How Resource Availability Modulates Biodiversity Effects On Ecosystem Functioning

Dissertation

zur Erlangung des
Doktorgrades der Naturwissenschaften (Dr. rer. nat.)
der
Naturwissenschaftlichen Fakultät I - Biowissenschaften -

der Martin-Luther-Universität
Halle-Wittenberg,

vorgelegt

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geboren am 10.01.1984 in Erfurt

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Datum der Verteidigung: 30.06.2016

People are beginning to realize that we need to live in accordance with the law of ecology, the law of finite resources, and if we don't, we're going to go extinct.

~ Paul Watson ~

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TABLE OF CONTENTS

SUMMARY

Ecosystem functioning is important for human well-being and the sustenance of ecosystems. Biodiversity is one of the major components of ecosystem functioning but is increasingly threatened by direct or indirect human influences. However, the exact mechanisms modulating the relationship between ecosystem functioning and biodiversity under spatially and temporally heterogeneous environmental conditions are not easy to predict. This is because not only ecosystem processes themselves, but species traits, which determine community characteristics and likely shape their effect on ecosystem function, are varying with the availability of resources like light, soil nutrients, and water. In order to understand the impact of the reduction in species richness and climate change on ecosystem functioning, it is crucial to understand the specific effects of variation of species traits and functional composition on the productivity of communities of varying species richness under changing resource conditions. A common means of trying to disentangle these complex processes is studying the effects of manipulated resource availability on artificially created communities of specifically selected species. Many studies have conducted experiments in grasslands because they are an important ecosystem. However, experiments aiming at understanding the mechanisms and consequences of species loss by establishing communities of varying species richness often did not manipulate resource availability or did not include the manipulation of more than one resource. Moreover, most studies focused on biomass production or aboveground traits, while only few studies analysed belowground plant characteristics. Another point missing from several studies while including species of different functional groups (like grasses, forbs or legumes) is that they do not vary these factors independently, which complicates the analysis of the origins of observed effects.

This work aims at filling in gaps left by former studies to contribute to a better understanding of the underlying processes by an experimental approach choosing eight perennial grassland species with certain morphological and physiological features.

Specifically, I focused on three research questions:

(1) How do nutrient and light availability affect the magnitude and the direction of functional trait variation in above- and belowground traits of species belonging to different functional groups (grasses or forbs) and being of different growth statures (tall or small)? Is functional trait variation to nutrient and light availability affected by the species richness of communities?

(2) Is trait dissimilarity increased by interactive effects of species richness and resource availability and can this be attributed to the functional composition of communities (functional groups, growth statures)?

(3) Which impacts do the combined effects of plant diversity and resource availability have on ecosystem functioning, such as above- and belowground biomass production, and diversity effects, and which role does functional trait variation play in this context?

This thesis comprises two experiments studying the variation in a large number of plant traits both aboveground and belowground to manipulation in nutrient and light availability of single plants in a greenhouse as well as in a field experiment including three diversity levels. Moreover, I analyse how this trait variation modulates diversity effects which shape ecosystem functioning. Additionally, the experiments aim at differentiating between the effects of species richness and functional composition on species productivity in mixed communities. The major findings were:

(1) Different availability of nutrients and light induced trait variation in above- and belowground traits of species belonging to different functional groups (grasses and forbs) that additionally differed in inherent growth statures. Grasses and forbs differed in most traits as well as in the magnitude of trait variation in traits mostly associated with nutrient acquisition and biomass allocation. Grasses were in general better competitors through better space and resource use aboveground and belowground, resulting in higher shoot biomass production.

While shade had a large impact on trait variation of individual plants in the greenhouse and plants in communities in the field, nutrient effects were mostly visible in the greenhouse experiment while only aboveground biomass production, overyielding and standing root biomass production in dependence on shading were affected in the field.

Whereas individually grown plants of different functional groups in the greenhouse showed large differences, plants growing in communities had larger trait differences depending on their inherent growth stature.

(2) Resource availability increased trait dissimilarity on several levels. However, the observed effects were mostly restricted to traits associated with nutrient acquisition. Dissimilarity between dominant and subordinate species increased with shading due to larger plasticity of subordinate species and because subordinate species had higher nitrogen tissue concentrations in fertilized communities. This led also to community-level trait dissimilarity in tissue nitrogen concentration under fertilization.

(3) Mixtures in my experiment were on average more productive than monocultures. The effect of resource availability on diversity effects was mainly due to fertilization, which decreased positive complementarity and net diversity effects, while variation in selection effects was not due to resource effects. Root standing biomass development was mainly affected by shading, but increased species richness or fertilization had no effects.

I furthermore analysed if trait dissimilarity can alter biodiversity effects and concluded that in traits that are connected to nutrient acquisition and respiration a smaller dissimilarity between species with large trait values is more beneficial for complementary resource use than large dissimilarity which would result in competitive inequalities.

The experiments underline that the functional composition of communities is more important than species richness to promote biodiversity effects and insure higher productivity of mixtures opposed to monocultures. This thesis shows that a varying extent of trait variation among species representing different growth forms, functional groups and varying in dominance may affect their functional dissimilarity under different resource supply which in turn shapes diversity effects on ecosystem functioning.

ZUSAMMENFASSUNG

Ökosystemfunktionen sind sowohl für den Menschen als auch für den Fortbestand von Ökosystemen wichtig. Die Biodiversität ist eine der wichtigsten Grundlagen für Ökosystemfunktionen, wird aber zunehmend durch direkte oder indirekte menschliche Einflüsse bedroht. Allerdings sind die genauen Mechanismen, die die Beziehung zwischen Ökosystemfunktionen und Biodiversität unter räumlich und zeitlich heterogenen Umweltbedingungen steuern, nicht leicht vorherzusagen. Dies liegt daran, dass nicht nur Ökosysteme selbst, sondern auch Pflanzenmerkmale, die die Eigenschaften von Pflanzengemeinschaften bestimmen und sich dadurch auch auf Ökosystemeigenschaften auswirken, mit der Verfügbarkeit von Ressourcen wie Licht, Nährstoffe im Boden und Wasser variieren. Um die Auswirkungen des Rückgangs der Artenvielfalt und des Klimawandels auf Ökosystemfunktionen besser erfassen zu können, ist es wichtig, die konkreten Auswirkungen der Veränderung der Artmerkmale unterschiedlicher Arten und der funktionellen Zusammensetzung von Pflanzenbeständen auf die Bestandsproduktivität unter sich ändernden Ressourcenbedingungen zu verstehen.

Eine übliche Methode diese komplexen Prozesse zu erforschen ist die Untersuchung in experimentellen Gemeinschaften speziell ausgewählter Arten. Viele Studien haben Experimente in Grasländern durchgeführt, da diese ein wichtiges Ökosystem darstellen. Allerdings wurden bei Untersuchungen zum Verständnis der Mechanismen und Folgen von Artenverlust oft keine Ressourcenverfügbarkeit oder zum Großteil nur die Verfügbarkeit einer Ressource manipuliert. Darüber hinaus konzentrieren sich die meisten Studien auf die Produktion von Biomasse oder auf oberirdische Pflanzenmerkmale, während nur wenige Studien unterirdische Pflanzeneigenschaften analysieren. Obwohl in mehreren Studien Arten unterschiedlicher funktioneller Gruppen (wie Gräser, Kräuter oder Leguminosen) einbezogen wurden, wurde die Artenzahl meist nicht unabhängig von der Anzahl der funktionellen Gruppen variiert. Dies erschwert die Analyse der Ursache der beobachteten Effekte.

Diese Arbeit hat das Ziel, die oben genannten Lücken zu füllen und zu einem besseren Verständnis der zugrunde liegenden Prozesse durch zwei Experimente mit acht mehrjährigen Grünlandarten mit bestimmten morphologischen und physiologischen Eigenschaften beizutragen.

Genauer gesagt, konzentrierte ich mich auf drei Forschungsfragen:

(1) Wie beeinflusst die Verfügbarkeit von Licht und Nährstoffen das Ausmaß und die Richtung der Merkmalsvariation ober- und unterirdischer funktioneller Artmerkmale von verschiedenen funktionellen Gruppen (Gräser oder Kräuter) und verschiedenen Wuchsformen (groß oder klein)? Wird die Variation funktioneller Merkmale aufgrund

veränderter Nährstoff- und Lichtverfügbarkeit durch die Artenzahl in Beständen beeinflusst?

(2) Wird die Unterschiedlichkeit von Pflanzenmerkmalen durch interaktive Effekte der Artenzahl und Ressourcenverfügbarkeit erhöht und kann dies durch die funktionelle Zusammensetzung von Pflanzengemeinschaften (funktionelle Gruppen, Wuchsformen) erklärt werden?

(3) Welche Auswirkungen haben die kombinierten Effekte der Artenzahl und Ressourcenverfügbarkeit auf Ökosystemfunktionen, wie beispielsweise ober- und unterirdische Biomasseproduktion, und Diversitätseffekte, und welche Rolle spielt die Variation funktioneller Merkmale in diesem Zusammenhang?

Die vorliegende Arbeit basiert auf zwei Experimenten, die die Variation in einer großen Anzahl von sowohl oberirdischen und unterirdischen Artmerkmalen von Einzelpflanzen durch Manipulationen der Nährstoff- und Lichtverfügbarkeit in einem Gewächshaus, sowie in einem Feldversuch in Beständen unterschiedlicher Artenzahl untersuchen. Außerdem wird analysiert, wie diese Merkmalsvariation Diversitätseffekte auf Ökosystemfunktionen beeinflusst. Darüber hinaus sollen die Experimente eine Differenzierung zwischen den Auswirkungen der Artenzahl und funktioneller Zusammensetzung ermöglichen, die die Produktivität in Gemeinschaften unterschiedlicher Arten fördern.

Zusammengefasst resultierten folgende Ergebnisse:

(1) Verschiedene Licht- und Nährstoffverfügbarkeit induzierte Variationen in ober- und unterirdischen funktionellen Merkmalen von Pflanzen unterschiedlicher funktioneller Gruppen (Gräser und Kräuter), die sich zusätzlich in ihren Wuchsformen unterschieden. Gräser und Kräuter unterschieden sich in den meisten Merkmalen und im Ausmaß der Merkmalsvariation in Eigenschaften, die mit Nährstoffaufnahme und Biomasseallokation zusammenhängen. Gräser waren im Allgemeinen durch eine bessere oberirdische und unterirdische Raum- und Ressourcennutzung gekennzeichnet, was eine größere Biomasseproduktion zur Folge hatte. Während Beschattung einen großen Einfluss auf die Merkmalsvariation sowohl von Einzelpflanzen im Gewächshaus als auch von Pflanzen in experimentellen Feldgemeinschaften hatte, waren Einflüsse veränderter Nährstoffverfügbarkeit auf die Variation der Pflanzenmerkmale meist nur im Gewächshausversuch sichtbar. Im Feldexperiment wurden die Effekte von Nährstoffverfügbarkeit auf oberirdische Biomasseproduktion und Wurzelbiomasseproduktion durch Beschattung beeinflusst.

Während einzeln gewachsene Pflanzen unterschiedlicher funktioneller Gruppen im Gewächshaus große Unterschiede zeigten, unterschieden sich im Feld zumeist Pflanzen großer und kleiner Statur.

(2) Veränderte Ressourcenverfügbarkeit führte zu einer Merkmalsunähnlichkeit auf mehreren Ebenen. Allerdings waren die beobachteten Effekte meist auf Pflanzenmerkmale beschränkt, die mit Nährstoffaufnahme verbunden sind. Die Unähnlichkeit zwischen dominanten und nicht dominanten Arten wurde durch Beschattung durch größere Merkmalsvariation nicht dominanter Arten erhöht, da sie höhere Stickstoffkonzentrationen im Gewebe in gedüngten Beständen zeigten. Dies führte auch zu Merkmalsunterschieden auf Bestandsebene in Stickstoffkonzentrationen der oberirdischen Gewebe unter Düngung.

(3) In meinem Experiment waren Pflanzenmischungen im Durchschnitt produktiver als Monokulturen. Die Wirkung der Ressourcenverfügbarkeit auf Diversitätseffekte erklärt sich vor allem durch die Düngung, welche positive Komplementaritätseffekte und Netto-Diversitätseffekte verminderte, während Variation in Selektionseffekten nicht auf Ressourcenänderung zurückzuführen war. Die Wurzelbiomasseproduktion wurde vor allem durch Beschattung beeinflusst; eine größere Artenzahl oder Düngung hatten keine Auswirkungen.

Ich untersuchte außerdem, wie Merkmalsunterschiede Artenzahleffekte verändern können. Hier ergab sich, dass in Merkmalen, die mit Nährstoffaufnahme verbunden sind, eine höhere Ähnlichkeit zwischen Arten mit großen Merkmalswerten günstiger für komplementäre Ressourcennutzung ist als große Verschiedenheit, welche zu Wettbewerbsungleichheiten führen würde.

Die Experimente unterstreichen, dass die funktionelle Zusammensetzung der Gemeinschaften wichtiger als die Artenzahl ist um Diversitätseffekte und eine höhere Produktivität von Mischungen im Verhältnis zu Monokulturen zu ermöglichen. Diese Arbeit zeigt weiterhin, dass das unterschiedliche Ausmaß der Merkmalsvariation von Arten unterschiedlicher Wuchsformen und funktioneller Gruppen sowie unterschiedlicher Dominanz ihre funktionelle Unterschiedlichkeit unter veränderter Ressourcenverfügbarkeit erhöhen kann, was wiederum die Auswirkungen von Diversitätseffekten auf Ökosystemfunktionen beeinflussen kann.

CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Biodiversity and ecosystem functioning relationships

People have become increasingly aware how important natural ecosystems and ecosystem functioning are in supporting human societies. Ecosystem functions encompass ecological processes that control fluxes of organic matter, nutrients, and energy, like biomass production, carbon and nitrogen cycling (Cardinale et al. 2012). The balances and functioning of ecosystems are threatened by various factors like intensified land-use, eutrophication (e.g. the increased deposition of nitrogen from the atmosphere or through fertilization), increasing atmospheric CO_2 levels, and the introduction of novel species from other geographical origins (Sala et al. 2000; Hautier et al. 2014). Consequently, we observe a rapid loss in biodiversity, which encompasses ecosystem diversity, species richness and diversity as well as genetic diversity (Balvanera et al. 2006; Cardinale et al. 2006, 2012). The diversity of vascular plants as primary producers of biomass has been shown to increase ecosystem resistance against invasive species (Hector et al. 2001; Fargione and Tilman 2005), climate extremes like drought (Isbell et al. 2015), and is widely acknowledged as one important determinant of ecosystem functioning (Naeem et al. 1999; Balvanera et al. 2014; Tilman et al. 2014). More specifically, a number of studies in experimental plant communities have led to the consensus that species richness enhances the multi-functionality of ecosystem functioning (Cardinale et al. 2012; Lefcheck et al. 2015) and that higher plant species richness induces higher aboveground biomass production in grassland ecosystems (Hooper et al. 2005; Díaz et al. 2006; Marquard et al. 2009). However, despite decades of research, the mechanisms underlying the complex interactions between plant diversity and ecosystem functioning are still not satisfactorily understood (Cardinale et al. 2012).

The extrapolation of general principles across ecosystems is complicated because ecosystem processes are variable, e.g. determined by abiotic conditions such as availability of space, light, water, and soil nutrients - the resources plants require for growth and reproduction. Depending on the particular ecosystem, these resources are available in varying abundances, e.g. in temperate grasslands with ample precipitation, the most limiting resources are light and soil nutrients. In Europe, semi-natural grasslands make up for approximately 15% of the territory (Ciais et al. 2010). Even more than forests (Ciais et al. 2010), temperate grasslands function as - at least temporary - carbon sinks (Hu et al. 2001; Jones and Donnelly 2004; Smith 2014), thus contributing to balancing atmospheric CO_2 levels. Additionally, grasslands are in general relatively rich in plant species (Wilson et al. 2012). Hence, it is critical to understand natural dynamics in grasslands in order to successfully apply management and conservation measures (Jones and Donnelly 2004).

Biodiversity experiments

Studies in natural grassland communities examining the impacts of species loss (Zobel et al. 1994; Wardle et al. 1999) and management (species removal, fertilization, mowing: e.g. Lepš 2004; fertilization: Hautier et al. 2014) are argued to allow a better direct estimate of future implications. However, despite having been criticized for their partly arbitrary species composition (e.g. Lepš 2004), experimentally set-up communities have their advantages. Since natural mechanisms are very complex, experimental communities enable a stepwise approach for disentangling causes and effects more easily. For example, they allow the selection of specific species to study composition and interaction effects more closely, while providing the possibility of simulating future conditions by manipulating environmental factors. As a model system, experimental grasslands have the benefit of a fast plant establishment, they are easy to maintain, and it is more feasible to manipulate abiotic conditions than e.g. in forests, for example by applying fertilizer, construction of roofs to decrease water and light availability or increasing CO_2 availability by open top chambers. Hence, several long-term grassland experiments have been conducted in the past years, in which plant species diversity was manipulated (Cedar-Creek experiment, e.g. Tilman et al. 1996; BIODEPTH sites, Hector et al. 1999; the Jena experiment, Roscher et al. 2005), although several experiments focus on forest communities as well (BIOTREE, Scherer-Lorenzen et al. 2007; BEF China, Bruelheide et al. 2014). In addition to species diversity, other experiments simulated the effects of variation in abiotic conditions (e.g.: drought: Jentsch et al. 2011; CO_2: Jasper Ridge Global Change experiment, Field et al. 1996; CO_2 and fertilization: Cedar-Creek BioCON experiment, Reich et al. 2001; fertilization: Lanta and Lepš 2007; Wacker et al. 2009; shading and fertilization: Fridley 2002, 2003).

Diversity effects

Positive effects of diversity on biomass production have been observed both aboveground (e.g. Fornara and Tilman 2008; Marquard et al. 2009) and belowground (Mueller et al. 2013; Cong et al. 2014; Ravenek et al. 2014). More specifically, *overyielding* could be shown: the biomass production of species growing in mixtures is significantly higher compared to the biomass of the same species growing in monocultures combined (Loreau 1998). The so-called *net diversity effect* (Loreau and Hector 2001; Cardinale et al. 2007) quantifies these differences in biomass production between mixtures and monocultures of their component species. But not only complementary use of resources in mixed communities due to functionally different species can result in a higher biomass production of plant mixtures opposed to monocultures (=*complementarity effect*). The *selection effect* results from the increasing possibility of more diverse communities to contain a dominant species, which is highly productive in monocultures as well as mixtures (Aarssen 1997; Huston 1997). Both effects thus originate from different ecological mechanisms but both depend on the functional traits of the present species and can simultaneously contribute to an increased biomass production with increasing species richness and lead to overyielding (Harper 1977).

However, diversity effects vary with changes in resource availability. Fertilization has been shown to increase diversity effects and overyielding (Reich et al. 2001; He et al. 2002; Fridley 2002, 2003). However, the effects of increased nutrient availability may depend on the simultaneous manipulation of other resources such as light (Fridley 2003) or CO_2 (He et al. 2002). Additionally, it has been shown that diversity effects are not time consistent (Lanta and Lepš 2007). Specifically, selection effects have been demonstrated to decrease while complementarity effects have been shown to increase in long-term experiments (Cardinale et al. 2007; Fargione et al. 2007), while the general positive effect of species richness on aboveground biomass production increased over time (Cardinale et al. 2007; Marquard et al. 2009).

