soil fungal abundance (Zak et al. 2003; Lange et al. 2014). Functional groups

can also influence soil microbial community composition. For example, the presence of legumes was related to a decrease in the soil fungal: bacterial ratio in the Jena Experiment, due to a decrease in soil fungi (Habekost et al. 2008; Lange et al. 2014). To better understand how plant diversity alters decomposition, future research should address how plant diversity alters the decomposer food web, via shifts in community traits due to species abundance or plasticity, and how this influences root decomposition. In summary, plant community composition influences root decomposition predominantly via changes in litter quality. Root traits and functional group composition explain root decomposition better than plant diversity.

Outlook

Trait-based approaches are essential to better characterise the ‘roots’ of diversity-ecosystem functioning relationships. In this thesis, the effect of plant diversity on two key ecosystem functions- belowground productivity and decomposition- were shown to be mediated by beneficial belowground interactions (i.e. complementarity effects, chapter 2), and by root traits (chapters 4, 5). The diversity or plasticity in vertical root distribution did not facilitate complementarity effects (chapters 2, 3, respectively). However, it is likely that the diversity and plasticity in other root traits do facilitate complementarity effects; as plant traits have been shown to explain resource uptake strategies (Freschet et al. 2010; Reich 2014), plant competition (Kunstler et al. 2016), and plant-soil feedbacks (Baxendale et al., 2014; Cortois et al., 2016). In this outlook, two factors that could increase the success of trait based approaches in explaining complementarity effects: better established root trait-function relations and incorporation of intraspecific trait variation, will be discussed.

Trait based approaches should include ‘all traits that are important for the function of interest’ (Petchey & Gaston 2006). To find links between the diversity in or dominant root traits and complementarity effects, the root trait(s) considered must perform or largely contribute to a growth limiting functions, e.g. resource uptake, defence metabolism, interactions with the microbial community. Therefore, well described trait-function relations are necessary to inform the selection of traits for trait-based models that aim to predict complementarity effects or ecosystem functions. Aboveground, leaf traits (e.g.

specific leaf area) have been closely related to functions (e.g. rate of carbon assimilation and respiration) (Poorter & Bongers 2006). Belowground, trait-function relations are not as well described as they are aboveground. This could be one of the reasons that trait-based approaches, such as the resource economic spectrum (a suite of traits which predict a plant species' resource uptake and growth strategy), are well established aboveground (Wright et al. 2004), but not belowground (Reich 2014; Weemstra et al. 2016). A well-established root economic spectrum, related to resource (Weemstra et al. 2016) and carbon economies (Roumet et al. 2016) could improve predictions of how root traits influence belowground interactions (e.g. complementarity effects, chapter 2), and ecosystem functions such as belowground community productivity (chapter 2), and root decomposition (chapters 4, 5). A whole plant economic spectrum has been established in some biomes, e.g. the subarctic (Freschet et al. 2010). The establishment of consistent correlations between leaf and root traits could alleviate the need to quantify root traits. However, roots reside in a vastly different environment than leaves, and are therefore not ubiquitously analogous in terms of trait-function relations. Grassland species' root traits have been shown to mirror leaf traits in terms of trait-function relations in some studies (Craine et al. 2002a), but not in others (Craine et al. 2005; Orwin et al. 2010), and the degree of correlation differs between traits (Tjoelker et al. 2005). Thus, the establishment of consistent root trait-function relations, with consideration of interactions with the soil abiotic and biotic environment, is necessary to inform trait based approaches. Moreover, most plants rely to some extent on relations with soil dwelling microbes, i.e. arbuscular mycorrhizal fungi (AMF), for resource acquisition (e.g. Klironomos 2003). AMF, and the mycelium networks it forms between plants, can mediate plant competitive interactions (Wagg et al. 2011b), community structure (Reynolds et al. 2003), and facilitation between plants (van der Heijden & Horton 2009). Therefore, plant response to AMF should be considered an important root functional trait (Hempel et al. 2013), and included in trait based approaches to elucidate the mechanisms underlying complementarity effects.