As an explanation for this, complementarity effects are proposed to be largely explained by belowground processes (Cardinale et al. 2007) but aboveground biomass production is still the major focus of most studies. It is well known that up to 80% of total plant biomass in temperate grasslands are allocated to belowground biomass (Jackson et al. 1996; Poorter et al. 2012). The uptake of belowground resources essential for plant growth, such as nutrients and water, is the main function of roots and thus a major contributor to ecosystem functioning (Bardgett et al. 2014). The relationship between plant species richness and belowground biomass production has been examined, although not extensively, both with (Spehn et al. 2005; Bessler et al. 2009) and without including the analysis of aboveground biomass (Gastine et al. 2003; Mueller et al. 2013; Ravenek et al. 2014). Furthermore, in experiments that only examined belowground biomass, contrasting results were found. For example, in a short-term experiment on grassland biodiversity carried out within the BIODEPTH project (e.g. Gastine et al. 2003; Spehn et al. 2005), no effects of increased species richness on belowground biomass production could be proven at single sites. Yet, other short-term biodiversity experiments demonstrated positive species richness effects on belowground biomass (Spehn et al. 2005; von Felten and Schmid 2008; Mommer et al. 2010; Bessler et al. 2012; Mueller et al. 2013). Few studies analysed resource effects like light availability (single plants: Ryser and Eek 2000; communities: Edwards et al. 2004) or nutrient availability (Berendse 1982) or both (Olff et al. 1990) on root biomass production of individuals, but not in biodiversity experiments (but see Mommer et al. 2010).

Plant functional traits and trait variation

Not only species richness, but also the community composition in terms of species functional identity has been proven important for biomass production and ecosystem functioning (Naeem et al. 1995; Symstad et al. 1998; Hector 2011). Consequently, community characteristics are not only dependent on the number of species, but also on species' differences in terms of growth rate, survival and reproduction (Violle et al. 2007) as reflected in functional dissimilarities among species. Hence, it is promising to include inherent species characteristics that reflect functional diversity to explain underlying mechanisms within communities and on the ecosystem level more precisely (McGill et al. 2006).

Functional traits are morphological, physiological or life-history characteristics measured at the level of individuals, which contribute to a plant's fitness by affecting survival, growth and reproduction (Violle et al. 2007). Hence, they have a large influence on how individual plant species react to variation in the biotic and abiotic environment and thus contribute to community characteristics and influence processes at the community-level (McGill et al. 2006; Roscher et al. 2012). Trait-based approaches also have been increasingly applied in analysis of ecosystem functioning (Grime 1998; Lavorel and Garnier 2002). However, while trait-based approaches have increased in complexity by attempting to scale from communities to ecosystems or including many traits (Lavorel et al. 1997; Dyer et al. 2001; Suding et al. 2008; Kraft et al. 2015) and some attempt to account for interspecific variation, there is still a large tendency of using species mean traits. Thus, intraspecific variation is not accounted for but is often neglected or minimized by analysing 'robust' traits with less observed variation (McGill et al. 2006). However, the consideration of trait variation in ecological studies has also been suggested and applied (Jung et al. 2010; Messier et al. 2010; Albert et al. 2011; Violle et al. 2012). It has been shown that this is necessary because intraspecific variation can even exceed interspecific variation (Messier et al. 2010; Lepš et al. 2011; Siefert and Ritchie 2016).

Intraspecific trait variation reflected as change in the phenotype expressed by a single genotype is dependent on biotic (e.g. plant neighbours, parasites, symbionts) as well as abiotic conditions (e.g. resource availability) (Schlichting and Levin 1986; Coleman et al. 1994; DeWitt et al. 1998; Hughes et al. 2008). In consequence, plant species differ in the magnitude or direction of the variation in morphological, physiological or life history traits in response to different resource availability (Albert et al. 2011). Plants need to balance resource acquisition for metabolism and growth to uphold a 'functional equilibrium' (Bloom et al. 1985; Poorter et al. 2012) which is dependent on the availability of resources. To reach this equilibrium, plants invest more biomass into organs, which are required for acquiring the most limiting resources ('optimal allocation theory', Bloom et al. 1985; Poorter and Nagel 2000). For example, decreased nitrogen availability leads to an increased biomass allocation to belowground organs, whereas light limitation induces increased allocation of biomass to aboveground organs (Valladares and Niinemets 2008). In dense canopies, light intensity is lower and the spectral composition of incident radiation is changed, which has been shown to instigate changes in photosynthetic rate, leaf physiology and leaf architecture (Givnish 1988; Valladares and Niinemets 2008). It can further lead to structural changes in the whole individual (Valladares and Niinemets 2008). Morphological adjustments to reduced light availability, such as increased apical dominance and the production of leaves with larger specific leaf area, have also been related to increased plant diversity in communities (e.g. Daßler et al. 2008; Gubsch et al. 2011).

The importance of root characteristics for ecosystem processes such as nutrient and carbon cycling has been emphasized increasingly in research on diversity-ecosystem functioning relationships (Fornara et al. 2009; Bardgett et al. 2014; Cong et al. 2014). Differences in root traits due to the functional composition of communities may promote complementary resource use and thus increase biomass production of mixed communities opposed to species monocultures (Gastine et al. 2003; Dimitrakopoulos and Schmid 2004; Reich et al. 2004; Fornara and Tilman 2008; Mommer et al. 2010; Mueller et al. 2013). In particular, specific root length (root length per unit of root biomass; Ostonen et al. 2007) and root length density (Atkinson 2000) have been identified as important characteristics linked to water regulation and nutrient uptake as well as to rhizodeposition and to respiration (Bardgett et al. 2014). However, trait variation in morphological root traits of grassland species in response to nutrient availability or plant species richness and functional diversity is not as well investigated as for aboveground traits apart from studies focusing on nutrient effects on a number of root traits (e.g. Fransen et al. 1999; Craine et al. 2001; Wahl et al. 2001; Leuschner et al. 2013). Reduction in soil nutrient availability or spatially heterogeneous nutrient distribution has been shown to promote root morphological and physiological changes, such as changes in respiration rates and root architecture (Hodge 2004).

However, usually, plant trait variation is caused by multiple environmental factors simultaneously and is inconsistent between traits (Harpole et al. 2011; Kazakou et al. 2014; Siefert et al. 2015). Most experiments examined the effects of single resources on trait variation, such as nutrients (e.g. Al Haj Khaled et al. 2005; Mokany and Ash 2008; Pontes et al. 2010; Kazakou et al. 2014) or light (Ryser and Eek 2000; Semchenko et al. 2012). Only few studies focused on trait variation in response to manipulation of more than one resource, e.g. both light and nutrients, or studies only examined effects on specific sets of traits (Olff et al. 1990; Shipley and Almeida-Cortez 2003; Freschet et al. 2013).

Thus, it is still difficult to predict to what extent intraspecific trait variation caused by changes in resource availability contributes to whole ecosystem responses. There is also a lack of evidence on differences between the extent and direction of trait variation of species with inherent trait differences. Variation in traits associated with resource use may be the key mechanism to promote the co-occurrence of species in diverse communities (Ashton et al. 2010). However, considering species-specific responses in trait variation, it is difficult to predict how changes in resource availability affect species dissimilarity, i.e. if species converge (trait values become more similar) or diverge (trait values become more dissimilar) in their traits. Moreover, it is challenging to disentangle if observed variation is a consequence of differences in species richness or the variation in resource availability.

Functional groups and growth statures

A way to simplify trait-based approaches is to cluster a large number of species into smaller groups (Craine et al. 2001). Plant species are often aggregated into functional groups based on their phylogeny, phenology, morphology and similarities in their functional traits (Gitay and Noble 1997), assuming that species within a functional group are more similar than species of different groups (Dyer et al. 2001).

The main functional groups distinguished in grassland ecosystems are grasses, non-legume forbs and nitrogen-fixing legumes, which differ in both their above- and belowground characteristics such as resource acquisition and resource use (Körner 1993; Craine et al. 2001; Dyer et al. 2001; Roscher et al. 2004). Combining several functionally different species in communities is thought to increase biomass production compared to monocultures or to communities with less species as the result of a more efficient use of available resources or facilitative interactions (Roscher et al. 2005; Spehn et al. 2005; Cardinale et al. 2007; Marquard et al. 2009). For example, complementary use of belowground resources could occur in communities comprising different functional groups because of differences in rooting depth (Dimitrakopoulos and Schmid 2004; von Felten and Schmid 2008), root biomass (Gastine et al. 2003; Bessler et al. 2012; Mueller et al. 2013) and root morphological traits (Grime et al. 1997; Craine et al. 2001; Siebenkäs et al. 2015). Additionally, legumes have had a large role in explaining complementarity effects due to their ability of increasing nitrogen availability for surrounding species and thus promoting their biomass production (Marquard et al. 2009). Aboveground, grass species have been characterized by lower leaf nitrogen concentration, greater leaf thickness and greater leaf tissue density as forbs (Grime et al. 1997; Craine et al. 2001; Reich et al. 2003; Poorter et al. 2012), implying differing resource use strategies.

The local abundance of species and plant community structures are mainly determined by resource competition (Tilman 1990). Plants compete for resources above- and belowground simultaneously as result of increased spatial and temporal heterogeneity in resource availability in communities (Tilman 1990). Specifically, in grasslands, the majority of species is of short stature and therefore has less access to sunlight. Tall growing species intercept a disproportionally large fraction of the incident radiation, resulting in a competitive advantage via size-asymmetric competition by additionally shading smaller plants (Weiner 1990). Therefore, small-statured species are often not able to produce as much biomass as taller species due to the resulting carbon limitation (Werger et al. 2002; Mariotte 2014). Belowground competition for resources is still a disputed topic (Schenk 2006), as different experiments gave evidence for symmetric competition (Wilson 1988; Weiner et al. 1997) in environments with homogeneously distributed nutrients as well as asymmetric competition in experiments where nutrients were heterogeneously distributed (Fransen et al. 2001; Rajaniemi 2003). However, increased nutrient availability might alter competition effects (Grime 1973; Fridley 2003; Hautier et al. 2009). Fertilization can increase size-asymmetric aboveground competition by allowing tall species to invest more in aboveground biomass, thereby shadowing the shorter plants even more. Additionally, fertilization may simultaneously increase belowground competition as species try to exploit nutrient rich soil patches (Fransen et al. 1999; Hodge 2004).

Functional composition of communities

The expectation that species exhibit differences in their resource acquisition, in their functional traits and in their reaction to variation in resource availability is commonly regarded as a prerequisite for complementary resource use (Naeem et al. 1994; Hooper 1998; Loreau and Hector 2001; Roscher et al. 2012). Thus, the inclusion of different functional groups is usually an important factor in explaining effects of biodiversity (Reich et al. 2004; Hooper et al. 2005; Balvanera et al. 2006; Lanta and Lepš 2007).

Since *a priori* defined functional groups do not show consistent differences in all traits and have been found to exhibit large variation within groups (Grime et al. 1997; Tjoelker et al. 2005; Wright et al. 2006), recent approaches stress the importance of continuous variables which quantify the difference of trait values of species in a community (Mouchet et al. 2010) and thus reflect its functional composition. As such, community weighted mean traits (CWM) and Rao's quadratic diversity index FD_Q (Rao's Q, Rao 1982) were shown to adequately predict aboveground biomass production in semi-natural grasslands (Díaz et al. 2007; Mokany et al. 2008; Schumacher and Roscher 2009). Additionally, the importance of these diversity measures for ecosystem functioning could be asserted (Valencia et al. 2015). CWMs can identify the effects of dominant trait values in a community (Grime 1998), i.e. community characteristics mainly depend on the trait values of species that make the greatest contribution to the community biomass. FD_Q represents the sum of pairwise distances between species that are weighted by their relative abundance (Petchey et al. 2004). Therefore, it reflects trait dissimilarities within a community, which are assumed to correlate positively with the complementary use of resources. Further, FD_Q is largest when species with larger trait dissimilarity have similar abundances (Mouchet et al. 2010). However, as these indices are based on individual trait values, it is to be expected that they are likewise subjected to environmental variation (de Bello et al. 2006; Díaz et al. 2007; Li et al. 2015).

Objectives and Outline

To conclude, despite a large amount of research on functional trait variation and the relationships between biodiversity and ecosystem functioning, there are still a number of aspects, which remain unresolved. Since there are inherent trade-offs in plant trait variation, it is necessary to explore the effects of changes in resource availability on both aboveground and belowground traits simultaneously, and to consider the additional effects of other individuals in communities of varying species richness on trait variation. However, studies on aboveground and belowground trait variation in biodiversity experiments that also manipulate different resources are to my knowledge not existing in the literature. Additionally, studies connecting trait variation and functional dissimilarity often do not account for the differences in the magnitude and direction of trait variation between functional groups or species of different sizes and between species of varying contribution to community biomass production. The experiments in this thesis address these points with the benefits of conducting two experiments specifically designed to disentangle if environmental effects lead to more or less trait dissimilarity between species of different inherent sizes and functional groups as result of manipulation of resources and species richness and how these effects influence biodiversity effects on ecosystem functioning.

The aim of this thesis is to investigate the importance of the variation in plant functional traits of perennial grassland species induced by experimental manipulations of resource availability, and additional variation in species richness and functional composition.

The analyses of plant functional traits and their variation due to the interaction of resource availability and species richness elucidate if and how the modulation of functional traits will lead to a complementary resource use and increased biomass production at the community level.

Specifically, I address the following research questions in this thesis:

(1) How do nutrient and light availability affect the magnitude and the direction of functional trait variation in above- and belowground traits of species belonging to different functional groups (grasses or forbs) and being of different growth statures (tall or small)? Is functional trait variation to nutrient and light availability affected by the species richness of communities?

(2) Is trait dissimilarity increased by interactive effects of species richness and resource availability and can this be attributed to the functional composition of communities (functional groups, growth statures)?

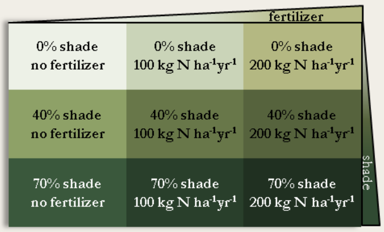
(3) Which impacts do the combined effects of plant diversity and resource availability have on ecosystem functioning, such as above- and belowground biomass production, and diversity effects, and which role does trait dissimilarity play in this context? To answer these questions, I conducted two experiments:

In a greenhouse experiment, plants of eight temperate grassland species of differing growth statures belonging to different functional groups (two small forbs, two tall forbs, two small grasses, two tall grasses, Box 1) were grown separately at nine crossed different levels of resource supply. The aboveground as well as the belowground plant material was harvested after four months and a large number of traits were measured to examine trait variation of the plants without competition.

A field experiment was established, in which monocultures as well as two- and four-species mixtures of the same species were grown at different resource availability of four crossed levels of light and nutrients (Box 1). In congruence with typical extensive land-use practice in the area, all plots were mown twice a year and hay was removed. After the first establishment period, shade treatments were installed from spring to autumn and fertilizer applications administered in spring and after first mowing in summer of each experimental year. In the second experimental year, aboveground traits were analysed at peak biomass in May in August. In the third year, aboveground biomass was sampled and sorted to species level while root core samples were taken for each experimental community to analyse belowground biomass production, distribution and root trait variation.

Greenhouse Experiment

Single plants were grown in a greenhouse in Bad Lauchstädt with natural light and a roof, which automatically retracts in under rainy conditions. The used substrate was topsoil from a location adjacent to the field experiment. Five replicates per treatment combination were randomly assigned to one of eighteen blocks representing the shading treatment (three levels) and one of three fertilizer levels (Fig. B2-1). Water was given in regular intervals to insure equal water supply.



0% shade **no fertilizer**	**0% shade** **100 kg N ha^{-1}yr^{-1}**	**0% shade** **200 kg N ha^{-1}yr^{-1}**
40% shade **no fertilizer**	**40% shade** **100 kg N ha^{-1}yr^{-1}**	**40% shade** **200 kg N ha^{-1}yr^{-1}**
70% shade **no fertilizer**	**70% shade** **100 kg N ha^{-1}yr^{-1}**	**70% shade** **200 kg N ha^{-1}yr^{-1}**

Figure B2-1: Treatment combinations of the greenhouse experiment

Field Experiment

A field experiment was established at a field site at the experimental station at Bad Lauchstädt including all species from Table B2-1. Species were grown in monocultures and mixtures of two- or four species of each pool These communities were randomly assigned to one of four treatment combinations of fertilization or shading (Fig. B2-3). The two four species mixtures per pool were replicated twice for each treatment combination, while monocultures and all possible two-species combinations in each pool were grown once per treatment combination. Shading (55%) was added block-wise. Shading scaffolds were installed April to September and fertilizer (total 120 kg N ha^{-1}yr^{-1}) applied in March and June in 2012 and 2013. Plots were mown twice a year and weeds were removed several times per year.

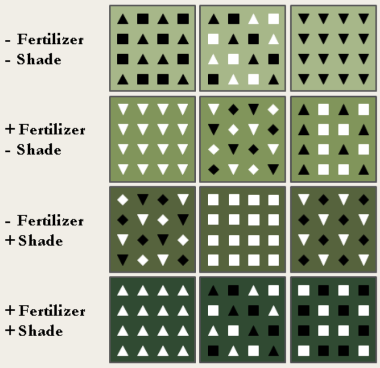


Figure B2-3: Treatment combinations of the field experiment and example of the field experimental set-up. Different symbols represent different species, black symbols represent forbs, white symbols grasses; square symbols show tall-statured and triangular symbols small-statured species

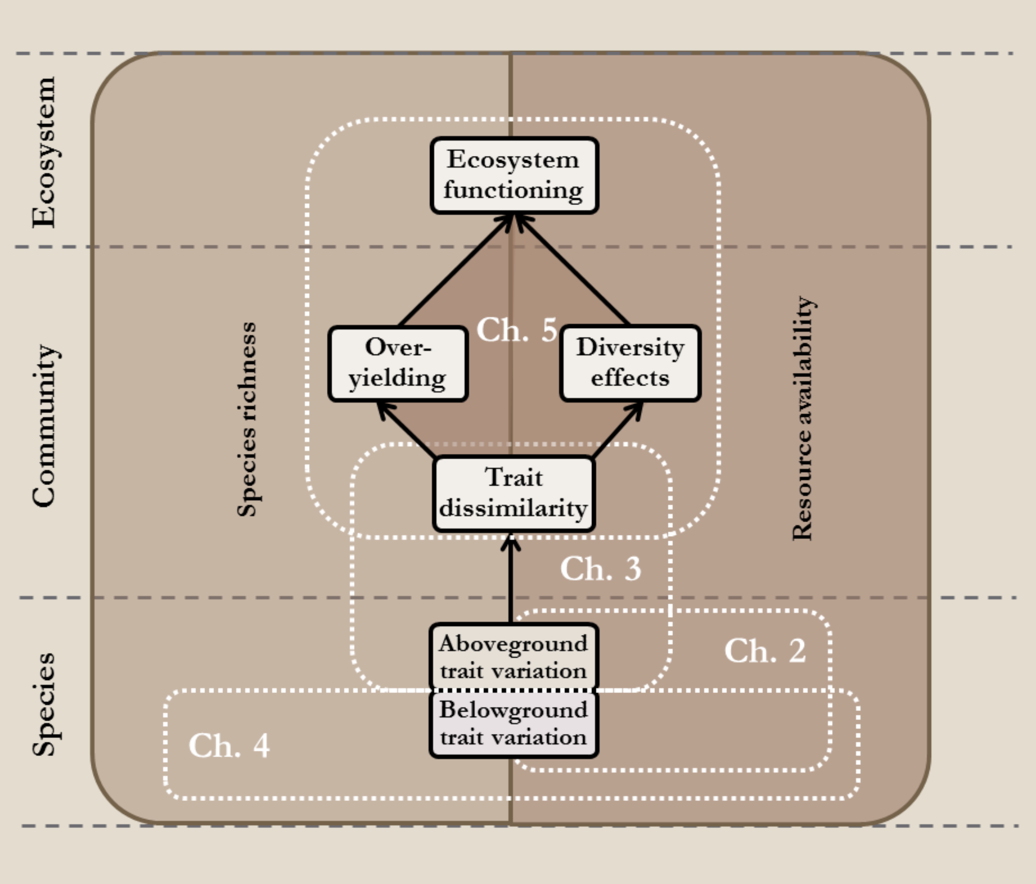
Figure B2-2: Greenhouse experiment

Table B2-1: Experimental species pools (pools serve as a replicate)

Pool A		Pool B	
Centaurea jacea ssp. jacea L.	(tall forb)	*Knautia arvensis* (L.) Coult.	(tall forb)
Plantago lanceolata L.	(small forb)	*Prunella vulgaris* L.	(small forb)
Arrhenatherum elatius l.	(tall grass)	*Dactylis glomerata* L.	(tall grass)
Anthoxanthum odoratum L.	(small grass)	*Lolium perenne* L.	(small grass)

Figure B2-4: Field experiment

Box 1: Short summary of the design of the experiments conducted in the framework in this thesis



Box 2: Schematic diagram of the processes studied in this thesis showing the focus of each chapter (Ch.) in the broader context

The results of these experiments serve as the basis for the following chapters to answer the aforementioned questions in the contexts described in Box 2:

In **chapter 2**, I present results of the short-term greenhouse experiment. I investigate how separately grown plants belonging to the eight perennial grassland species vary in their aboveground and belowground traits at nine different combinations of light and nutrient levels (Box 2). I address how the direction and the magnitude of trait variation differ between species of different functional groups and growth statures. Furthermore, I present the traits, which show greater variation in response to differences in resource availability. Lastly, I show in which environments the ranking of species is consistent or dependent on the relationship between trait variation and interspecific trait differences.

Chapter 3 puts the aboveground trait variation of each species in response to different availability of light and nutrients (two levels each) into a community context based on the results of the field experiment. Thus, I analyse trait variation in response to crossed factors of shade, fertilization and species richness. Moreover, I present that dominant species are less variable in their response to changes in resource availability than subordinate ones, and how trait differences between dominant and subordinate species are affected by increasing competition for resources.

Chapter 4 focusses on the effects of resource manipulation and of species richness on the variation in root morphological traits, root biomass production, root-shoot-ratios, and the vertical root distribution in the field. I show that communities of different species richness and functional composition (functional groups or growth statures) exhibit the potential for complementary resource use and vertical root segregation.

In **chapter 5**, I investigate the effects of functional composition, species richness and resource availability on aboveground biomass production in species mixtures versus monocultures, i.e. overyielding, net effects, complementarity and selection effects. Furthermore, this chapter bridges the gap between the chapters 3 and 4 and puts the analysed traits into context with the observed biodiversity effects (complementarity effect, selection effect).

Chapter 6 summarizes and discusses the main results and highlights the links between the individual chapters. Moreover, it puts the results into the context of the overarching research questions, derives a general conclusion and discusses implications for future research.

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CHAPTER 2

PHENOTYPIC PLASTICITY TO LIGHT AND NUTRIENT AVAILABILITY ALTERS FUNCTIONAL TRAIT RANKING ACROSS EIGHT PERENNIAL GRASSLAND SPECIES

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AobPlants 7:plv029

AoB PLANTS
The open-access journal for plant sciences

Research Article

Phenotypic plasticity to light and nutrient availability alters functional trait ranking across eight perennial grassland species

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Received: 17 November 2014; **Accepted:** 13 March 2015; **Published:** 27 March 2015

Associate Editor: Ulo Niinemets

Citation: Siebenkäs A, Schumacher J, Roscher C. 2015. Phenotypic plasticity to light and nutrient availability alters functional trait ranking across eight perennial grassland species. *AoB PLANTS* **7**: plv029; doi:10.1093/aobpla/plv029

Abstract. Functional traits are often used as species-specific mean trait values in comparative plant ecology or trait-based predictions of ecosystem processes, assuming that interspecific differences are greater than intraspecific trait variation and that trait-based ranking of species is consistent across environments. Although this assumption is increasingly challenged, there is a lack of knowledge regarding to what degree the extent of intraspecific trait variation in response to varying environmental conditions depends on the considered traits and the characteristics of the studied species to evaluate the consequences for trait-based species ranking. We studied functional traits of eight perennial grassland species classified into different functional groups (forbs vs. grasses) and varying in their inherent growth stature (tall vs. small) in a common garden experiment with different environments crossing three levels of nutrient availability and three levels of light availability over 4 months of treatment applications. Grasses and forbs differed in almost all above- and belowground traits, while trait differences related to growth stature were generally small. The traits showing the strongest responses to resource availability were similarly for grasses and forbs those associated with allocation and resource uptake. The strength of trait variation in response to varying resource availability differed among functional groups (grasses > forbs) and species of varying growth stature (small-statured > tall-statured species) in many aboveground traits, but only to a lower extent in belowground traits. These differential responses altered trait-based species ranking in many aboveground traits, such as specific leaf area, tissue nitrogen and carbon concentrations and above-belowground allocation (leaf area ratio and root : shoot ratio) at varying resource supply, while trait-based species ranking was more consistent in belowground traits. Our study shows that species grouping according to functional traits is valid, but trait-based species ranking depends on environmental conditions, thus limiting the applicability of species-specific mean trait values in ecological studies.

Keywords: Above- and belowground traits; forbs; functional groups; functional traits; grasses; growth stature; light; nutrients; trait variation.

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Published by Oxford University Press on behalf of the Annals of Botany Company.

Introduction

There is a growing consensus that the use of functional traits has the potential to gain a better understanding of the functioning of organisms, how they relate to the environment and to address unresolved issues of community ecology and ecosystem research (Lavorel and Garnier 2002). It is mostly assumed that trait variation between species is much larger opposed to intraspecific trait variability (Diaz and Cabido 1997; McGill *et al.* 2006). This assumption is reflected in the a priori classification of plant species into functional groups, i.e. grouping of species according to similarities in their functional characteristics, as well as the application of more recently developed trait-based approaches (Lavorel *et al.* 1997; Dyer *et al.* 2001). However, both genetic differentiation and environmental variation are well-known factors, which may affect the phenotypic expression of functional traits (Coleman *et al.* 1994; Violle *et al.* 2012). In natural environments, plants are exposed to variation in multiple environmental factors and simultaneously compete for resources above- and belowground (Chapin *et al.* 1987). Trait variation at different levels of plant organization, ranging from physiological and biochemical to morphological characteristics, and allocation between plant organs enable plant species to adjust to a wide range of ecological conditions. Light availability and thus carbon acquisition via photosynthesis as well as soil nutrient availability are the most limiting factors for plant growth in temperate grasslands. Variation in traits associated with light acquisition and carbon assimilation, especially morphological and physiological leaf traits [e.g. specific leaf area (SLA) and leaf nitrogen concentrations (LNCs)], shoot traits associated with a better positioning of plant organs for light interception in dense canopies (e.g. height growth, allocation between leaves and supporting tissue) and biomass allocation between above- and belowground plant organs [root : shoot ratio (RSR) and leaf area ratio (LAR), i.e. leaf area per total dry mass] are typical responses to variation in light availability (Givnish 1988; Valladares and Niinemets 2008). In turn, variation in morphological root characteristics associated with nutrient uptake (e.g. specific root length (SRL), i.e. root length per unit root mass) and altered allocation between roots and shoots may result from changes in the availability of belowground resources (Ryser and Lambers 1995; Hill *et al.* 2006). However, different levels of nutrient availability may also induce an alteration in leaf morphological traits such as leaf dry matter content (LDMC) and SLA (Chapin *et al.* 1987; Hodgson *et al.* 2011). The close integration of plant carbon and nutrient metabolism requires a balance of various resources for growth. Thus, the acquisition of a single resource (e.g. carbon) is not independent of the availability of others (e.g. nutrients), and it is commonly assumed that plants allocate proportionally more resources to organs, which determine the capture of the most limiting resource to achieve a 'functional equilibrium' (Bloom *et al.* 1985; Poorter *et al.* 2012). An alternative explanation, however, is based on the observation that allocation into different plant organs is a function of plant size following non-linear allometric relationships (Coleman *et al.* 1994; Müller *et al.* 2000). Therefore, the degree of plasticity in changing allometric allocation is important to reduce resource imbalances (Shipley and Meziane 2002).

The most commonly accepted classification of non-legume herbaceous species into functional groups distinguishes between monocots (grasses) and dicots (forbs), mainly due to their taxonomy, phylogeny and differences in their growth forms (Dyer *et al.* 2001; Reich *et al.* 2003*a*). It has been shown that grasses have lower LNCs, greater leaf thickness and leaf tissue density, as well as smaller root diameters (RDs) and invest a smaller proportion of total biomass into leaves than forbs (Grime *et al.* 1997; Craine *et al.* 2001; Reich *et al.* 2003*b*; Poorter *et al.* 2012). Higher tissue density correlates with greater leaf longevity and plays a central role in plant strategies of nutrient acquisition and use (Grime *et al.* 1997; Aerts and Chapin 2000). Species with greater tissue density are thought to minimize nutrient loss and to maintain growth at low resource supply (= conservative species), while species with low tissue density are capable of fast resource acquisition and are more responsive in terms of growth to increased nutrient availability (= exploitative species) (Chapin 1980; Reich *et al.* 2003*b*). However, many ecological characteristics do not differ consistently between grasses and forbs, but display a large variation within these functional groups (Grime *et al.* 1997; Craine *et al.* 2001; Tjoelker *et al.* 2005). It has been hypothesized that exploitative species show greater trait plasticity than conservative species in response to nutrient availability (Crick and Grime 1987), but to our knowledge the few experimental studies testing this hypothesis on herbaceous species focussed exclusively on grass species and did not obtain consistent results (Van de Vijver *et al.* 1993; Grassein *et al.* 2010).

In temperate grasslands, usually a small number of species with an inherent taller growth achieves dominance and contributes the largest fraction of community biomass. A larger number of species with an inherent small growth stature is therefore more likely restricted to grow in low light conditions because taller growing species intercept a disproportionately larger fraction of light (Weiner 1990). Consequently, competition for light is asymmetric. Although soil resources are less likely pre-emptable, competition belowground has also been suggested to be size-asymmetric (Rajaniemi 2003). This size-asymmetry in competition raises the question how inherently tall- and small-statured species differ in their potential of responding plastically to varying light and nutrient availability.

Although it is well known that plants need to adjust to multiple local environmental factors, most experimental research focussing on functional trait variation to resource availability has manipulated single resources, such as nutrients (e.g. Al Haj Khaled *et al.* 2005; Mokany and Ash 2008; da Silveira Pontes *et al.* 2010; Kazakou *et al.* 2014) or light (Ryser and Eek 2000; Semchenko *et al.* 2012), while the interaction of light and nutrient availability on functional trait expression has rarely been studied or has been restricted to a smaller set of traits (Olff *et al.* 1990; Shipley and Almeida-Cortez 2003; Freschet *et al.* 2013). Previous studies manipulating single resources have shown that some aboveground traits are more plastic than others (Poorter *et al.* 2012; Kazakou *et al.* 2014) and that species ranking according to trait values is often consistent across environments (Mokany and Ash 2008; Kazakou *et al.* 2014). However, these results need to be extended to variation in multiple environmental factors covering species which may be assumed to play a different ecological role in natural communities and involving a larger set of traits.

In the present study, we grew eight perennial grassland species (four grasses, four forbs) of varying growth stature under controlled resource supply over 4 months at different combinations of light and nutrient availability to test the following hypotheses. (i) Grass species possess trait values associated with a more conservative use of resources and forb species show trait values indicative for a more exploitative use of resources, while differences between small- and tall-statured species in traits not directly related to height growth are small if grown as single plant individuals. (ii) The magnitude of trait variation in response to resource availability differs among traits and is greatest in traits related to allocation and the uptake of light and nutrients to maintain the 'functional equilibrium' at varying resource availability. (iii) The direction of trait variation in response to light and nutrient availability does not differ between grasses and forbs or small- and tall-statured species, but the extent of trait variation differs between forbs and grasses due to their different resource-use strategies. (iv) Trait-based species ranking varies across environments, but may be consistent for traits with similar plasticity across species or if differences in trait plasticity do not exceed interspecific trait differences.

Methods

Experimental design

Eight perennial species were chosen for the experiment. The four forb species and four grass species, including both small-statured and tall-statured species, are typical representatives of Central European semi-natural temperate grasslands (Molinio-Arrhenatheretea; Ellenberg 1988) (Table 1). Seeds were acquired from a commercial supplier (Rieger-Hoffman GmBH, Blaufelden-Raboldshausen, Germany). Seeds were pre-germinated in petri-dishes on moistened filter paper in an unheated glasshouse in April 2011. Seedlings in the stage of cotyledon emergence were separated and transferred into quickpots of 20 cm^3 volume (Hermann Meyer KG, Rellingen, Germany). At the time of primary leaf emergence, single seedlings were transplanted into pots (volume 3 L, diameter 16.5 cm, height 18.5 cm) from 30 to 31 May 2011. Sieved topsoil from a close-by field (0–30 cm, czernozem; Altermann *et al.* 2005) at the experimental field station Bad Lauchstädt (Germany, 51°23′38″N, 11°52′45″E) was used as substrate [soil texture: loamy sand; pH 7.29; nitrogen 1.18 mg N g^{-1}, carbonate 1.27 %, organic carbon 15.14 mg C g^{-1}, C : N ratio 12.83, phosphorus (from double lactate extracts) 15.86 mg P kg^{-1}, potassium (from calcium acetate lactate extracts) 51.7 mg K kg^{-1}]. Plants were cultivated at ambient temperatures in a greenhouse with a roof, which automatically closes at rain.

Three weeks after transplanting, plants were randomly assigned to orthogonally crossed shade × fertilizer treatments of three levels each. The levels for shade treatments were full sunlight (= control), 40 % shade (= medium) and 70 % shade (= high), each replicated in six blocks arranged on tables (98 × 200 cm size). Shading was accomplished by fastening one (for 40 % shade) or two layers (for 70 % shade) of green shading cloth (polyethylene, aperture size 2 × 10 mm, Hermann Meyer KG, Rellingen, Germany) to aluminium frames at 96 cm height and closed on all sides. All plants received micronutrient solution [1 mL Hoagland A-Z solution, **see Supporting Information—Table S1**] and 1 mL of $FeCl_3$ in 100 mL distilled water at the beginning of the experiment. NPK fertilization was applied equivalent to 62.5 mg nitrogen in total, spread over eight applications administered biweekly in 50 mL custom mixed fertilizer solution containing 6.9 g L^{-1} $CaHPO_4$, 8.35 g L^{-1} K_2SO_4, 12.68 g L^{-1} $MgSO_4 \cdot 7H_2O$ and 3.57 g L^{-1} NH_4NO_3 for high-level fertilization, equivalent to 200 kg ha^{-1} year^{-1} nitrogen. Half the dosage was applied for medium-level fertilization, equivalent to 100 kg ha^{-1} year^{-1} nitrogen. These resemble the commonly applied fertilizer intensities in agriculturally managed semi-natural grasslands in Europe (Olff *et al.* 1990). Other plants did not receive additional nutrients throughout the whole duration of the experiment (= control).

Each species was cultivated with five replicates per treatment combination except for *Prunella vulgaris* L., for which one treatment combination (high shade ×

Table 1. Studied species, plant height (Jäger 2011), grouping into functional groups (grasses vs. forbs) and growth stature (small vs. tall).

Species	Family	Height (cm)	Functional group	Stature
Anthoxanthum odoratum L.	Poaceae	20–50	Grass	Small
Lolium perenne L.	Poaceae	10–60	Grass	Small
Arrhenatherum elatius (L.) P. Beauv. ex J. Presl & C. Presl	Poaceae	60–120	Grass	Tall
Dactylis glomerata L.	Poaceae	50–120	Grass	Tall
Plantago lanceolata L.	Plantaginaceae	10–50	Forb	Small
Prunella vulgaris L.	Lamiaceae	5–30	Forb	Small
Centaurea jacea ssp. jacea L.	Asteraceae	15–80	Forb	Tall
Knautia arvensis (L.) Coulter	Dipsacaceae	30–80	Forb	Tall

high fertilization) was lost due to seedling mortality. In total, 344 plants were grown. All plants were manually watered with tap water on a regular basis according to the estimated pot weight for 60 % water capacity and accounting for increasing plant size throughout the experiment. Plants assigned to different shading treatments were randomly assigned to blocks and re-arranged within blocks every 4 weeks.

Data collection

Four months after initiating treatments with different light and nutrient availability, all plants were harvested from 10 to 20 October 2011. Shortly before the harvest, stomatal conductance (SC-1 Leaf Porometer, Decagon Devices Inc.) and leaf greenness (= unitless measure of foliar chlorophyll content; SPAD 502 Plus Chlorophyll Meter, Spectrum Technologies, Inc.) were measured (5–7 October 2011, between 10:00 and 15:00 h). Stomatal conductance was measured at one fully expanded leaf per individual using the auto mode of the porometer (taking the first 30 s of stomatal conductance data to predict the final stomatal conductance occurring under true steady state conditions). Leaf greenness was measured with five replicates on fully expanded leaves and averaged per individual. At the point of harvest, maximum (stretched) plant height was recorded. Plants were cut at ground level and stored overnight in a cooling chamber at 4 °C in wet paper towels to achieve water saturation. The following day, aboveground plant parts were separated into inflorescences, leaves and stems (including leaf sheaths in the case of grasses) and dead material (leaves with less than two-third of green tissue). Ten fully expanded leaves per plant were chosen, blotted dry using tissues to remove any surface water and immediately weighed. Then, leaf area of the bulk sample was measured with a LI-3100 Area Meter (LI-COR Inc., Lincoln, NE, USA). If grass individuals had more than 10 tillers, only 10 were randomly chosen for separation and measurements. At the time of harvest, single individuals (<10 %) of *A. elatius* and *K. arvensis* and ~40 % of *P. lanceolata* plants had reached the reproductive stage. Belowground biomass was cleaned by rinsing off all soil over a 0.5 mm sieve. Root material was weighed and subsamples of 0.5–1 g fresh weight, representing the typical distribution of different root sizes of the plant individual, were stored at −20 °C. The root subsamples were thawed at a later point and scanned in deionized water on a flatbed scanner at 800 dpi and analysed with image analysis software (WinRHIZO; Regent Instruments, Quebec City, Canada). Root diameters calculated with this software are weighted by the overall length of analysed roots, thus attenuating the potential effect of thicker tap roots, if present. For each plant compartment, dry weight was determined after drying at 70 °C for 48 h. Total belowground biomass was derived by extrapolating dry mass of the scanned subsamples from the fresh mass to dry mass ratio of the remaining root system.

Three individuals per species and treatment combination were randomly selected for chemical analyses. Leaves (used for leaf area measurements), residual aboveground and root material of these individuals were separately chopped, finely ground with a ball mill and C and N concentrations were determined using an elemental analyser (Vario EL III Element Analyzer, Elementar, Hanau, Germany). All variables derived from these measurements are summarized in Table 2.

Data analysis

Data analysis was conducted with the statistical software R 3.0.2 (R Core Team 2013) including the package *lme4* (Bates *et al.* 2013). The software Canoco 4.5 (Biometris International, Wageningen) was used for multivariate analysis.

Linear mixed-effects models were used to determine to what extent differences among species assigned to different functional groups (forbs vs. grasses) and varying

Table 2. Summary and description of variables investigated in this study.