Root traits can change in the presence of interspecific neighbours (chapter 3, and references therein). This trait plasticity (or intraspecific variation) presents a challenge for belowground trait based approaches, as root traits of individuals cannot be measured

in mixtures (with certain exceptions, see Baxendale et al. 2014). Intraspecific trait variation in leaf traits can be substantial (Messier et al. 2010; Auger & Shipley 2013; Kichenin et al. 2013). Including variation in leaf and stem traits has improved predictions of a plant community's response to changes in the environment (Jung et al. 2010; Albert et al. 2012; Kichenin et al. 2013). New analytical techniques that can include intraspecific trait variation without measuring traits of every individual (Ross et al., 2017) offer an option for belowground trait based approaches (discussed in greater detail above in 'Root-root interactions and root trait plasticity'). Root traits are highly plastic (e.g. Hodge 2004), and have been shown to explain a variety of ecosystem processes (Bardgett et al. 2014). Thus, considering intraspecific root trait variation in trait-based approaches could better explain diversity-ecosystem functioning relations than trait-based approaches that only include interspecific variation.

Finally, taking a lesson from roots themselves, gaining a mechanistic understanding of diversity-ecosystem functioning relations will require complementarity in biodiversity studies in the field and greenhouse or laboratory. Field experiments provide a study system which is closer to 'reality', from which relations can be observed and considered. Hypotheses can be formed on why these relations occur, and tested in a controlled setting in the greenhouse to determine the factors which contribute to the relations observed in the field. For instance, isolating and testing hypotheses on the underlying causes of complementarity effects observed in the field (chapter 2) on experimental plant communities in a more controlled setting. Candidate mechanisms could be tested, for example the presence of certain soil organisms (e.g. mycorrhizal fungi, plant growth promoting bacteria, meso- and macro- fauna involved in nutrient cycling such as earthworms), changes in root traits due to neighbour interactions, and increases in nutrient uptake or nutrient use efficiency across a diversity gradient. Perhaps, like combining multiple species leads to greater root productivity, combining field and greenhouse studies will result in greater research productivity. This thesis contributes a belowground, field perspective to research on how root interactions and plasticity contribute to diversity-ecosystem functioning relations. This is imperative for describing patterns that happen in an environment that is experimentally manipulated, but is still close to 'reality'. One way in which the research in this thesis could be strengthened is

by considering patterns found in the field in a more controlled environment, to better elucidate underlying mechanisms. Indeed, “extracting generalities from laboratory experiments is as challenging as extracting causalities from field experiments” (Lubbers 2014).

Concluding remarks

Biodiversity positively affects ecosystem processes (see reviews: Balvanera et al. 2006; Cardinale et al. 2006; Díaz et al. 2006), and thus, biodiversity loss can impair ecosystem functioning (Hooper et al. 2005, 2012; Cardinale et al. 2012). Understanding these relations from a belowground plant perspective can give rise to a mechanistic understanding how plant diversity influences ecosystem functioning. This thesis showed that the positive belowground diversity-productivity relation can be attributed to belowground complementarity effects, paralleling patterns aboveground. A potential mechanism underlying complementarity effects, vertical root segregation, was found to be unrelated to complementarity effects. It was not the diversity, nor the plasticity in vertical root distributions that led species to overyield in mixtures. Links between root traits and complementarity effects should be further explored with consideration for root trait plasticity and root trait-function relations. Well-established root trait-function relations would better enable trait selection for belowground trait-based models. Plasticity in response to neighbouring species occurs, and may also contribute to complementarity effects. Finally, the role of root associated microbes in complementarity effects should also be further explored (see above sections).

Plant diversity did not have a clear and consistent effect on root decomposition. Instead, there was a consistent effect of functional group composition on decomposition via its influence on litter quality: grasses reduced root decomposition. The effects of the plant community on root decomposition were shown to be mediated by root traits, highlighting that root traits are important predictors of root decomposition. Feedbacks between plant diversity, litter quality, and the soil decomposer community also influenced root decomposition. By litter quality through changes in species composition or root traits, plant diversity can influence the decomposer community, leading to changes in root decomposition. Exploration of these feedbacks could further uncover the

mechanisms by which the plant community (its diversity or functional group composition) alters root decomposition. This would improve predictions of how species loss could influence decomposition of root litter, and associated effects on carbon cycling and storage.