Variable	Unit	Description	Variable group	Abbreviation
Specific leaf area	$m_{leaf}^2\ kg_{leaf}^{-1}$	Leaf area per unit leaf dry mass	Leaf	SLA
Leaf dry matter content	$mg_{dw\ leaf}\ g_{fw\ leaf}^{-1}$	Leaf dry mass per water-saturated leaf fresh weight	Leaf	LDMC
Leaf nitrogen concentration	$mg\ N\ g_{leaf}^{-1}$	Leaf nitrogen concentration	Leaf	LNC
Leaf carbon concentration	$mg\ C\ g_{leaf}^{-1}$	Leaf carbon concentration	Leaf	LCC
Leaf greenness		Unitless measure of leaf chlorophyll concentration	Leaf	LeafG
Stomatal conductance	$mmol\ m^{-2}\ s^{-1}$	Stomatal conductance per leaf area	Leaf	g_s
Leaf mass fraction	$g_{leaf} g_{shoot}^{-1}$	Leaf mass per aboveground shoot mass	Shoot	LMF
Shoot nitrogen concentration	$mg\ N\ g_{shoot}^{-1}$	Shoot nitrogen concentration	Shoot	SNC
Shoot carbon concentration	$mg\ C\ g_{shoot}^{-1}$	Shoot carbon concentration	Shoot	SCC
Plant height	cm	Stretched plant height	Shoot	Height
Leaf area ratio	$mm_{leaf}^2\ mg_{plant}^{-1}$	Leaf area per total plant biomass	Allocation	LAR
Root:shoot ratio	$g_{root}\ g_{shoot}^{-1}$	Root biomass per aboveground biomass	Allocation	RSR
Root nitrogen concentration	$mg\ N\ g_{root}^{-1}$	Root nitrogen concentration	Root	RNC
Root carbon concentration	$mg\ C\ g_{root}^{-1}$	Root carbon concentration	Root	RCC
Specific root length	$m_{root}\ g_{root}^{-1}$	Root length per root mass	Root	SRL
Root length density	$cm_{root}\ cm_{soil}^{-3}$	Root length per soil volume	Root	RLD
Root diameter	mm	Average root diameter	Root	RD
Total biomass	g	Total plant biomass	Performance	BM

in growth stature (small vs. tall) as well as variation in resource availability (shade as linear term with 1 = full light, control, 2 = 40 % shade, 3 = 70 % shade; fertilizer as linear term with 1 = no fertilization, control, 2 = medium fertilization, 3 = high fertilization) and their interactions explained variation in the measured variables. Starting from a constant null model with block and species identity as random terms, the fixed effects were added stepwise in the following sequence: fertilization, shade, fertilization × shade interaction, functional group identity (grass vs. forb) and growth stature (tall vs. small) and their respective interaction with fertilization and shade. In order to evaluate the statistical significance of model improvement by sequential addition of fixed effects, the maximum likelihood method and likelihood ratio tests were applied. Data were transformed to logarithms to approach a better normal distribution except for stomatal conductance, leaf greenness, leaf N and C concentrations (no transformation), RSR (cubic root transformation) and height (square root transformation).

To decompose the variability attributable to model terms, mixed-effect models were fitted with the restricted maximum likelihood method. Variance components associated with random effects (block, species and residual) were estimated from the full model. To assess the fraction of variability associated with the fixed effects, a series of hierarchical models was fitted. For each individual effect, the share of explained variability was estimated as the difference between the total variability attributed to random effects in models not including and models including the respective fixed effect.

Resource availability is a critical determinant for inflorescence development and plant individuals must reach a critical size to initiate flowering in many herbaceous species (Sugiyama and Bazzaz 1998). To control for possible effects of plant developmental stage on trait differences, we introduced developmental stage (vegetative vs. reproductive) as a covariate before the experimental factors in additional models. The inclusion of developmental stage had only minor effects on the outcome of statistical analyses.

Additionally, the above-mentioned model was modified following a suggestion by Shipley and Meziane (2002) to test for non-linear allometric allocation in LAR and RSR as a function of plant size. The natural logarithm of total leaf area (or shoot biomass respectively) was modelled by additionally fitting the natural logarithm of total biomass (or root biomass, respectively) and its interaction with the previously described terms. The random term including species identity was modified by accounting for species-specific differences in the allometric relationship to plant size, i.e. the natural logarithm of total biomass and root biomass, respectively. Significant

Table 3. Summary of mixed-effects model analyses for functional traits combining all species. Models were fitted by stepwise inclusion of fixed effects. Likelihood ratio tests (χ^2) were used to assess model improvement and the statistical significance of the explanatory terms (*P* values). For abbreviations and description of variables see Table 2.

	SLA		**LDMC**		**LNC**		**LCC**		**LeafG**		**g_s**	
	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*
Fertilizer	0.27	0.606	1.24	0.265	63.15	<0.001	1.25	0.263	87.91	<0.001	4.72	0.030
Shade	52.75	<0.001	32.84	<0.001	15.45	<0.001	6.43	0.011	31.63	<0.001	1.93	0.165
Fertilizer × shade	1.57	0.210	0.02	0.877	0.52	0.470	0.48	0.488	3.11	0.078	0.18	0.673
Functional group (FG)	1.14	0.285	14.34	<0.001	8.61	0.003	6.50	0.011	10.74	0.001	22.56	<0.001
FG × fertilizer	14.77	<0.001	9.27	0.002	0.93	0.334	1.60	0.206	21.49	<0.001	3.01	0.083
FG × shade	4.18	0.041	27.30	<0.001	1.54	0.215	0.78	0.378	70.01	<0.001	2.70	0.100
Growth stature (GS)	3.15	0.076	0.64	0.425	3.93	0.047	3.97	0.046	3.43	0.064	3.13	0.077
GS × fertilizer	1.15	0.284	0.89	0.346	0.37	0.543	1.04	0.309	0.89	0.344	0.18	0.671
GS × shade	0.67	0.413	6.82	0.009	2.96	0.085	10.24	0.001	0.36	0.550	4.08	0.043
FG × GS	0.32	0.575	2.22	0.136	1.67	0.196	3.94	0.047	0.15	0.701	5.77	0.016
	LMF		**SNC**		**SCC**		**Height**		**LAR**		**RSR**	
	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*
Fertilizer	0.24	0.627	74.40	<0.001	0.71	0.401	18.58	<0.001	21.02	<0.001	121.78	<0.001
Shade	26.90	<0.001	27.60	<0.001	5.62	0.018	26.98	<0.001	56.29	<0.001	37.25	<0.001
Fertilizer × shade	3.85	0.050	0.67	0.411	5.66	0.017	0.00	0.980	0.85	0.357	3.53	0.060
Functional group (FG)	14.48	<0.001	17.69	<0.001	1.47	0.225	12.24	<0.001	1.48	0.224	0.77	0.380
FG × fertilizer	1.17	0.280	0.39	0.534	1.37	0.241	1.33	0.249	20.31	<0.001	13.30	<0.001
FG × shade	8.70	0.003	13.69	<0.001	5.27	0.022	6.14	0.013	13.66	<0.001	5.19	0.023
Growth stature (GS)	0.29	0.592	3.15	0.076	1.62	0.203	1.72	0.190	<0.01	0.951	8.93	0.003
GS × fertilizer	4.77	0.029	0.38	0.539	<0.01	0.972	2.45	0.118	0.04	0.842	8.99	0.003
GS × shade	15.03	<0.001	20.17	<0.001	<0.01	0.974	12.32	<0.001	5.84	0.016	0.23	0.631
FG × GS	1.02	0.313	5.34	0.021	0.35	0.552	2.05	0.152	0.35	0.554	2.96	0.085

	RNC		RCC		SRL		RLD		RD		Biomass	
	χ^2	***P***	χ^2	***P***	χ^2	***P***	χ^2	***P***	χ^2	***P***	χ^2	***P***
Fertilizer	68.00	$<$0.001	16.79	$<$0.001	33.72	$<$0.001	0.23	0.630	11.43	$<$0.001	47.68	$<$0.001
Shade	23.74	$<$0.001	5.06	0.024	29.12	$<$0.001	31.04	$<$0.001	2.64	0.104	30.61	$<$0.001
Fertilizer × shade	2.55	0.110	13.50	$<$0.001	0.70	0.404	1.93	0.165	0.89	0.347	0.38	0.539
Functional group (FG)	20.18	$<$0.001	0.61	0.435	4.61	0.032	14.67	$<$0.001	7.28	0.007	12.20	$<$0.001
FG × fertilizer	7.28	0.007	0.10	0.750	0.60	0.438	0.34	0.558	3.33	0.068	5.93	0.015
FG × shade	14.65	$<$0.001	$<$0.01	0.996	1.12	0.290	0.14	0.706	1.30	0.255	2.08	0.149
Growth stature (GS)	0.23	0.630	0.26	0.613	14.33	$<$0.001	1.20	0.274	2.98	0.084	0.17	0.684
GS × fertilizer	5.45	0.020	0.05	0.830	1.27	0.261	4.71	0.030	1.01	0.314	0.99	0.319
GS × shade	7.56	0.006	0.00	0.989	0.08	0.772	0.09	0.760	0.71	0.399	0.06	0.812
FG × GS	1.22	0.269	0.56	0.455	0.02	0.883	0.33	0.565	0.30	0.585	0.06	0.813

interactions of total biomass (or root biomass) with the experimental factors indicate deviations of the allometric slope dependent on resource availability, functional group identity or growth stature, while significance of the main experimental factors shows differences in the allometric intercept.

Furthermore, standardized principal component analysis (PCA) was applied to trait data of all species in combination to elucidate the major sources of variation in multiple traits. Data were corrected for block effects and transformed if necessary to achieve normal distribution before multivariate analyses. The resulting scores describing the distribution of plant individuals along the leading principal components were subjected to variance decomposition as described above for single traits.

The magnitude of intraspecific trait variation was estimated by calculating coefficients of variation (CVs, standard deviation over mean) across shade × nutrient treatments for each species based on mean values per treatment. In order to test for differences in the magnitude of trait variation between functional groups (grasses vs. forbs) and dependent on growth stature (tall vs. small), a two-factorial ANOVA was utilized. The consistency of species ranking in trait values across fertilizer × shade treatments was tested with Spearman's rank correlation. A high correlation coefficient ($\rho > 0.75$, $n = 36$) resembles a consistent ranking of species independent of resource availability.

Results

Leaf traits

Fertilization had positive effects on LNCs and leaf greenness and negative effects on stomatal conductance, while fertilization did not impact SLA, LDMC and leaf carbon concentrations (LCCs) **[see Supporting Information—Fig. S1A–F]** (Table 3). Reduced light availability due to shading affected all leaf traits with exception of stomatal conductance (Table 3). Specific leaf area and LNC increased, while LDMC, LCC and leaf greenness decreased under shading **[see Supporting Information—Fig. S2A–F]** (Table 3). Nutrient and light availability did not interact in their effects on leaf traits.

Grass and forb species differed in all leaf traits with the exception of SLA (Table 3). Forbs had greater LNC, leaf greenness and stomatal conductance and had lower LDMC and LCC than grasses. Variation in SLA, LDMC and leaf greenness in response to nutrient and light availability also differed between grasses and forbs. While in forb species SLA tended to decline and LDMC tended to increase with increasing fertilization, the opposite was observed in grass species. The positive effects of fertilization on leaf greenness were stronger in grasses than in forbs, whereas the decrease of leaf greenness in response to shading was more pronounced in forbs than in grasses. In contrast, the responsiveness of SLA (increase) and LDMC (decrease) to shading was more pronounced in grasses than in forbs. Leaf traits barely differed among species with tall and small growth stature, except for LNC and LCC, which were larger in tall-statured species. However, in small-statured species, shade had a stronger negative effect on LDMC and LCC. The opposite was the case for stomatal conductance (g_s); g_s of tall-statured species showed a greater variation in response to shade than g_s of small-statured species.

Shoot traits

Fertilization increased plant height and shoot nitrogen concentrations (SNCs), but did not affect leaf mass fraction (LMF) and shoot carbon concentrations (SCCs) **[see Supporting Information—Fig. S1G–J]** (Table 3). Shading decreased SCC and increased LMF, SNC and height **[see Supporting Information—Fig. S2G–J]**. Nutrient and light availability showed interactive effects on SCC. With both increasing nutrients and shade SCC declined. Forb and grass species differed in all aboveground shoot traits with exception of SCC. Grass species allocated less shoot mass to leaves (lower LMF), grew taller and had lower SNC than forb species. The effects of shading on shoot traits also differed between grasses and forbs. Grasses showed a stronger increase in LMF, SNC and height than forbs in response to shading, but shading led to a more pronounced reduction of SCC in forbs than in grasses. Aboveground shoot traits did not differ among species with tall and small growth stature, but the increase in plant height, SNC and LMF in response to shading was more pronounced in small-statured than in tall-statured species.

Above- and belowground allocations

Fertilization as well as shading increased allocation in favour of aboveground plant organs [higher LAR, smaller RSR; **Supporting Information—Figs S1K and L and S2K and L**, Table 3]. Root : shoot ratio decreased with increasing shade because shading reduced root mass stronger than shoot mass. Nutrient and light availability did not interact in their effects on LAR and RSR. Above- and belowground allocations did not differ between functional groups, but the increase in LAR and the decrease in RSR in response to fertilization and shading were more pronounced in grasses than in forbs. Tall-statured species had a greater RSR than small-statured species, and fertilization led to a stronger decline in RSR in small-statured than in tall-statured species. In contrast, LAR did not depend on the growth stature. The increase in LAR in response to shading was more pronounced in small-statured than that in tall-statured species.

The inclusion of root mass in analyses of shoot mass to test for size-dependent variation in root : shoot allocation **[see Supporting Information—Table S2]** showed that the allometric slope varied between grasses and forbs [significant interaction FG × log(root mass)]: forbs had a steeper allometric slope (α) than grasses ($\alpha_{\text{forbs}} = 0.74$ vs. $\alpha_{\text{grasses}} = 0.51$). Different resource availability and growth stature, however, did not affect allometric slopes in root : shoot allocation. Testing the size dependency of variation in LAR **[see Supporting Information—Table S2]** rendered that the allometric slope varied dependent on nutrient availability, i.e. fertilization led to a steeper slope ($\alpha_{\text{control}} = 0.40$ vs. $\alpha_{\text{fertilized}} = 0.78$), while shading or differences between functional groups or growth statures did not affect allometric slopes.

Belowground traits

Fertilization increased root nitrogen concentrations (RNCs) and RD and decreased SRL and root carbon concentrations (RCCs) **[see Supporting Information—Fig. S1M–Q]** (Table 3). Shading led to larger SRL and RNC, but resulted in lower root length density (RLD) and RCC **[see Supporting Information—Fig. S2M–Q]** (Table 3). Shading and fertilization interacted in their effects on RCC; in shade and at high nutrient availability RCC declined to the lowest levels.

Grass and forb species differed in all belowground traits except for RCC: forbs had lower SRL and RLD than grasses, but RNC and RD were greater in forbs than in grasses. The increase of RNC in response to shade was larger in grasses but the positive effect of fertilization on RNC was greater in forbs. Species of different growth stature differed in their SRL; small-statured species exhibited a higher SRL. Fertilization led to a larger increase in RLD in small-statured compared with tall-statured species. The effects of shading and fertilization on RNC were stronger in small-statured than in tall-statured species.

Plant biomass

Performance expressed as total plant biomass increased with fertilization **[see Supporting Information—Fig. S1R]** and decreased with shading **[see Supporting Information—Fig. S2R]**, but both factors did not interact in their effects on total plant biomass (Table 3). Grass species produced more biomass than forbs, and positive effects of fertilization were more pronounced in grasses than in forbs. Total plant biomass did not differ among species with tall and small growth stature.

Attribution of sources of variation in multiple traits

The two leading axes of a standardized PCA of trait values across all species (Fig. 1) accounted for almost 60 % of variation. The first axis explaining 37 % of variation had

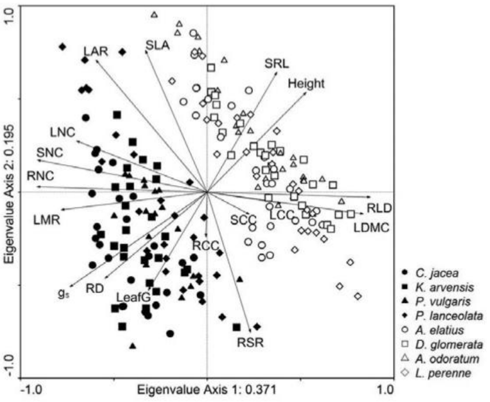

Figure 1. Standardized PCA of trait values across all studied species at different levels of resource availability. Abbreviations: SLA, specific leaf area; LDMC, leaf dry matter content; LNC, leaf nitrogen concentration; LCC, leaf carbon concentration; LeafG, leaf greenness; g_s, stomatal conductance; LMF, leaf mass fraction; SNC, shoot nitrogen concentration; SCC, shoot carbon concentration; LAR, leaf area ratio; RSR, root : shoot ratio; RNC, root nitrogen concentration; RCC, root carbon concentration; RLD, root length density; SRL, specific root length; RD, root diameter.

high positive loadings for LDMC and RLD, opposed to high negative loadings for tissue N concentrations (SNC, RNC and LNC), LMF and stomatal conductance. The second axis explaining 19 % of variation had high positive loadings for SLA, LAR and SLR and high negative loadings for RSR. The major sources of variance explaining variation in multiple traits along the two leading axes were differences between functional groups (first axis 69 %, second axis 15 %) and due to shading (first axis 15 %, second axis 53 %) **[see Supporting Information—Fig. S3]**. The third axis mainly represented trait variation due to species identity (40 %) and fertilization (10 %).

Magnitude of intraspecific trait variation

Intraspecific trait variation quantified as CV across treatments differed greatly among traits, while differences among grasses vs. forbs and small vs. tall-statured species in intraspecific trait variation were often non-significant (Table 4). Overall, grasses had a greater intraspecific variation in stomatal conductance, LNC, LDMC, SNC and RSRs than forbs (Fig. 2). Small-statured species varied more in their LDMC and less in their leaf greenness than tall-statured species.

In general, carbon concentrations (SCC, LCC and RCC) showed the smallest intraspecific variation in response to different levels of resource availability (Fig. 2). Intraspecific variation in nitrogen concentrations (SNC, LNC and RNC) was greater, but comparable in different plant organs. Leaf dry matter content and leaf greenness were less variable than SLA, LNC and stomatal conductance among leaf traits, while LMF was less variable than height and SNC among shoot traits. Intraspecific variation in RNC and SRL were similar, but smaller than intraspecific variation in RLD. Characteristics related to above-belowground allocation (LAR and RSR), RLD as well as performance quantified as total biomass had the greatest intraspecific variation in response to varying resource availability.

Attribution of sources of variation in single traits

Traits with a great plasticity in the response to shading were SLA, LAR (>50 % of variance), while the environment-induced variation in traits related to nitrogen-acquisition (LNC, leaf greenness, SNC and RNC), RSR and plant biomass were attributable to additive effects of shading and fertilization (>20 % of variance in total) (Fig. 3). A large proportion of variance in LDMC, leaf greenness, g_s, plant biomass as well as all whole-shoot and root traits with exception of C concentrations was due to differences between functional groups. Differences due to growth statures mostly explained a minor proportion of variance with exception of RSR and SRL. Species identities often explained >10 % of residual variance with exception of tissue nitrogen concentrations, LCC and g_s.

Consistency of species ranking in trait values

Species ranking across all shade × fertilizer combinations remained conserved for LDMC, g_s, plant height, root-morphological traits (RLD, SRL and RD) and plant biomass (Fig. 4). These traits had in common that >50 % of trait variation was attributable to the summed effects of functional group, growth stature and species identity (Fig. 3). The inconsistent ranking of species in other traits was mostly manifested through all treatment combinations, with exception of leaf greenness, where deviating species ranking was mainly caused by deep shade (not shown).

Discussion

Interspecific trait differences (Hypothesis 1)

The classification of grasses and forbs into distinct functional groups is widely applied in ecological studies. Grasses and forbs are known to differ in their shoot and root architecture and anatomy; however, it was repeatedly shown that this grouping may not be based on single characteristics, but that trait combinations including both above- and belowground traits distinguish between grasses and forbs most efficiently (Craine *et al.* 2001; Reich *et al.* 2003*a*; Tjoelker *et al.* 2005). Plant resource-use strategies may be described along a fundamental trade-off between resource acquisition and resource

Table 4. Summary of two-factorial analysis of variance (ANOVA) of trait variation in response to resource availability estimated as coefficient of variation (CV, standard deviation over means) across treatments. Given are *F*-values and statistical significance of the explanatory terms (*P* values). For abbreviations and description of variables see Table 2.

	SLA		**LDMC**		**LNC**		**LCC**		**LeafG**		**g_s**	
	F	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
Functional group (FG)	2.72	0.174	11.46	0.028	8.24	0.045	1.72	0.260	0.57	0.493	7.81	0.049
Growth stature (GS)	0.12	0.743	9.44	0.037	2.12	0.219	1.67	0.266	13.47	0.021	0.48	0.526
FG × GS	0.33	0.598	1.48	0.291	0.03	0.876	0.28	0.625	0.64	0.467	0.12	0.744

	LMF		**SNC**		**SCC**		**LAR**		**RSR**		**Height**	
	F	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
Functional group (FG)	1.45	0.295	24.43	0.008	6.56	0.063	5.42	0.080	14.62	0.019	0.62	0.475
Growth stature (GS)	2.24	0.209	7.10	0.056	1.14	0.346	1.01	0.371	2.71	0.175	5.43	0.080
FG × GS	0.78	0.427	0.11	0.753	<0.01	>0.999	0.10	0.769	2.15	0.216	0.06	0.814

	RNC		**RCC**		**SRL**		**RLD**		**RD**		**Biomass**	
	F	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
Functional group (FG)	1.02	0.371	2.20	0.212	0.79	0.426	1.26	0.324	3.40	0.139	2.04	0.227
Growth stature (GS)	4.78	0.094	0.04	0.858	0.02	0.897	0.04	0.848	1.02	0.369	1.01	0.372
FG × GS	0.02	0.889	0.06	0.815	1.59	0.276	<0.01	0.971	0.60	0.483	0.71	0.448

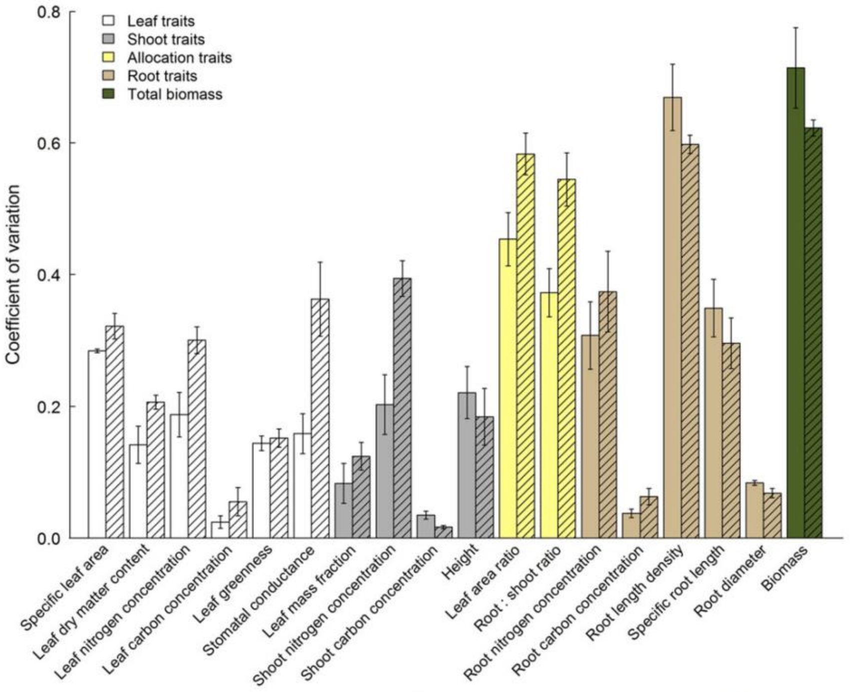

Figure 2. Intraspecific trait variation in response to resource availability estimated as coefficient of variation (CV, standard deviation over means) across treatments. Shown are means (±1 SE) across species. Hatched bars represent grass species.

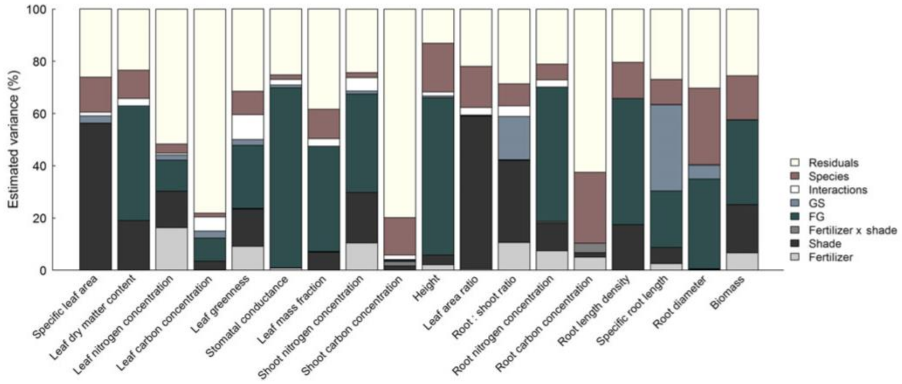

Figure 3. Estimated variance decomposition based on mixed-effects model analyses shown in Table 3. Note the variance components for block effects and residuals were combined in the graph, as well as the respective interactive effects of FG, GS × resource availability (as 'interactions').

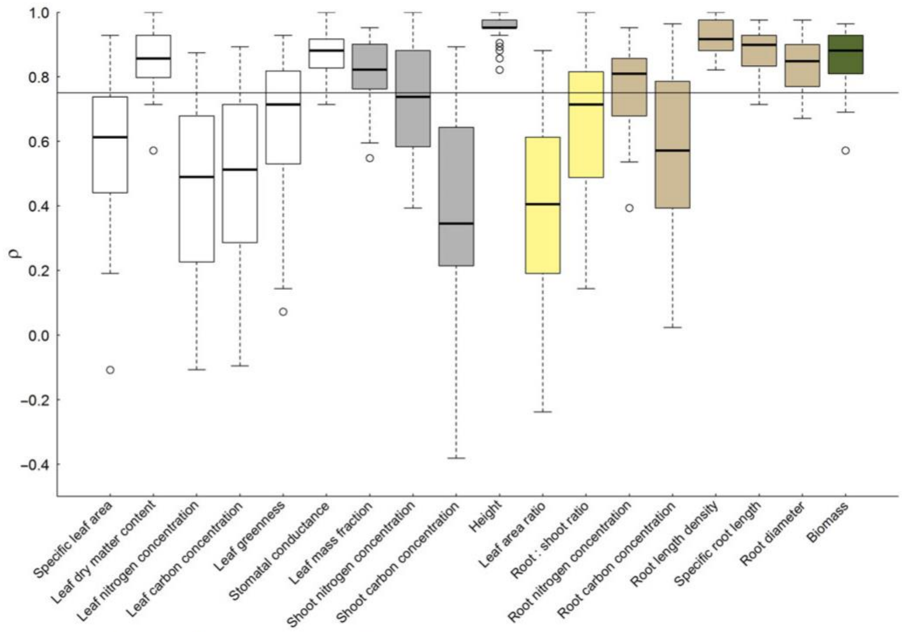

Figure 4. Species ranking in trait values across shade × fertilizer treatments, tested with Spearman's rank correlation, where a high correlation coefficient ($\rho > 0.75$) indicates a consistent ranking of species independent of resource availability. For colours of trait groups see Figure 2.

conservation and related to a 'fast-slow' plant economic spectrum (Reich 2014). High root N concentrations and SRLs are mechanistically related to root respiration, reflecting patterns of high metabolic activity associated with nutrient uptake and assimilation, and fast growth (Reich *et al.* 2003*a*; Tjoelker *et al.* 2005; Roumet *et al.* 2006). High leaf N concentrations correlate positively with leaf respiration and net photosynthesis rates and negatively with tissue longevity (e.g. Lambers and Poorter 1992; Reich *et al.* 2003*a*), while low tissue N concentrations indicate high nutrient retention (Aerts and Chapin 2000). In our study, grass species were characterized by greater LDMC, lower tissue N concentrations and had thinner roots, which is in line with our Hypothesis 1 that grasses possess trait combinations indicating a more conservative use of resources, while forbs were characterized by trait combinations associated with a more exploitative use of resources (greater tissue N concentrations, higher stomatal conductance and leaf greenness). In contrast, grass species had greater SRLs than forbs, which is supposed to be associated with an exploitative use of resources and greater rates of nutrient uptake. Species with 'fast' traits are assumed to grow best in higher resource conditions, while 'slow' species are thought to be superior when resource are scarce and conservation of resources results in better growth (Reich 2014). Nevertheless, grown as single plants, grass species accumulated more biomass than forb species in all treatments, which might be explained by greater costs for 'fast' traits of forb species. Trait differences between inherently small- and tall-statured species were generally small, which is also consistent with Hypothesis 1. Small-statured species had higher SRL, but invested less biomass into belowground organs (smaller RSR) than tall-statured species, resulting in comparable values for RLD. There was no evidence for differences in height growth and total biomass production after 4 months, showing that stature did not matter for growth when resource supply was externally controlled and not limited by competition. The greater RSR of tall-statured compared with small-statured species suggests that growth of tall species in their natural habitats is more likely limited by belowground resources (nutrients) and evolutionary processes selecting for greater investment into roots. It could be argued that differences in RSR were due to non-linear allometric allocation dependent on plant size (e.g. Shipley and Meziane 2002). Mixed-model analyses, however, showed that growth stature only affected the intercept of root : shoot allometry, while having no significant effects on the slope of this relationship **[see Supporting Information—Table S2]**.

Effects of light and nutrient availability on trait expression (Hypothesis 2)

In accordance with results from earlier studies, shading resulted in greater investment into aboveground plant organs (higher LAR and lower RSR), higher tissue N concentrations, but lower tissue C concentrations (lower C/N ratios due to reduced growth) and the formation of leaves with larger SLA (Ryser and Lambers 1995; Ryser and Eek 2000; Evans and Poorter 2001). Less is known about the effect of shade on belowground morphology. Ryser and Eek (2000) showed that SRL of *D. glomerata* increased, when light availability was reduced to 20–30 % of full daylight, which is consistent with our results. A better nutrient availability is known to increase allocation to aboveground plant organs (higher LAR and lower RSR) (Elberse and Berendse 1993; Ryser and Lambers 1995). Root-morphological changes, i.e. a decreased RD and increased SRL, have been described in response to phosphorus deficiency, but less concordant effects have been observed for nitrogen deficiency (Hill *et al.* 2006). In our study, using NPK fertilization, we found decreasing SRL and increasing RD with fertilization in congruence with other studies (Fransen *et al.* 1999; Grossman and Rice 2012). Despite shading- or fertilization-induced plastic changes in all studied traits (Table 3), the magnitude of trait responsiveness varied greatly among traits. Allocation between above- and belowground plant organs (LAR and RSR), traits related to resource uptake (SLA and SRL) and RLD, which is closely related with SRL, were the most plastic characteristics in all studied species (Fig. 2) as predicted by our Hypothesis 2. However, plasticity in tissue nitrogen concentrations also showed considerable variation in response to resource availability suggesting that C and nutrient metabolism were not balanced at different levels of resource supply.

Extent and structure of trait variation as affected by functional groups and growth stature (Hypothesis 3)

Albeit similar responses across all studied species, significant interactions between functional group identity and resource availability (shade, fertilization) in several leaf traits (SLA, LDMC and leaf greenness), above- and belowground allocations (RSR and LAR), height and shoot C and N concentrations showed that aboveground traits of grasses and forbs differed in their responsiveness to environmental variation as suggested by our Hypothesis 3, while this was not the case for root-morphological traits (Table 3). Although studies comparing pairs of grass species from fertile and infertile habitats suggested that exploitative species show greater plasticity to nutrient availability than conservative species (Crick and Grime 1987; Grassein *et al.* 2010), we found that grass species having traits characteristic for a conservative use of resources were more plastic in several traits (LDMC, LNC, g_s, SNC and RSR) than forb species (Fig. 2, Table 4). In accordance with our expectations, we also found that trait variation of small-statured species in response to light availability was greater than in tall-statured species in many aboveground traits, suggesting that inherently small species, which often suffer from light competition in their natural habitats, have been selected for greater responsiveness to shade (Table 3).

Effects of light and nutrients on trait-based species ranking (Hypothesis 4)

A number of recent studies have pointed out that intraspecific trait variation might be equally important to consider in ecological studies than interspecific trait differences (Albert *et al.* 2011; Violle *et al.* 2012). The consistency of trait-based species ranking in different environments depends on the direction of trait variation in response to environmental variation and the relative magnitude of inter- vs. intraspecific variation (Garnier *et al.* 2001). Several studies, mostly conducted at varying nutrient supply, delivered heterogeneous results. For example, consistent species rankings have been found for leaf traits such as SLA and LDMC (Al Haj Khaled *et al.* 2005; Mokany and Ash 2008; Kazakou *et al.* 2014), while Rose *et al.* (2013) reported less consistent species rankings in these traits. However, it has also been emphasized that the stability of species rankings depends on the considered trait (Kazakou *et al.* 2014). Our study manipulating nutrient supply in combination with different levels of shading, provided clear evidence that trait-based species ranking is only consistent across environments, when trait variation in response to resource availability is small compared with differences between functional groups, growth statures or dependent on species identity (Figs 2 and 3) or if the magnitude and direction of trait variation does not differ between species, confirming Hypothesis 4. For example, species ranks in all root-morphological traits (RLD, SRL and RD) were largely consistent although these traits differed greatly in their intraspecific variation in response to resource availability (Fig. 3), but plasticity in these traits did not differ between functional groups or dependent on growth stature (Table 3). In contrast, species ranking in LDMC and plant height was also consistent, probably due to great differences in trait values between species buffering against the differential effects of resource availability dependent on functional group identity or growth statures.

Conclusions

The usefulness of trait-based definitions of plant functional groups depends on their repeatability (Gitay and Noble 1997) implying that environment-induced variation in trait expression is similar across species. Our study showed that species assigned to the predefined functional groups of grasses and forbs differed in most studied traits. The differentiation between grasses and forbs based on multiple traits remained robust irrespective of light and nutrient availability (Fig. 1), and the identity of traits most responsive to variation in resource availability was similar among grasses and forbs (Fig. 2) justifying species classification into the commonly used functional groups (Dyer *et al.* 2001; Tjoelker *et al.* 2005). However, small interspecific differences in combination with varying plasticity in response to the environmental conditions altered trait-based ranking among species in several traits. The varying consistency in trait-based ranking may limit the usefulness of functional groups as well as the applicability of species-specific mean trait values in predicting species or community responses to environmental variation.

Sources of Funding

This work was supported by the German Science Foundation (RO2397/4).

Contributions by the Authors

All authors designed the experiment, A.S. and C.R. performed the experiment, A.S., J.S. and C.R. analysed the data, A.S. drafted the manuscript and C.R. and J.S. contributed to writing the manuscript.

Conflict of Interest Statement

None declared.

Acknowledgements

We thank B. Sawall and A. Thondorf for sample preparation and chemical analyses, all student helpers, especially T. Henning, for their endurance during harvests and the technicians of the field station Bad Lauchstädt, who helped maintaining the experiment. Additionally, we are grateful to N. Buchmann and E.-D. Schulze for discussing results, and comments from the reviewers and editors that helped to improve the manuscript.

Supporting Information

The following additional information is available in the online version of this article –

Table S1. Composition of micronutrient solution.

Table S2. Summary of mixed-effects model analyses across all studied species testing for non-linear allometric allocation in leaf area ratio and root to shoot ratio.

Figure S1. Trait values of eight studied grassland species in response to three different levels of fertilization (control = no fertilizer addition, medium and high).

Figure S2. Trait values of eight studied grassland species in response to different levels of shade (control = full light, medium and high).

Figure S3. Estimated variance decomposition based on mixed-effects model analyses for leading principal components based on multiple traits.

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Supplementary material

Table S1: Summary of chemical concentrations for Hoagland A-Z micronutrient solution applied before the beginning of the experiment

Component solution a)	mg l^{-1}	Component solution b)	mg l^{-1}
$Al_2(SO_4)_2$	56	As_2O_3	5.6
$B(OH)_3$	611	$BaCl_2$	33
$Co(NO_3)_2$ x 6 H_2O	56	$CdCl_2$	5.6
CuSO4 x 5 H_2O	56	$Bi(NO_3)_3$	6.8
KBr	28	$RbSO_4$	5.6
KI	28	K_2CrO_4	28
LiCl	28	KF	5.6
$MnCl_2$ x 4 H_2O	389	$PbCl_2$	5.6
$NiSO_4$ x 7 H_2O	56	$HgCl_2$	5.6
$SnCl_2$ x 2 H_2O	28	MoO_3	28
TiO_2	56	H_2SeO_4	5.6
$ZnSO_4$ x 7 H_2O	99	$SeSO_4$	28
		HWO_4	5.6
		VCl_3	7.2

Table S2: Summary of mixed-effects model analyses across all studied species testing for non-linear allometric allocation in leaf area ratio (relating leaf area to total biomass) and root:shoot ratio (relating shoot biomass to root biomass)

	Leaf area			Shoot biomass	
	χ^2	P		χ^2	P
Fertilizer	**46.73**	**<0.001**	Fertilizer	**181.18**	**<0.001**
Shade	**54.76**	**<0.001**	Shade	**13.73**	**<0.001**
Functional group (FG)	1.09	0.300	Functional group (FG)	3.79	0.051
Growth stature (GS)	3.16	0.075	Growth stature (GS)	**9.34**	**0.002**
Total biomass (BM)	**35.84**	**<0.001**	Root biomass (BM)	**28.08**	**<0.001**
Fertilizer x Shade	2.30	0.129	Fertilizer x Shade	**5.66**	**0.017**
FG x Fertilizer	**35.12**	**<0.001**	FG x Fertilizer	**27.11**	**<0.001**
FG x Shade	**5.99**	**0.014**	FG x Shade	0.15	0.700
GS x Fertilizer	0.44	0.508	GS x Fertilizer	**8.63**	**0.003**
GS x Shade	**7.37**	**0.007**	GS x Shade	0.07	0.791
Fertilizer x Total BM	**10.45**	**0.001**	Fertilizer x Root BM	3.51	0.060
Shade x Total BM	1.04	0.309	Shade x Root BM	2.60	0.107
FG x Total BM	0.97	0.325	FG x Root BM	**5.96**	**0.014**
GS x Total BM	0.45	0.502	GS x Root BM	0.03	0.870

Models were fitted by stepwise inclusion of fixed effects. Likelihood ratios tests (χ^2) were used to assess model improvement and the statistical significance of the explanatory terms (P values).