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Summary

Roots are plants' connection to Earth, providing stability, acting as a vector for resource uptake, and as a mediator of interactions between microbes and neighbouring plants belowground. Tangled and elusive, roots are a difficult muse to study. Nevertheless, research and practice has begun to unearth the ways in which roots function, and the mechanisms that underlie the interactions between roots and their biotic and abiotic environment. The life of a root may at first seem dark and dull; however, closer examination reveals the vibrant, dynamic, and unique nature of a root's existence. This thesis headed belowground in the Jena Biodiversity Experiments, to find out the effect of plant diversity on root production and decomposition. The role of root traits as underlying factors in these relations was tested.

Increasing plant species richness led to increasing root standing biomass; i.e. there was a positive net effect of biodiversity on productivity (**chapter 2**). The positive net effect of biodiversity on productivity can be partitioned into complementarity effects and selection effects. Complementarity and selection effects are not mutually exclusive, i.e. both can be positive or negative at the same time. Positive complementarity effects occur when, on average, species produce more biomass in mixture than expected, based on their biomass production aboveground (i.e. a higher than expected relative yield). Selection effects are positive when species that are highly productive in monocultures have the highest relative yields in mixtures. In the Jena Trait Based Experiment, the positive diversity-productivity relation could be attributed to complementarity effects (**chapter 2**).

Resource partitioning and niche differentiation are often proposed to facilitate complementarity effects. Segregation of vertical root distribution could allow species to occupy distinct areas (niches) in the soil profile, reducing spatial overlap in nutrient uptake. Vertical root segregation could facilitate complementarity effects, and has been hypothesized to be a potential mechanism underlying the diversity-productivity relation. Vertical root segregation could occur if species in a plant community had diverse root distributions. For example, if a mixture is composed of a species with a shallow root distribution, mainly occupying the topsoil, and a deep-rooted species (perhaps with a tap-root) which places roots more evenly throughout the soil, it could be

expected that niche differentiation would take place. A greater volume of soil is explored, more nutrients are taken up from the same area of land by the two-species community than when the species are grown in monocultures, and thus, the mixture yields more than is expected, based on monoculture yield. Complementarity effects could arise as both species may benefit from reduced intra-specific competition belowground. To test if this was the case, species-specific vertical root distribution was measured in monoculture, and weighted by species relative abundance in mixtures to calculate functional diversity. In contrast to what was expected, no relation between the functional diversity of vertical root distribution and complementarity effects was found (**chapter 2**). This may be because: (1) species alter their vertical root distribution in mixtures, (2) species have other strategies for resource partitioning in mixtures, or (3) niche differentiation via vertical root segregation is less important than other factors for overyielding in mixtures.

The first option was further explored. Do species alter their vertical root distribution in mixtures? Indeed, species do (**chapter 3**). Vertical root distribution is plastic, aligning with previous research that shows that plants respond to the soil biotic and abiotic environment by altering their root traits. The direction of shift in vertical root distribution differed between species: in mixtures, grasses became shallower, forbs became deeper (**chapter 3**). How species responded to their neighbours also differed between functional groups. Forbs rooted deeper when growing with deep rooting neighbours, evidence for root aggregation; grasses did not respond to the vertical root distribution of their neighbours. Forbs over-yielded belowground, i.e. forbs had a higher mean belowground relative yield than expected, based on their yield in monoculture. Grasses, on the other hand, did not (**chapter 3**). Were forbs able to over-yield due to their change in vertical root distribution? It may be that species alter their vertical root distribution in order to place their roots in areas that are beneficial in terms of nutrient uptake. However, the change in vertical root distribution was not linked to an increase in the species' belowground relative yield (**chapter 3**).