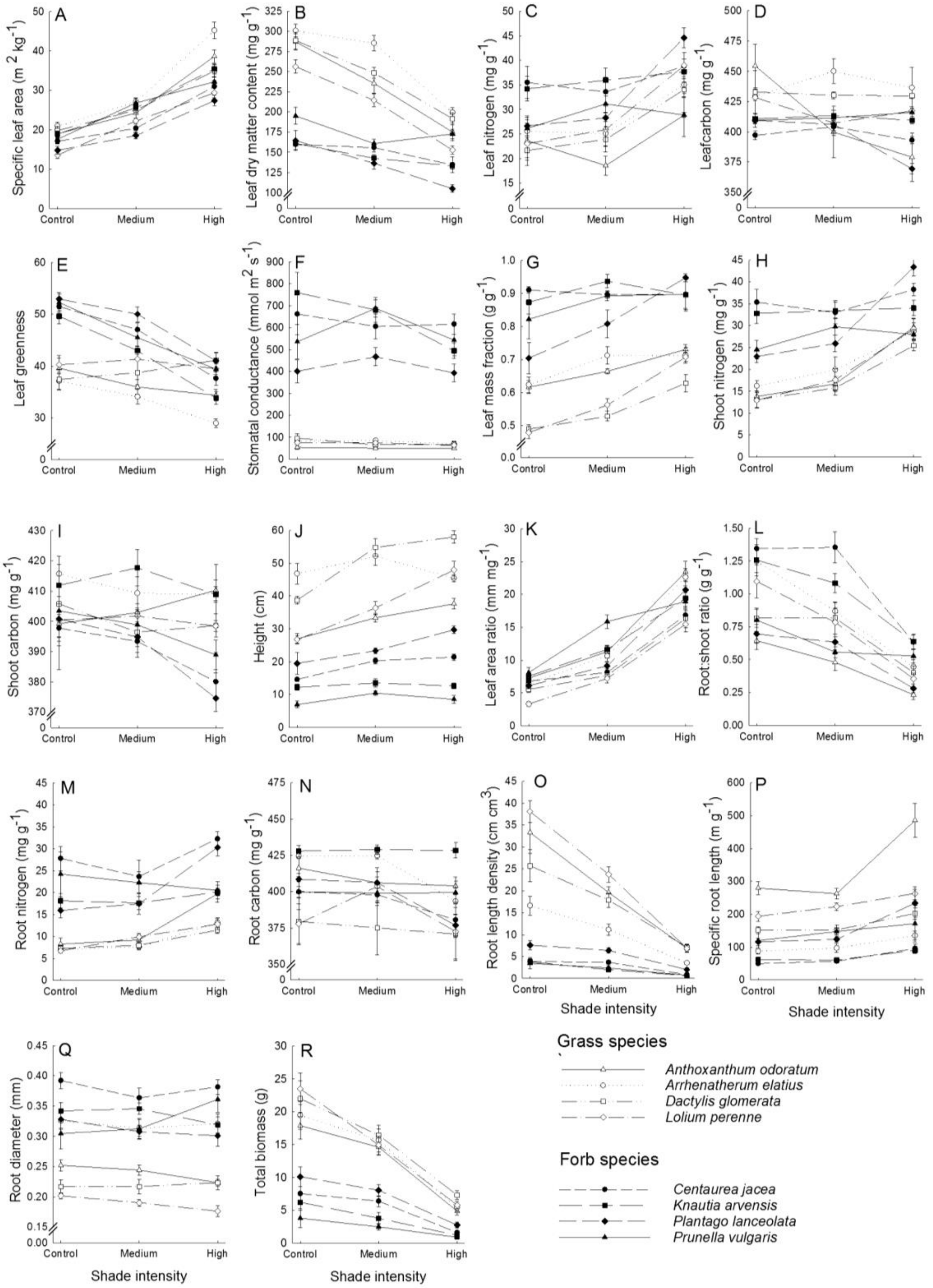

Figure S1: Specific leaf area (A), leaf dry matter content (B), leaf nitrogen concentration (C), leaf carbon concentration (D), leaf greenness (E), stomatal conductance (F), leaf mass fraction (G), shoot nitrogen concentration (H), shoot carbon concentration (I), plant height (J), leaf area ratio (K), root to shoot ratio (L), root nitrogen concentration (M), root carbon concentration (N), root length density (O), specific root length (P), root diameter (Q), and total biomass (R) of eight studied grassland species in response to three different levels of fertilization (control = no fertilizer addition, medium, high). Shown are species mean values (± 1SE) across different levels of shading.

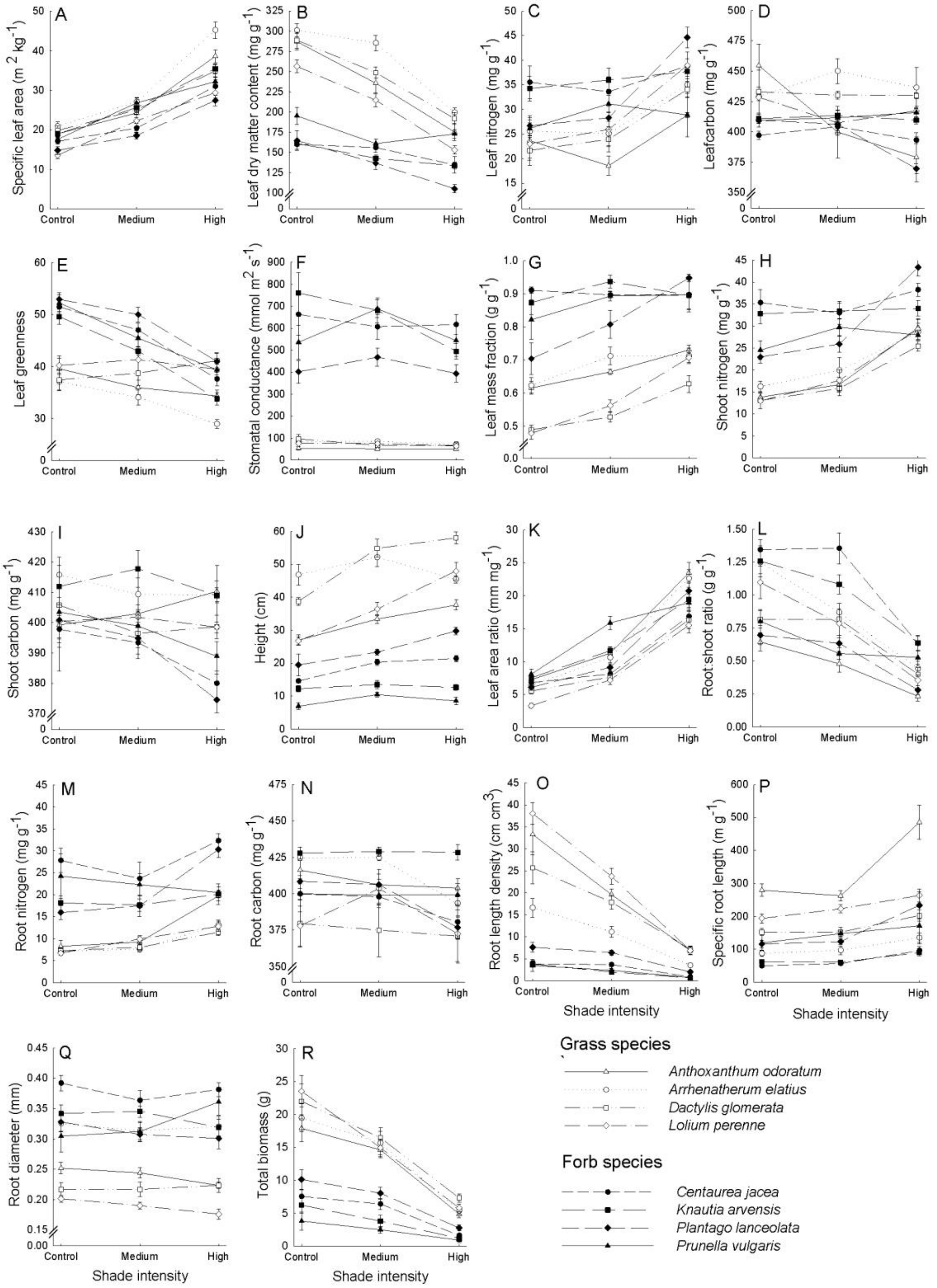

Figure S2: Specific leaf area (A), leaf dry matter content (B), leaf nitrogen concentration (C), leaf carbon concentration (D), leaf greenness (E), stomatal conductance (F), leaf mass fraction (G), shoot nitrogen concentration (H), shoot carbon concentration (I), plant height (J), leaf area ratio (K), root:shoot ratio (L), root nitrogen concentration (M), root carbon concentration (N), root length density (O), specific root length (P), root diameter (Q), and total biomass (R) of eight studied grassland species in response to different levels of shade (control = full light, medium, high). Shown are species mean values (± 1SE) across different levels of fertilization.

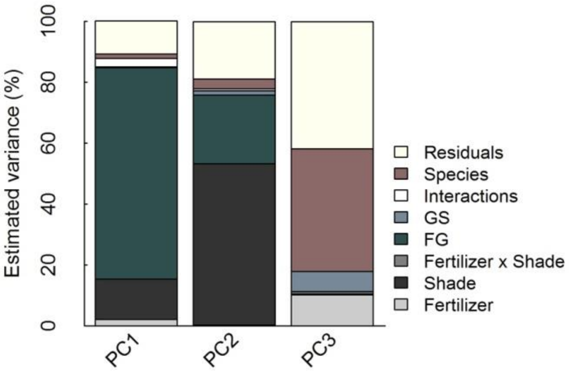

Figure S3: Estimated variance decomposition based on principal components analysis shown in Figure 4. (Note that variance components for the interactive effects of FG, GS x resources were combined in the graph as 'interactions').

CHAPTER 3

TRAIT PLASTICITY IN RESPONSE TO RESOURCE AVAILABILITY AND BIODIVERSITY MODULATES FUNCTIONAL DISSIMILARITY AMONG SPECIES IN EXPERIMENTAL GRASSLANDS

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Journal of Plant Ecology (under review)

Abstract

Aims: Functional trait differences among species are thought to be a prerequisite for niche differentiation. Plant traits are known to vary plastically in response to environmental conditions and different plant neighbourhoods. However, it is not clear to which extent the direction and magnitude of trait variation differ among species representing different growth forms or varying in dominance in different environments and how this trait variation affects community-level trait dissimilarity.

Methods: In a field experiment, we studied shoot and leaf traits of eight perennial grassland species assigned to two functional groups (grasses vs. forbs) and varying in growth stature (small vs. tall) in monocultures, two- and four-species mixtures that were exposed to two levels of light and nutrient availability.

Important findings: Fertilization increased leaf greenness and specific leaf area (SLA) as well as leaf and shoot nitrogen concentrations. Under shading, the formation of longer shoots with elongated internodes, increased biomass allocation into leaves, higher SLA and stomatal conductance (g_s) indicated larger efforts to acquire light. Increased leaf nitrogen concentrations (LNC) and shoot nitrogen concentrations (SNC) and decreased shoot carbon concentrations (SCC) suggested that plant growth was carbon-limited under shading. Increasing SLA and SNC, but decreasing SCC and shoot biomass with increasing species richness revealed an accelerated carbon limitation at higher species richness irrespective of the external manipulation of light and nutrient supply. Overall, the direction of trait variation in response to resource availability (shade, fertilization) and species richness did not differ between functional groups or growth statures. The magnitude of variation in several traits at different resource availability, however, was larger in grasses than in forbs (shade: shoot length, leaf greenness, LNC; fertilization: LNC) as well as in small-statured than in tall-statured species (shade: SLA, g_s; fertilization: LNC, SNC). In general, dominant species in terms of aboveground biomass production allocated less biomass into leaves, had taller shoots with higher SCC but lower SNC, and formed leaves with lower SLA but greater leaf greenness than subordinates. Shading accentuated differences in SLA, while fertilization increased differences in SNC and biomass allocation into leaves between dominants and subordinates. The differential plasticity of functional groups and growth statures to fertilization led to an increased dissimilarity in tissue nitrogen concentration under fertilization, while resource supply did not alter community-level dissimilarity in other traits. Our study shows that a varying extent of trait variation depending on functional group or growth stature identity and species dominance may modulate community-level trait dissimilarity at different resource availability, and therefore emphasizes the context-dependency of trait-based approaches.

Key words: biodiversity, functional groups, functional traits, growth stature, trait dissimilarity

Introduction

Functional trait differences among species are expected to increase the potential for niche differentiation and thereby promote species coexistence (Levine and HilleRisLambers 2009). In this context, functional traits are morphological, physiological or life-history characteristics expressed in the phenotype of plant individuals, which are relevant for plant fitness due to their effects on growth, survival or reproduction (Violle et al. 2007).

In natural environments, plants are exposed to multiple environmental factors and temporal and spatial variation in resource availability (Chapin et al. 1987). Nitrogen and light are among the main resources limiting plant growth in semi-natural temperate grasslands (Whitehead 2000; Hautier et al. 2009). As a consequence of the vertical canopy structure of plant stands and due to the reduced light availability in lower strata, plants of varying growth height receive different fractions of the incoming radiation. Large individuals intercept disproportionately more light, resulting in their competitive advantage via size-asymmetric competition (Weiner 1990). Conversely, belowground competition for resources is mostly assumed to be symmetric when resources are distributed evenly (Wilson 1988; Weiner et al. 1997) and thus independent of plant size. In grasslands, usually a few tall-growing species gain dominance and produce the largest fraction of aboveground biomass, while the majority of species are constrained to remain subordinate and thus contribute only little to community biomass (Werger et al. 2002; Mariotte 2014).

Plasticity in functional traits is important, as it allows plants to cope with environmental variation. For example, the formation of longer shoots, often associated with greater allocation of biomass to aboveground organs, may optimize light interception, while morphological and physiological adjustment of leaves in order to maximize carbon gain may increase the potential to tolerate low-light conditions (Valladares and Niinemets 2008). Plasticity in morphological traits like increased apical dominance and the formation of leaves with larger specific leaf area, known as typical adjustments to reduced light availability, have also been found in studies along experimental plant diversity gradients and have been explained by increased competition for light due to denser and taller canopies in more diverse plant communities (e.g. Daßler et al. 2008; Gubsch et al. 2011; Roscher et al. 2011b). However, the magnitude and to some degree the direction of trait variation in response to single resources (Pontes et al. 2010; Rose et al. 2013), the combined effects of several resources (Olff et al. 1990; Freschet et al. 2013) and along plant diversity gradients (Gubsch et al. 2011; Roscher et al. 2011c; Lipowsky et al. 2015) vary greatly among species and dependent on the considered traits.

Given these species-specific responses, it is challenging to predict how changes in resource availability affect trait dissimilarity among species, i.e. whether species converge (become more similar) or diverge (become more dissimilar) in their traits. So far, the few available studies addressing this issue in natural communities did not produce consistent results (Spasojevic and Suding 2012; Price et al. 2014). Furthermore, it remains unclear whether increased or decreased trait dissimilarity at varying resource availability depends on plant species diversity or functional composition of the investigated plant communities.

Using similarities in functional traits for a classification into functional groups is a common approach of aggregating the great variety of species into a predictive framework (Gitay and Noble 1997). Species assigned to a particular functional group are expected to be more similar in their responses to environmental variation than species assigned to different functional groups (Grime et al. 1997; Lavorel and Garnier 2002). Grasses and forbs are two commonly distinguished functional groups in grasslands, which are characterized by differences in their morphology, physiology, resource utilization and acquisition strategies (Körner 1993; Hector et al. 1999; Roscher et al. 2004). However, due to the vertical structure of plant stands and the reduced light availability in the lower strata, it is also likely that inherent differences in growth statures (i.e. tall- or small-statured species) affect the magnitude and direction of trait variation in grassland species.

In the present study, we evaluated to which extent resource availability and plant diversity (species richness, functional composition) influence functional trait variation and trait dissimilarity among species in a field experiment with monocultures, two- and four-species mixtures grown at manipulated levels of light supply (shading vs. no shade) and nutrient availability (fertilized vs. no fertilization) in the first year of treatment applications. We used eight common perennial grassland species representing two functional groups (grasses and forbs) as well as two growth statures (tall and small). In a previous short-term common garden experiment with the same species grown as single individuals at different levels of light and nutrient availability, we found that the magnitude of trait variation in response to resource availability differed between functional groups (grasses > forbs) and between growth statures (small > tall), while the direction of trait variation was similar across species (Siebenkäs et al. 2015). However, it is not clear to which degree trait variation in response to external resource supply is modified when plants interact with other plants of the same or other species and what the consequences for trait dissimilarity among these species are. We ask the following questions:

(1) Do the direction and magnitude of trait variation in response to resource availability and varying growth conditions in mixtures of increasing species richness differ between species representing different growth forms (grasses vs. forbs, tall vs. small)?

We expected that the direction of trait variation in response to light and nutrient availability does not differ between functional groups and species with inherently different growth statures. We assumed that the magnitude of trait variation in response to increased nutrient availability through fertilization is greater in grasses than in forbs. Furthermore, we expected that a reduced light availability under shading results in a greater magnitude of trait variation in small-statured than in tall-statured species. We also expected that the altered availability of above- and belowground resources in communities of increasing species richness has additional effects on trait variation.

(2) Do the traits of species being dominant in terms of aboveground biomass production in mixture vary dependent on resource availability and mixture species richness?

We expected that the traits of dominant species are similar irrespective of resource availability in our short-term study, but that trait differences between dominants and subordinates are accentuated under conditions increasing asymmetric competition for aboveground resources.

(3) Do differential effects of resource availability on trait variation of different species affect community-level trait dissimilarity in mixtures of varying species richness?

Based on our assumptions concerning the direction and magnitude of trait variation in species belonging to different functional groups and representing different growth statures, we expected that trait dissimilarity is greater when the availability of belowground resources is increased and that of aboveground resources is reduced and that the effects of resource availability on trait dissimilarity are higher in mixtures with more species.

Material and Methods

Experimental design

The experiment was established at the Experimental Field Station of the Helmholtz Centre for Environmental Research (UFZ) in Bad Lauchstädt, Germany (51°23'38'' N, 11°52'45'' E, 118 m a.s.l.) in April 2011. Prior to the establishment of the experiment, the site was agricultural cropland. The mean regional annual temperature is 9.5 °C, and the mean annual precipitation is 492 mm (1981-2010; weather data from intensive monitoring experiment in Bad Lauchstädt, working group C/N dynamics, UFZ, http://www.ufz.de/index.php?de=940). The prevalent soil is a chernozem (Altermann et al. 2005). The soil texture (0-30 cm depth) is loamy sand; soil chemical properties are summarized in Table S1.

Four perennial forb species and four perennial grass species common in mown temperate grasslands (*Arrhenatherion* communities, Ellenberg 1988) were chosen for the experiment (Table 1). Species were randomly assigned to two experimental pools, each containing one small- and one tall-statured forb and one small- and one tall-statured grass species (Table 1). The experiment consisted of 96 plots of 2 × 2 m size. These encompassed monocultures of all species and all possible two-species combinations of both pools (each with four replicates), and the four-species mixtures from both pools (each with eight replicates). Plots were arranged in eight blocks, each comprising four monocultures, six two-species mixtures and two four-species mixtures with an equal number of plots per pool and equal occurrences of each individual species. Seeds were acquired from a commercial supplier (Rieger-Hoffman GmbH, Blaufelden-Raboldshausen, Germany). Initial sowing density in April 2011 was approximately 1000 viable seeds per m^2 (adjusted for germination rates determined in laboratory trials). In the mixtures, species were sown in equal proportions. Following the first mowing in September, all plots were re-sown in October 2011 with a total density of 500 viable seeds per m^2 to imitate a more diverse age structure within populations.

Table 1: Studied species with their taxonomy, plant height (Jäger 2011), and assignment to functional groups, growth statures and experimental species pools

Species	Family	Height (cm)	Functional group	Stature	Species pool
Anthoxanthum odoratum L.	Poaceae	20 - 50	grass	small	A
Lolium perenne L.	Poaceae	10 - 60	grass	small	B
Arrhenatherum elatius L. P.Beauv. ex J.Pres & C.Presl	Poaceae	60 - 120	grass	tall	A
Dactylis glomerata L.	Poaceae	50 - 150	grass	tall	B
Plantago lanceolata L.	Plantaginaceae	10 - 50	forb	small	A
Prunella vulgaris L.	Lamiaceae	5 - 30	forb	small	B
Centaurea jacea ssp. jacea L.	Asteraceae	15 - 80	forb	tall	A
Knautia arvensis (L.) Coulter	Dipsacaceae	30 - 80	forb	tall	B

After one year of growth, plots were assigned to the following treatments manipulating nutrient and light availability: (F-S-) no fertilization, no shading, (F-S+) no fertilization, shading, (F+S-) fertilization, no shading, and (F+S+) fertilization, shading.