In diverse mixtures, more biomass is accumulated belowground (**chapter 2**), which is a product of production, but also decomposition. Greater biomass production sequesters more carbon from the atmosphere through photosynthesis. In combination with carbon

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assimilation through plant biomass production, the release of carbon to the atmosphere through decomposition of plant litter also informs predictions of carbon storage in grasslands. Previously, plant diversity was found to have a negative effect on decomposition in the Jena Experiment. This was mainly due to an increase in the presence of grasses over the diversity gradient. Grasses produce relatively recalcitrant litter (compared to forbs or legumes), which has been shown in a number of studies to decompose slower than litter of other functional groups. Here, the same dataset was considered with structural equation modelling to uncover the role of root traits, and the soil abiotic and biotic environment. Plant diversity had a negative influence on root decomposition, more diverse mixtures decomposed slower (**chapter 5**). There were also large functional group effects. Grass presence had a strong negative effect on root decomposition, legume presence had a strong positive effect (**chapter 5**). In a biodiversity experiment without legumes, the Jena Trait Based Experiment, plant diversity did not have an effect on root decomposition, likely because it did not influence functional group composition (**chapter 4**). The abundance of grass roots was negatively related to root decomposition via changes in litter quality (**chapter 4**).

Plant community composition influenced root decomposition via changes in litter quality, specifically, changes in root traits (**chapter 4, 5**). In the Jena Main Experiment, plant diversity and the presence of grasses also influenced the abundance of oribatid mites. Root potassium concentration and the abundance of oribatid mites were positively related to plant diversity. More diverse communities produced root litter that had a higher potassium concentration, promoting decomposition. However, the overall effect of plant diversity on root decomposition was negative due to greater abundance of oribatid mites, which were negatively related to root decomposition. Plant diversity also had a negative influence on decomposition that was not explained by root traits or other factors considered (**chapter 5**). Legumes promoted decomposition as litter quality improved (low root lignin and C:N ratio) when legumes were present in the litter. Grasses reduced decomposition by reducing litter quality (increasing root lignin and root C:N, decreasing root potassium) (**chapter 5**). In the Jena Trait Based Experiment, root lignin and potassium data were unfortunately unavailable. Instead, the morphology of the root system was considered. A root's morphology determines its contact with the

soil, and the plethora of organisms who call the soil home. Specific root length and root diameter were found to play a role in mediating the effect of grass root abundance on root decomposition (**chapter 4**). The common thread between these chapters is that functional group composition can explain variation in root decomposition through shifts in litter quality (**chapters 4, 5**). This thesis shows that both root chemical and morphological root traits can aid in explaining the effects of plant community composition on root decomposition (**chapters 4, 5**).

To conclude, the belowground diversity-productivity relation was attributed to increasing complementarity effects. Research in this thesis highlighted the role of plant functional groups. Functional groups differed in how they altered their vertical root distribution in response to neighbours; forbs rooted deeper, grasses shallower. Forbs over-yielded belowground, grasses did not. Functional groups differed in their root traits, which led to differences in litter quality and root decomposition. Root traits were shown to be important in mediating plant community composition-root decomposition relations. However, the roles of root traits in facilitating complementarity effects and the diversity-productivity relation were not clearly elucidated in this thesis. Vertical root distribution was not an important factor underlying complementarity effects. Further research on how root traits relate to functions (i.e. resource uptake), and how the diversity or prominence of certain root traits in a plant community affects ecosystem functioning is needed. From this thesis, it can be recommended that to best explain and predict ecosystem functions such as productivity and decomposition, measures of plant community composition and trait based approaches should be considered.

Samenvatting

Plantenwortels vormen de connectie tussen plant en bodem. Ze zorgen voor stabiliteit, faciliteren grondstof opname en fungeren als intermediar in interacties tussen microben en buurplanten. Verstrengeld en ontastbaar, wortels zijn een moeilijke muze om te bestuderen. Desalniettemin is de wetenschap en praktijk begonnen de manier waarop wortels functioneren en het onderliggende mechanisme tussen de interactie van wortels en hun biotische en abiotische omgeving aan het licht te brengen. Het leven van een plantenwortel lijkt op het eerste gezicht donker en saai; nader onderzoek onthult echter een levendig, dynamisch en uniek bestaan. Dit proefschrift dook ondergronds in het Jena Biodiversiteits Experiment om het effect van plantendiversiteit op het ondergrondse functioneren van het ecosysteem, plantenwortel productie en afbraak te achterhalen. De rol van plantenworteleigenschappen als onderliggende factoren in deze relaties werd getest.

Wij hebben geconstateerd dat planten gemeenschappen met meer soorten, meer wortelbiomassa produceerden vanwege gunstige interacties tussen soorten, oftewel positieve 'complementariteits effecten' (**hoofdstuk 2**). In het algemeen produceerden soorten meer wortelbiomassa wanneer zij in mengsels groeiden dan wat op basis van hun groei in monoculturen verwacht werd. Dit kan komen door de grondstof verdeling. Verschillen in wortelplaatsing van soorten kan leiden tot een gunstige verdeling van de nutriënten over de verschillende plantensoorten in biodiverse gemeenschappen. Soorten nemen in dat geval grondstoffen op uit verschillende gebieden in de bodem, wat resulteert in een hogere totale nutriënten opname over de gehele gemeenschap en ook een hogere productie. Een grotere diversiteit in plantenwortelverdeling in de bodem kan dus de grondstof verdeling faciliteren en de complementariteits effecten ten grondslag liggen. In tegenstelling tot wat wij verwachtten, werd er geen relatie tussen de functionele diversiteit van verticale wortelverdeling en complementariteits effecten gevonden (**hoofdstuk 2**). Soorten kunnen echter hun verticale wortelverdeling veranderen in reactie op hun burens. En dit doen ze inderdaad (**hoofdstuk 3**). In mengsels wortelden grassen oppervlakkiger en kruiden juist dieper. Alleen kruiden reageerden op de wortelverdeling van hun burens en verschoven hun wortelverdeling om te clusteren met hun burens. Was deze verschuiving in verticale wortelverdeling gerelateerd aan een

verhoogde productiviteit? Helaas, nee. Soorten veranderen hun wortelverdeling, maar hun groei heeft hier geen baat bij (**hoofdstuk 3**).

Een hogere ondergrondse biomassa in biodiverse mengels samen met de snelheid van wortelafbraak, beïnvloedt de koolstofopslag en cyclus. Wij constateerden dat functionele groepssamenstelling, en niet planten diversiteit, consistente effecten had op wortelafbraak. Grassen reduceerden de decompositie door een lagere kwaliteit in hun wortelstrooisel (**hoofdstuk 4**). De samenstelling van de plantengemeenschap beïnvloedde wortelafbraak via veranderingen in strooisel kwaliteit, d.w.z. veranderingen in worteleigenschappen (**hoofdstuk 4, 5**).

Dit proefschrift toonde aan dat plantendiversiteit de ondergrondse productiviteit verhoogt door complementariteitseffecten, oftewel gunstige interacties tussen soorten. De onderliggende oorzaken blijven onbekend. Verticale wortelverdeling was geen belangrijke factor die complementariteitseffect ten grondslag ligt. Nader onderzoek naar hoe worteleigenschappen betrekking hebben op functies (d.w.z. grondstof opname) en hoe de diversiteit of prominentie van bepaalde worteleigenschappen in een plantengemeenschap het functioneren van een ecosysteem beïnvloedt, is nodig. Veranderingen in de kwaliteit van wortelstrooisel bleek de belangrijkste reden voor een veranderingen in wortelafbraaksnelheid. Metingen gedaan aan plantengemeenschap samenstelling die gerelateerd zijn aan planteneigenschappen, d.w.z. functionele groepssamenstelling, kunnen dus mogelijk de wortelafbraak beter voorspellen dan soorten rijkdom op zichzelf.