From spring (16 April) until autumn (17 September) 2012, four blocks were shaded each by attaching one layer of green shading cloth (polyethylene, aperture size 2 × 10 mm, Hermann Meyer KG, Rellingen, Germany) to a 2.10 m high wooden scaffolding and fastening it to the ground on all sides. Blocks were arranged on the field-site ensuring that scaffoldings did not shade surrounding blocks. Based on continuous half-hourly measurements (SPK125, PAR Quantum Sensor; Skye Instruments Ltd, UK), photosynthetic active radiation (PAR) in the shaded blocks was reduced by 55% during the daytime in comparison to blocks without shading. In each block, an equal number of plots per species pool and species-richness level were chosen at random for the fertilization treatment. Fertilizer was applied as pellets (commercially available slow release NPK fertilizer 120:52:100 kg ha^{-1} yr^{-1}), resembling the commonly applied fertilizer amount in managed European semi-natural grasslands (Olff et al. 1990). Half of the dosage was distributed in spring (15 March 2012) and the other half after first mowing (18 June 2012).

Species, which were not part of the original plot species combinations, were regularly weeded. All plots were mown to 5 cm twice a year (early June and September) and mown plant material was removed, as is common practice for extensively managed hay meadows in the region.

Data collection

Leaf greenness and stomatal conductance were measured in spring (15 to 22 May 2012) and in summer (13 to 17 August 2012) between 10 a.m. and 3 p.m. Leaf greenness, a unit-less measure of foliar chlorophyll content, was assessed as the absorption of two different wavelengths (650 nm, 940 nm) with a portable chlorophyll meter (SPAD-502Plus Chlorophyll Meter, Konica Minolta, Inc., Japan) on five fully expanded leaves for each species per plot. Stomatal conductance was measured on three fully expanded leaves per species and plot with a leaf porometer (SC-1 Leaf Porometer, Decagon Devices Inc., Pullmann, USA) applying the auto mode of the device, which uses the first 30 s of stomatal conductance data to predict the final stomatal conductance occurring under true steady state conditions.

Single shoots of each species were sampled between 21 to 25 May 2012 and 20 to 24 August 2012 in one half of each plot, excluding the outer 40 cm of the plot margin. If only one life stage, either vegetative or reproductive, was predominant, five shoots per species were sampled along a transect taking the shoot rooting closest to the sampling point at every 25 cm. In species where both life stages were present, four vegetative and four reproductive shoots were chosen. Shoots were cut at their base close to the ground, and stored in plastic bags in a cooler. In the laboratory, maximum stretched shoot length of all samples was measured. Stem diameter and lengths of three central internodes of the main shoot axis were determined and the number of buds and inflorescences were counted on reproductive shoots. Inflorescences were defined as “flowering” when they displayed ripe anthers, produced seeds, or had already withered. Shoots were separated into leaves, stems (including leaf sheaths in case of grasses), and reproductive parts (inflorescences, fructescences). A leaf area meter (LI-3100 Area Meter, Li-COR, Lincoln, USA) was used to measure the area of five fully developed leaves (leaf blades in case of grasses), if available. Dry mass of all shoot compartments was determined after drying at 70°C for 48 h. For subsequent chemical analyses, the samples of measured leaves were pooled per species and plot (separately for each life stage) for each harvest and ground to fine powder with a ball mill (Mixer Mill MM200, Retsch, Haan, Germany).

In the remaining half of each plot, aboveground biomass in two randomly placed quadrats each of 20 × 50 cm size was harvested (29 May to 4 June, 27 to 31 August 2012). Samples were cut 3 cm above the soil surface, sorted to sown species, weeds and detached dead plant material. The number of shoots in vegetative and in reproductive stage was counted for each sown species. Aboveground biomass samples per species and plot from both harvests were shredded separately and subsamples were milled to fine powder. Nitrogen and carbon concentrations of leaf and shoot samples were measured with an elemental analyser (Vario EL Element Analyzer, Elementar, Hanau, Germany).

Data analyses

Traits and variables related to species performance derived from the described measurements are summarized in Table 2. Reproductive traits of grass species were only available in the spring harvest. For forb species, reproductive traits were either measured only in summer (*C. jacea*, *P. vulgaris*) or during both harvests (*K. arvensis*, *P. lanceolata*). For statistical analyses, reproductive traits for *P. lanceolata* and *K. arvensis* were averaged across seasons. Seasonal variation was considered in analyses of non-reproductive traits. To account for the possible effects of the experimental factors (plant diversity, resource availability) on the proportion of different life stages, values of traits recorded on vegetative and reproductive shoots were weighted by the proportion of shoots of each life stage derived from the determination of shoot density to get whole plot values.

Table 2: Summary and description of studied variables

Variable	**Unit**	**Description**	**Variable group**	**Abbreviation**
Specific leaf area	$m_{leaf}^{2} kg_{leaf}^{-1}$	leaf area per unit leaf dry mass	Light acquisition	SLA
Leaf greenness		unitless measure of leaf chlorophyll concentration	Light acquisition	LeafG
Stomatal conductance	mmol $m^{-2}s^{-1}$	stomatal conductance per leaf area	Light acquisition	g_s
Leaf mass fraction	$g_{leaf}\ g_{shoot}^{-1}$	leaf mass per total aboveground shoot mass	Light acquisition	LMF
Shoot carbon concentration	mg C g_{shoot}^{-1}	shoot carbon concentration	Light acquisition	SCC
Shoot length	cm	stretched shoot length	Light acquisition	Height
Internode length	cm	mean length of internodes	Light acquisition	Int_length
Stem diameter	cm	stem diameter	Light acquisition	Stem_dia
Leaf nitrogen concentration	mg N g_{leaf}^{-1}	leaf nitrogen concentration	Nitrogen nutrition	LNC
Shoot nitrogen concentration	mg N g_{shoot}^{-1}	shoot nitrogen concentration	Nitrogen nutrition	SNC
Shoot biomass	g	aboveground plant biomass	Performance	BM_{shoot}
Flower proportion		flowering inflorescences per total inflorescence number	Performance	$Phen_{shoot}$
Inflorescence mass fraction	$g_{infl}\ g_{shoot}^{-1}$	Inflorescence mass per total aboveground shoot mass	Performance	IMF

Data analysis was performed using the statistical software R 3.1.1 (R Core Team 2014). To assess whether the direction and magnitude of trait variation in response to resource availability and plant diversity depend on functional group or growth stature identity (question 1), we applied linear mixed-effects models with the *lmer* function in the R package *lme4* (Bates et al. 2014). Starting from a constant null model with block, plot nested in block, composition (= mixture identity), and species identity as random effects, fixed effects were added in the following order: fertilization (two factor levels), shade (two factor levels), species richness (SR, as logarithmic term), their possible two-way interactions (fertilizer x shade, fertilizer x SR, shade x SR), functional group identity (FG-ID, grass or forb), growth stature identity (GS-ID, tall or small), the respective two-way interactions of FG-ID and GS-ID with fertilizer, shade and species richness, and season (except for analyses of reproductive traits). The maximum likelihood method and likelihood ratio tests for model comparison were applied to evaluate the significance of the fixed effects. Logarithmic transformations were used to normalize the data, except for LNC, SCC, LMF and internode length, which remained untransformed. For visualization of treatment effects (see Figs. 1 and 2), trait data were standardized (z-transformed) and linear mixed-effects models with block and mixture identity as random effects were calculated separately for each species to estimate the variance attributable to the effects of fertilization, shade and species richness.

To test if functional trait variation of dominant species (in terms of biomass proportions in plant mixtures) depends on resource availability and if increased species richness affects traits differences between dominant and subordinate species (question 2), community means of trait values were computed as

$$CMT = \sum_{i=1}^{S} p_i t_i \qquad \text{(eqn. 1),}$$

where S is the number of species in the community, p_i is the relative abundance of the i-th species in a community and t_i is the trait value of the i-th species (Garnier et al. 2004). CMT were calculated as weighted community means (= CMT_w) using species biomass proportions and simple community means (= CMT_s) based on species presences-absences. Simple community means (CMT_s) attribute more importance to subordinate species by assigning the same weight to them as to dominant species, while weighted community means (CMT_w) emphasize traits of the dominant species. Thus, the ratio CMT_w/CMT_s represents how different the trait values of dominants and subordinates are in a mixture. Cases where CMT_w is smaller than CMT_s, i.e. $CMT_w/CMT_s < 1$ reflect larger trait values of subordinate species, whereas cases where CMT_w is greater than CMT_s reflect larger trait values of dominant species. If CMT_w/CMT_s is not significantly different from one, this may either indicate similar trait values across all species, or that realized biomass proportions are very close to the expected values based on sown species proportions (0.5 for two-species mixtures or 0.25 for four-species mixtures). The significance of $CMT_w/CMT_s \neq 1$ was tested with a two-sided t-test with a confidence level of 0.95 for each treatment combination. To exclude the possibility that realized biomass proportions were the major determinant of CMT_w/CMT_s close to 1 or in explaining treatment differences in CMT_w/CMT_s, the Simpson evenness index was computed using species-specific biomass data. Analyses of the ratio of realized over expected evenness showed that species abundance proportions in the mixtures were significantly different from sown proportions (= equal proportions) in all treatments, but realized evenness did not depend on resource availability (detailed analyses not shown). Thus, possible treatment effects on trait differences between dominants and subordinates were mainly due to differential effects of treatments on trait variation.

To test if resource availability influences trait dissimilarity of species in the mixtures (question 3), FD_Q (Rao's Q; Rao 1982) was calculated using the R package *FD* (Laliberté and Legendre 2010) separately for each trait related to light and nitrogen acquisition (see Table 2) for all two- and four- species mixtures,

$$FD_Q = \sum_{i=1}^{S}\sum_{j=1}^{S} p_i p_j d_{ij} \qquad \text{(eqn. 2)},$$

where S is the number of species in the mixture, p_i and p_j are the relative abundances of species i and j (presence-absence in our study), and d_{ij} is the trait distance between species i and j. Calculations were based on standardized trait data (mean = 0, variance = 1) and a Euclidean distance matrix.

Linear mixed-effects models were also applied to the indices characterizing community-level trait composition (FD_Q, CMT_w/CMT_s). Again starting from a constant null model with block, plot nested in block and composition (= mixture identity) as random effects, fixed effects were added stepwise in the following sequence: fertilizer, shade, species richness (SR; two or four species), functional group richness (FGR; one or two functional groups), growth stature richness (GSR; one or two growth statures), the respective two-way interactions of these terms, and season. FD_Q data (except for LMF and g_s) and CMT_w/CMT_s (only for leaf greenness, g_s, and shoot length) were transformed to natural logarithms to fulfil the assumptions of normal distribution.

Results

Trait variation in response to resource availability and species richness

Means and variation in traits related to light acquisition

Fertilization increased specific leaf area (SLA) and leaf greenness, but did not influence other traits related to light acquisition (Fig. 1a-h, Table 3). Shaded plants had higher SLA, stomatal conductance (g_s), and leaf mass fraction (LMF), grew taller, formed longer internodes, and had lower shoot carbon concentrations (SCC) in comparison to those grown in full light. Leaf greenness and stem diameter were not affected by shading (Fig. 1a-h, Table 3). Shade and fertilization did not interact in their impact on the expression of light-acquisition traits. Increased species richness had positive effects on SLA but negative effects on SCC, while its negative effect on leaf greenness was only marginally significant (Fig. 2a-h, Table 3). Species richness effects on light-acquisition traits were independent of resource availability (Table 3). Forb species were characterized by higher g_s, larger LMF and shorter internodes than grass species, while other light-acquisition traits did not differ between functional groups (Table 3, Fig. 2a-h). Species of different growth statures differed in most traits related to light-acquisition except for SLA and internode length (Table 3). Tall-statured species were characterized by larger leaf greenness and g_s, had thicker and longer shoot axes, and higher SCC, but their LMF were smaller than in small-statured species (Fig. 2a-h).

Table 3: Summary of linear mixed effects models for functional traits combining all species

	SLA		**LeafG**		**g_s**		**LMF**		**SCC**	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Fertilizer	**6.519**	**0.011**	**11.680**	**0.001**	1.638	0.201	0.002	0.969	2.216	0.137
Shade	**28.655**	**<0.001**	0.718	0.397	**5.210**	**0.022**	**11.014**	**0.001**	**11.574**	**0.001**
SR	**4.941**	**0.026**	3.289	0.070	0.020	0.887	2.126	0.145	**4.382**	**0.036**
Fertilizer x Shade	0.060	0.807	3.609	0.057	0.020	0.888	2.999	0.083	3.201	0.074
Fertilizer x SR	0.156	0.693	0.104	0.747	0.539	0.463	0.305	0.581	0.448	0.503
Shade x SR	0.919	0.338	0.930	0.335	0.728	0.394	1.147	0.284	1.203	0.273
FG-ID	0.241	0.623	2.505	0.114	**27.772**	**<0.001**	**5.431**	**0.020**	<0.001	0.986
GS-ID	0.052	0.820	**5.431**	**0.020**	**10.200**	**0.001**	**7.272**	**0.007**	**5.087**	**0.024**
FG-ID x GS-ID	0.588	0.443	0.025	0.875	0.076	0.783	0.143	0.705	1.434	0.231
FG-ID x Fertilizer	0.176	0.675	0.012	0.912	0.425	0.514	0.789	0.374	0.194	0.659
FG-ID x Shade	1.437	0.231	**13.723**	**<0.001**	0.016	0.901	0.299	0.584	3.432	0.064
FG-ID x SR	0.361	0.548	0.159	0.690	0.087	0.767	1.829	0.176	**4.042**	**0.044**
GS-ID x Fertilizer	<0.001	0.986	0.121	0.728	0.203	0.652	2.863	0.091	0.966	0.326
GS-ID x Shade	**3.913**	**0.048**	0.891	0.345	**5.791**	**0.016**	2.657	0.103	1.183	0.277
GS-ID x SR	3.327	0.068	0.322	0.570	0.099	0.753	0.499	0.480	0.746	0.388
Season	**125.992**	**<0.001**	**150.270**	**<0.001**	**91.061**	**<0.001**	**144.981**	**<0.001**	**66.988**	**<0.001**

	Height		**Int_length**		**Stem_dia**		**LNC**		**SNC**	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Fertilizer	3.481	0.062	0.383	0.536	1.111	0.292	**33.008**	**<0.001**	**32.760**	**<0.001**
Shade	**12.350**	**<0.001**	**6.952**	**0.008**	0.397	0.529	**8.369**	**0.004**	**21.330**	**<0.001**
SR	1.797	0.180	0.109	0.741	1.967	0.161	0.026	0.871	**6.058**	**0.014**
Fertilizer x Shade	2.917	0.088	0.888	0.346	2.491	0.115	0.022	0.882	1.853	0.173
Fertilizer x SR	0.239	0.625	0.971	0.324	<0.001	0.997	0.146	0.702	<0.001	0.998
Shade x SR	0.155	0.694	1.240	0.266	1.691	0.194	0.164	0.686	0.209	0.647
FG-ID	2.076	0.150	**5.104**	**0.024**	1.722	0.189	3.273	0.070	0.877	0.349
GS-ID	**14.867**	**<0.001**	2.163	0.141	**12.028**	**0.001**	0.198	0.657	**8.441**	**0.004**
FG-ID x GS-ID	1.104	0.293	0.231	0.630	0.330	0.566	0.701	0.403	3.521	0.061
FG-ID x Fertilizer	0.002	0.966	1.473	0.225	0.005	0.942	**4.143**	**0.042**	0.252	0.616
FG-ID x Shade	**5.602**	**0.018**	0.229	0.632	0.763	0.382	**6.445**	**0.011**	0.552	0.457
FG-ID x SR	1.518	0.218	**5.604**	**0.018**	0.546	0.460	1.281	0.258	0.701	0.402
GS-ID x Fertilizer	1.110	0.292	1.823	0.177	0.654	0.419	**7.132**	**0.008**	**5.209**	**0.022**
GS-ID x Shade	1.370	0.242	3.722	0.054	0.595	0.441	2.949	0.086	0.275	0.600
GS-ID x SR	1.091	0.296	0.128	0.720	2.455	0.117	0.748	0.387	0.024	0.876
Season	**329.481**	**<0.001**	-	-	-	-	**56.696**	**<0.001**	**8.836**	**0.003**

	BM_{Shoot}		**$Phen_{Shoot}$**		**IMF**	
	χ^2	P	χ^2	P	χ^2	P
Fertilizer	1.437	0.231	0.230	0.631	2.101	0.147
Shade	3.546	0.060	**8.766**	**0.003**	**15.513**	**<0.001**
SR	**9.001**	**0.003**	0.156	0.692	0.487	0.485
Fertilizer x Shade	2.022	0.155	0.020	0.888	0.111	0.739
Fertilizer x SR	0.532	0.466	1.493	0.222	0.610	0.435
Shade x SR	<0.001	1.000	0.012	0.912	0.111	0.739
FG-ID	2.031	0.154	**9.050**	**0.003**	0.001	0.976
GS-ID	**13.384**	**<0.001**	3.010	0.083	0.316	0.574
FG-ID x GS-ID	1.304	0.254	**4.329**	**0.037**	0.609	0.435
FG-ID x Fertilizer	0.556	0.456	**3.939**	**0.047**	1.647	0.199
FG-ID x Shade	2.432	0.119	**7.599**	**0.006**	0.006	0.940
FG-ID x SR	3.171	0.075	0.004	0.951	0.973	0.324
GS-ID x Fertilizer	1.902	0.168	1.632	0.201	3.342	0.068
GS-ID x Shade	1.378	0.240	1.052	0.305	2.500	0.114
GS-ID x SR	0.278	0.598	3.443	0.064	0.264	0.608
Season	**165.222**	**<0.001**	-	-	-	-

Models were fitted by stepwise inclusion of fixed effects. Likelihood ratio tests (χ^2) were used to assess model improvement and the statistical significance of the explanatory terms (P values). Significant terms are highlighted in bold. Abbreviations are: FG-ID= functional group identity (grass or forb), GS-ID = growth stature identity (tall or small), SR= species richness. For abbreviations and description of traits see Table 2.

Trait differences due to different growth statures did not depend on functional group identity (non-significant interaction FG × GS; Table 3). The effects of fertilization and shade on the expression of light-acquisition traits did not differ between functional groups with the exception of shading effects on leaf greenness and shoot length. Shade led to an increase in leaf greenness in grasses, while this was not the case in forbs (Fig. 1b). Shoot length of grasses increased more strongly under shading than in forbs (Fig. 1f). The extent and direction of variation in light-acquisition traits in response to fertilization was also similar between plants of different growth statures. However, the strength of shade effects varied with growth stature, as increases in SLA and g_s in response to shade were larger in small-statured than in tall-statured species (Fig. 1a, c). Effects of species richness on internode length and SCC (Table 3) varied between functional groups. Grass species formed shoots with longer internodes and lower carbon concentrations, while forb species had shorter internodes and did not change SCC when growing in plant communities with higher species richness (Fig. 2e, g).

Means and variation in traits related to nitrogen nutrition

Fertilization as well as shading increased nitrogen concentrations in leaves (LNC) and in shoots (SNC) (Fig. 1i-k, Table 3). Increased species richness had a positive effect on SNC (Fig. 2k), while LNC was not affected (Fig. 2i). Tissue nitrogen concentrations did not differ between functional groups (Fig. 2i-k), but positive fertilizer and shade effects on LNC were more pronounced in grass species than in forb species (Fig. 1i). Tall-statured and small-statured species did not differ in LNC, but small-statured species had larger SNC than tall-statured species (Fig. 2i-k). In small-statured species, fertilization led to a greater increase in LNC and in SNC than in tall-statured species (Fig. 1i-k).