Op basis van dit proefschrift wordt aanbevolen dat voorspellingen over ecosysteem functies zoals productiviteit en afbraak, het beste een maat voor de samenstelling van de plantengemeenschap en op planteneigenschappen gebaseerde benaderingen overwogen kan worden.

Thank you

This book is the product of the combined efforts of many people, and with great honesty I can attest that there would be no book without them. The list of people who have contributed to this thesis and left a positive mark on me during this time is (thankfully!) quite long. So, the fear of forgetting someone (and the beauty in brevity!) lead me to first opt for a general thank you here, and a longer personal thank you later (over beers!). And so, I want to say, 'bedankt allemaal', this journey has been quite the experience (as I was promised by my mother during the skype conversation in August 2013, looking out the window where in true Dutch fashion, it was pouring rain). However, some of my near and dear have informed me that the acknowledgements section is likely the only part of this book that they will read, they encouraged me to go on a bit. I'd like to thank the following people for the opportunities, challenges, lessons, and joys that they have brought to my life during this PhD (and all the years that led to it).

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About the Author

Natalie Justine Oram was born in Moose Jaw, Canada on January 4, 1987. She grew up on a farm near Bridgeford, Saskatchewan. On the farm, Natalie especially liked working with the cattle. Checking pastures on hot windy days in an old and partially antiquated orange truck- with shoddy breaks and a metal stick hap-hazardly sticking out of the floor to set the beast into gear- remain one of the most calming and grounding memories she has. Perhaps leading to an affinity for natural grasslands?

Natalie holds a BSc. in Agricultural Science, with a minor in Rural Extension and Capacity Development from the University of Guelph, in Ontario, Canada, and a MSc. in Organic Agriculture, specialization Agroecology from Wageningen University, Wageningen, the Netherlands and ISARA Lyon, France. After working on priming and memory response in *Brassica rapa* at the Freie Universität, Berlin, Germany, Natalie returned to Wageningen for her PhD, which has resulted in many memories, cakes, long runs, and the book which you are holding.

Natalie enjoys trekking in the mountains, baking, running (two hobbies which she's found to be quite complementary), and time spent with friends and family.



Publications

- Chen, H., **Oram, N.J.**, Barry, K.E., Mommer, L., van Ruijven, J., de Kroon, H., Ebeling, A., Eisenhauer, N., Fischer, C., Gleixner, G., Gessler, A., González Macé, O., Hacker, N., Hildebrandt, A., Lange, M., Scherer-Lorenzen, M., Scheu, S., Oelmann, Y., Wagg, C., Wilcke, W., Wirth, C. & Weigelt, A. (2017) Root chemistry and soil fauna, but not soil abiotic conditions explain the effects of plant diversity on root decomposition. *Oecologia*, 185, 499–511.
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Affiliations of Co-authors

Kathryn Barry	Spezielle Botanik und Funktionelle Biodiversität, Institute of Biology, University of Leipzig, Germany
Hongmei Chen	Spezielle Botanik und Funktionelle Biodiversität, Institute of Biology, University of Leipzig, Germany
Hans de Kroon	Department of Experimental Plant Ecology, Institute for Water and Wetland Research, Radboud University, The Netherlands
Anne Ebeling	Population Ecology Group, Institute of Ecology, Friedrich-Schiller-University Jena, Germany
Nico Eisenhauer	German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany, Institute of Biology, University of Leipzig; Germany
Christine Fischer	Institute of Geosciences, Friedrich-Schiller-University, Jena, Germany, Department of Conservation Biology, Helmholtz Centre for Environmental Research, Leipzig, Germany
Arthur Gessler	Swiss Federal Institute for Forest, Snow and Landscape Research – WSL, Birmensdorf, Switzerland, Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), Berlin, Germany
Gerd Gleixner	Max-Planck-Institute for Biogeochemistry, Jena, Germany
Annette Gockele	Faculty of Biology – Geobotany, University of Freiburg; Germany
Odette González Macé	Animal Ecology Group, JFB Institute of Zoology and Anthropology, University of Göttingen; Germany
Nina Hakker	Geocology, University of Tübingen, Germany
Anke Hildebrandt	Max-Planck-Institute for Biogeochemistry, Jena, Germany, German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany, Institute of Geosciences, Friedrich-Schiller-University, Jena, Germany

Affiliations of Co-Authors (continued)

Markus Lange	Max-Planck-Institute for Biogeochemistry, Jena, Germany
Liesje Mommer	Plant Ecology and Nature Conservation Group, Wageningen University, The Netherlands
Yvonne Oelmann	Geoecology, University of Tübingen, Germany
Janneke Ravenek	Department of Experimental Plant Ecology, Institute for Water and Wetland Research, Radboud University, The Netherlands
Michael Scherer-Lorenzen	Max-Planck-Institute for Biogeochemistry; PO Box 100164, 07701 Jena, Germany
Stefan Scheu	Animal Ecology Group, JFB Institute of Zoology and Anthropology, University of Göttingen; Germany
Annemieke Smit-Tiekstra	Department of Experimental Plant Ecology, Institute for Water and Wetland Research, Radboud University, The Netherlands
Jan Willem van der Paauw	Plant Ecology and Nature Conservation Group, Wageningen University, The Netherlands
Jasper van Ruijven	Plant Ecology and Nature Conservation Group, Wageningen University, The Netherlands
Cameron Wagg	Department of Evolutionary Ecology and Environmental Studies, University of Zürich, Zürich, Switzerland
Alexandra Weigelt	Spezielle Botanik und Funktionelle Biodiversität, Institute of Biology, University of Leipzig, Germany, German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany
Wolfgang Wilcke	Institute of Geography and Geoecology, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany
Christian Wirth	Spezielle Botanik und Funktionelle Biodiversität, Institute of Biology, University of Leipzig, Germany, German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany, Max-Planck-Institute for Biogeochemistry, Jena, Germany

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)

- Root turnover in grassland biodiversity experiments: root traits and interactions with soil biota (2014)

Post-graduate courses (7.3 ECTS)

- Introduction to R; PE&RC (2014)
- Jena PhD R workshop; Jena PhDs (2014)
- Microbial ecology; PE&RC (2015)
- Soil ecology; PE&RC (2016)
- Root ecology; PE&RC (2016)

Invited review of (unpublished) journal manuscript (3 ECTS)

- Plant and Soil: Grass nutrient strategies, manure application soil processes (2015)
- Geoderma: The effect of invasive plants on soil properties (2016)
- Geoderma: Responses of root traits to drought (2017)

Competence strengthening / skills courses (4.9 ECTS)

- PhD Competence assessment; WGS (2014)
- Effective behaviour in your professional surroundings; WGS (2014)
- Jena PhD writing workshop; Jena PhDs (2015)
- Oral presentation skills; Jena PhDs/ProScience (2015)
- Scientific writing; Wageningen Into Languages (2017)

PE&RC Annual meetings, seminars and the PE&RC weekend (3.6 ECTS)

- PE&RC First year weekend (2014)
- PERC Day (2014-2016)
- PE&RC Middle year weekend (2015)

- PE&RC Last year weekend (2016)

Discussion groups / local seminars / other scientific meetings (4.5 ECTS)

- PhD Symposium; Wageningen (2013)
- PhD Carousal; Wageningen (2014)
- Crossroads in ecology (2014, 2015)
- Plant Soil Interactions Discussion Group; organizer and participant (2016-2017)
- Frontiers in ecology (2017)

International symposia, workshops and conferences (8.3 ECTS)

- NAEM Meeting; poster presentation (2014)
- GSBI Conference; poster presentation; Dijon, France (2015)
- Rhizo4 Conference; poster presentation; Maastricht, the Netherlands (2015)
- NAEM Meeting; oral presentation (2016)
- NAEM Meeting; oral presentation (2017)

Lecturing / supervision of practicals / tutorials (1.5 ECTS)

- Trending topics in biology and chemistry of soil and water (2017)

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