Means and variation in shoot mass and traits related to reproduction

Fertilization and shade did not affect shoot mass (BM_{shoot}) (Table 3, Fig. 1l), while BM_{shoot} decreased with increasing species richness (Fig. 2l). Functional groups did not differ in BM_{shoot}, but small-statured species had a smaller BM_{shoot} than tall-statured species (Table 3, Fig. 2l). On average, fertilization did not affect flowering phenology and inflorescence mass fraction (IMF) (Table 3). In shade, flowering phenology was delayed and IMF was lower than in full light (Table 3, Fig. 1m-n). Grass species had a more advanced flowering phenology than forb species during the pre-mowing sampling, but functional groups did not differ in IMF. The negative effect of shade on the onset of flowering was more pronounced in forbs than in grasses. Fertilization led to a more advanced flowering phenology in grasses, while it induced a delay in forbs (Fig. 1m).

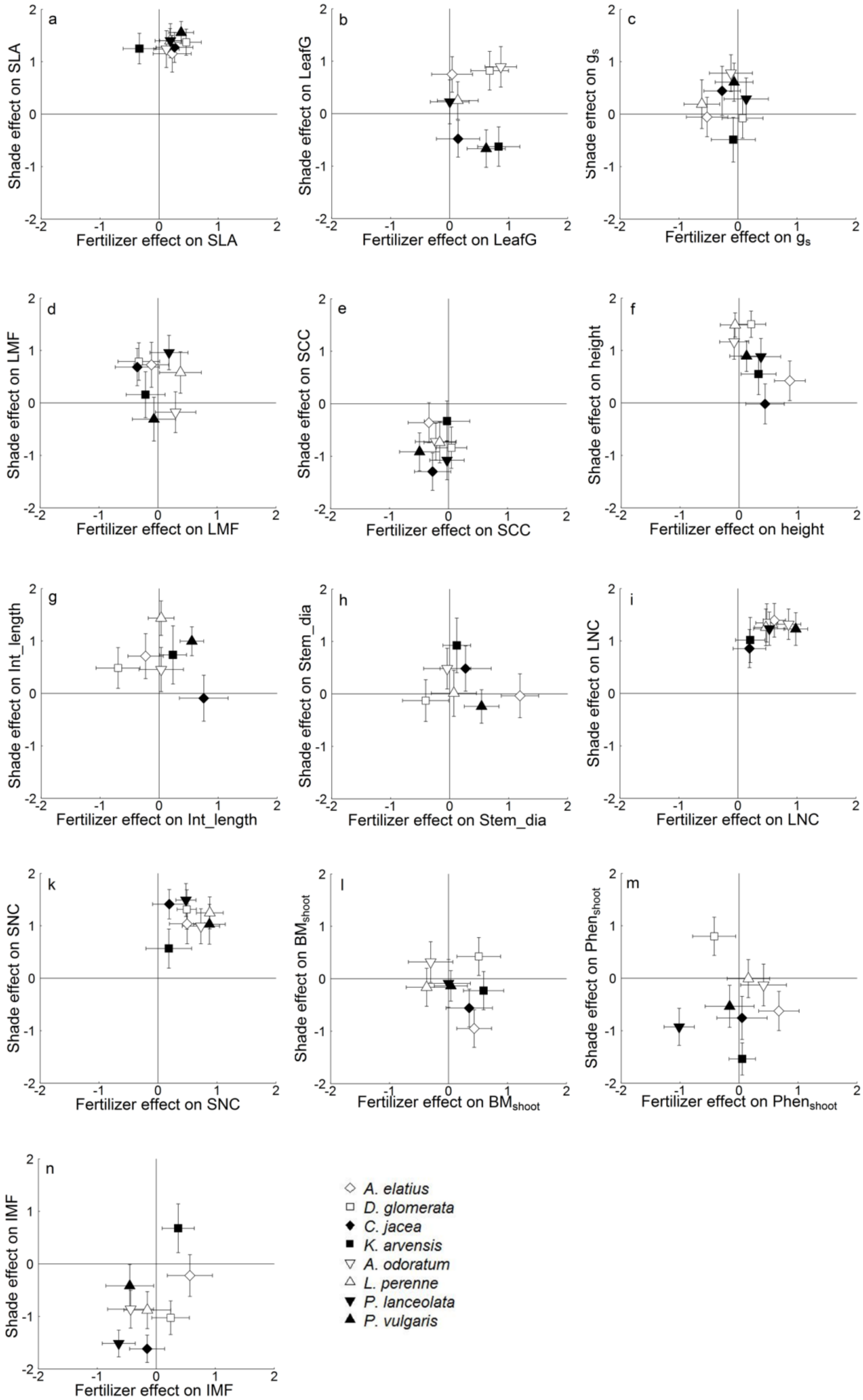

Figure 1: Trait variation in response to shade plotted against trait variation in response to fertilization for (a) specific leaf area, (b) leaf greenness, (c) stomatal conductance, (d) leaf mass fraction, (e) shoot carbon concentration, (f) shoot length, (g) internode length, (h) stem diameter, (i) leaf nitrogen concentration, (k) shoot nitrogen concentration (l) shoot mass, (m) flowering phenology, and (n) inflorescence mass fraction. Filled symbols represent forbs, open symbols grass species; square symbols show tall-statured and triangular symbols small-statured species. Trait variation is expressed as estimate of variance (± 1SE) in response to fertilization and shading, respectively, from linear-mixed effects models of standardized variables and averaged

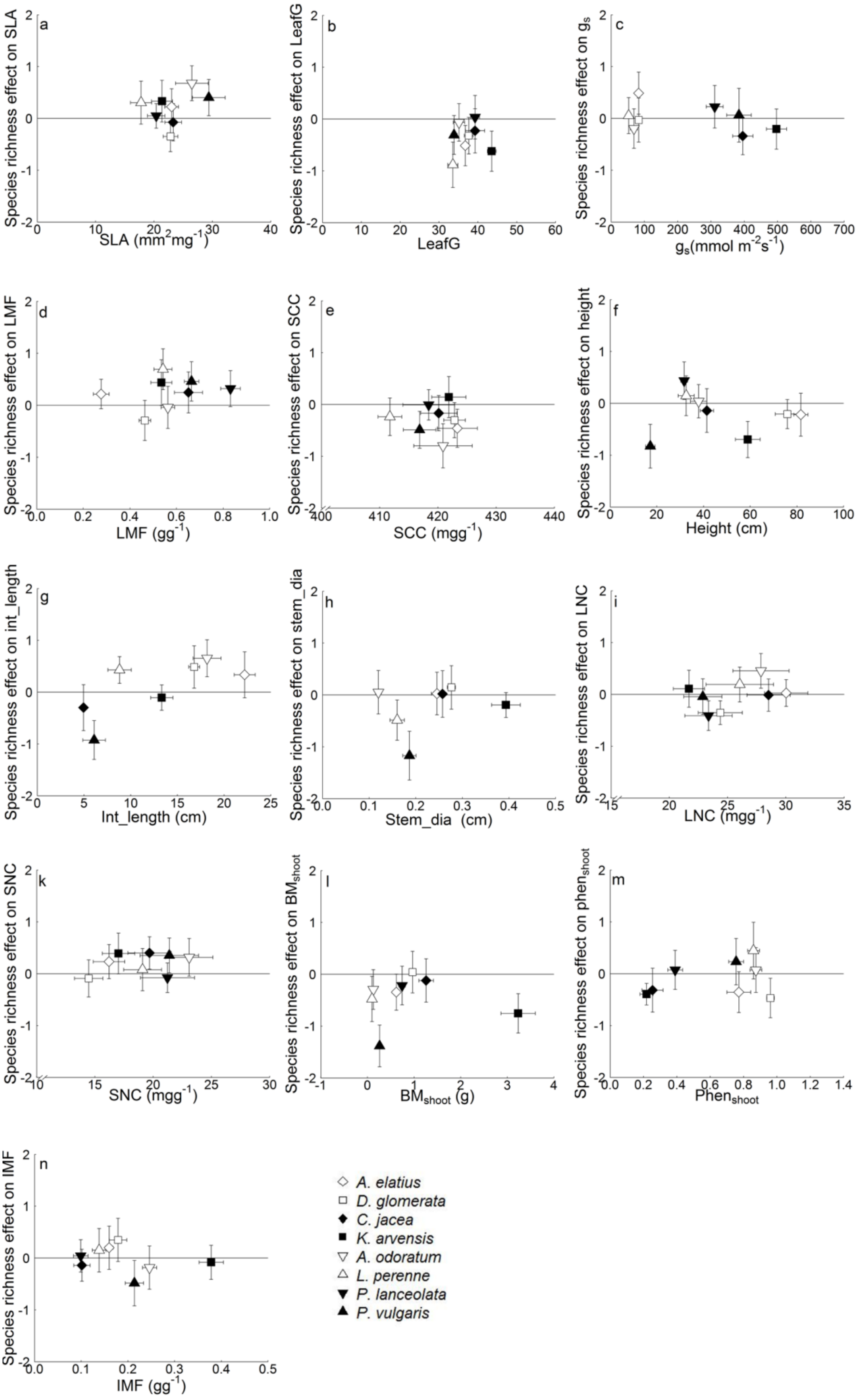

Figure 2: Trait variation in response to increased species richness plotted against trait means (± 1SE) across treatments for (a) specific leaf area, (b) leaf greenness, (c) stomatal conductance, (d) leaf mass fraction, (e) shoot carbon concentration, (f) shoot length, (g) internode length, (h) stem diameter, (i) leaf nitrogen concentration, (k) shoot nitrogen concentration (l) shoot mass, (m) flowering phenology, and (n) inflorescence mass fraction. Filled symbols represent forbs, open symbols grass species; square symbols show tall-statured and triangular symbols small-statured species. Trait variation is expressed as estimate of variance (± 1SE) in response to increased species richness from linear-mixed effects models of standardized variables and averaged across seasons. Cases above zero on the y-axis indicate increased trait values in response to increased species richness. For abbreviations and description of variables see Table 2.

Effects of resource availability and plant diversity on trait differences between dominant and subordinate species

Averaged across all treatments, dominant species had lower SLA, LMF, and SNC than subordinate species ($CMT_w/CMW_s < 1$; $p < 0.05$), while leaf greenness, SCC, and shoot length of dominants were larger than in subordinates ($CMT_w/CMW_s > 1$; $p < 0.05$). Stomatal conductance and LNC did not differ between dominants and subordinates ($p > 0.05$). Fertilization increased differences between dominants and subordinates in LMF and in SNC (Fig. 3d, h), while shading promoted differences between dominants and subordinates in SLA (Fig. 3a). Plant diversity in terms of species richness and functional group richness did not affect trait differences between dominants and subordinates. However, effects of fertilization on differences between dominants and subordinates in a mixture depended on growth stature richness (Table S2). Subordinate species had larger LMF than dominant species in fertilized mixtures with species of the same growth stature, while subordinate species in fertilized mixtures of tall- and small-statured species had lower LMF than dominant species. In fertilized mixtures containing species of both growth statures, the differences in shoot height and SNC between subordinates and dominants was larger than in mixtures of species of the same growth stature.

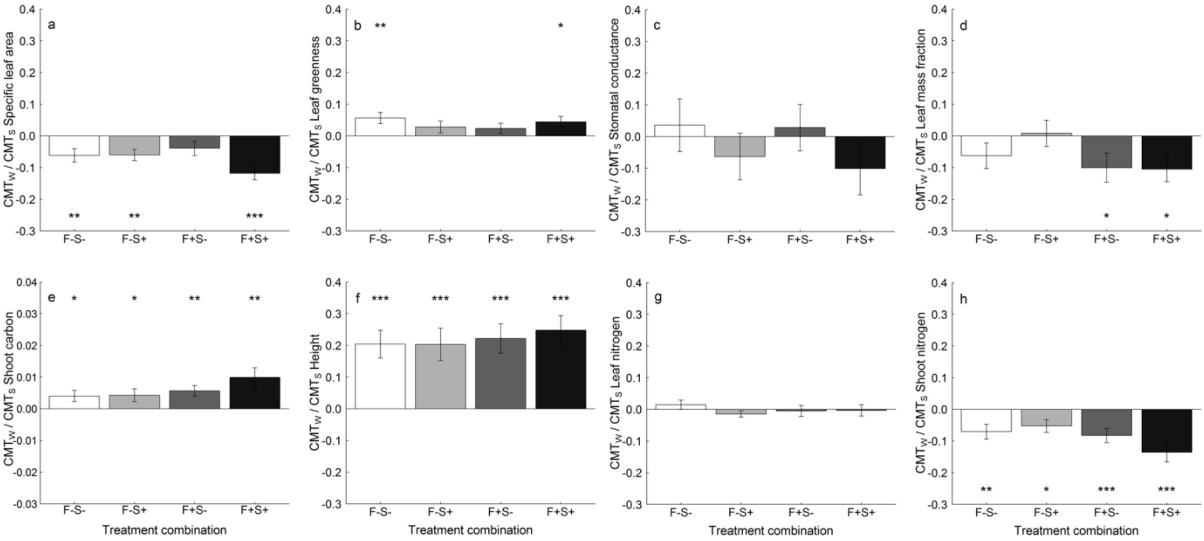

Figure 3: CMT_w/CMT_s ratios (weighted community means (= CMT_w) divided by simple community means (= CMT_s)) for (a) specific leaf area, (b) leaf greenness, (c) stomatal conductance, (d) leaf mass fraction, (e) shoot carbon concentration, (f) shoot length, (g) leaf nitrogen concentration, and (h) shoot nitrogen concentration in two-species and four-species mixtures grown at different combinations of light and nutrient availability. Values are averaged across seasons (spring, summer) and shown as (CMT_w/CMT_s - 1) per treatment (means ± 1SE). Significant deviations from zero are indicated as * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. Abbreviations of treatments are: F-S- = no fertilization, no shading, F-S+ = no fertilization, shading, F+S- = fertilization, no shading, and F+S+ = fertilization, shading.

Trait dissimilarity as affected by resource availability and plant diversity

Fertilization increased dissimilarity (FD_Q) in LNC and SNC, but did not affect dissimilarity in other traits (Table 4). On average, shading had no effect on trait dissimilarity. The four-species mixtures had a higher dissimilarity in LMF, SNC and SCC than two-species mixtures, but mixture species richness did not influence dissimilarity in other traits (Table 4).

Table 4: Summary of linear mixed-effects models for community-level trait dissimilarity (FD_Q) of two-species and four-species mixtures

	SLA		**LeafG**		**g_s**		**LMF**		**SCC**		**Height**		**LNC**		**SNC**	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Fertilizer	2.400	0.121	0.389	0.533	0.001	0.979	0.251	0.617	0.858	0.354	1.187	0.276	**12.754**	**<0.001**	**5.717**	**0.017**
Shade	0.050	0.824	2.527	0.112	0.592	0.442	0.239	0.625	1.070	0.301	1.118	0.290	0.951	0.329	0.068	0.794
SR	1.983	0.159	2.917	0.088	2.049	0.152	**6.440**	**0.011**	**5.321**	**0.021**	1.015	0.314	3.175	0.075	**3.953**	**0.047**
FGR	0.001	0.970	0.005	0.942	**21.973**	**<0.001**	0.432	0.511	0.008	0.931	0.046	0.830	0.186	0.667	0.116	0.734
GSR	1.428	0.232	0.211	0.646	0.052	0.820	1.865	0.172	4.257	0.039	0.414	0.520	0.527	0.468	**8.852**	**0.003**
Fertilizer x Shade	0.002	0.969	0.697	0.404	0.298	0.585	0.080	0.777	0.643	0.423	0.012	0.914	1.710	0.191	0.610	0.435
Fertilizer x SR	0.207	0.649	3.635	0.057	1.786	0.181	0.598	0.439	0.139	0.709	0.265	0.607	0.231	0.630	0.003	0.953
Shade x SR	0.082	0.775	0.021	0.885	0.620	0.431	1.942	0.163	0.010	0.919	0.585	0.444	1.180	0.277	0.666	0.414
Fertilizer x FGR	0.515	0.473	0.099	0.753	0.598	0.439	0.683	0.409	0.205	0.651	1.106	0.293	4.456	0.035	0.244	0.621
Shade x FGR	3.105	0.078	2.933	0.087	0.699	0.403	0.177	0.674	2.481	0.115	1.346	0.246	**7.311**	**0.007**	0.666	0.414
Fertilizer x GSR	2.590	0.108	0.474	0.491	3.586	0.058	0.236	0.627	0.080	0.777	0.408	0.523	0.527	0.468	1.916	0.166
Shade x GSR	2.333	0.127	2.606	0.106	1.193	0.275	1.666	0.197	2.246	0.134	0.124	0.724	0.294	0.587	0.867	0.352
Season	0.678	0.410	3.224	0.073	3.633	0.057	2.348	0.125	0.739	0.390	**6.139**	**0.013**	1.389	0.239	**32.883**	**<0.001**

Models were fitted by stepwise inclusion of fixed effects. Likelihood ratio tests (χ^2) were used to assess model improvement and the statistical significance of the explanatory terms (P values). Significant terms are highlighted in bold. Abbreviations are: SR = species richness, FGR = functional group richness, GSR = growth stature richness. For abbreviations and description of traits see Table 2.

Functional group or growth stature richness mostly did not affect trait dissimilarity with exception of increased dissimilarity in g_s at higher functional group richness, and increased dissimilarity in SNC and SCC at higher growth stature richness. Effects of fertilization on dissimilarity in LNC were larger in mixtures with one functional group than in grass-forb mixtures. Effects of shade on dissimilarity in LNC also depended on functional group richness. In shade, dissimilarity in LNC was larger in grass-forb mixtures than in mixtures of one functional group, while in full light, mixtures representing a single functional group had a greater dissimilarity in LNC.

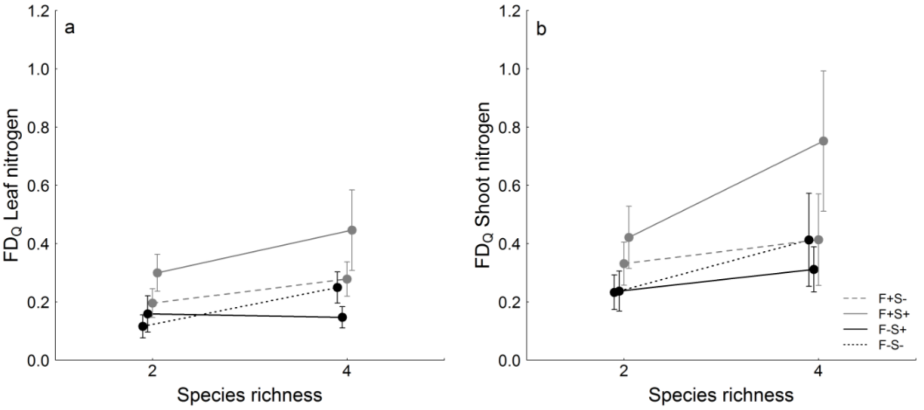

Figure 4: Community-level trait dissimilarity (FD_Q) in (a) leaf nitrogen concentration and (b) shoot nitrogen concentration in two-species and four-species mixtures grown at different combinations of light and nutrient availability. Values are averaged across seasons (spring, summer) and shown as means (±1 SE) per treatment and species-richness level. Abbreviations for treatments are: F-S- = no fertilization, no shading, F-S+ = no fertilization, shading, F+S- = fertilization, no shading, and F+S+ = fertilization, shading.

Discussion

Do the direction and magnitude of trait variation in response to resource availability and plant diversity differ between functional groups and growth statures?

The concept of functional groups assumes that species assigned to a particular functional group are relatively similar in their trait values or respond similarly to environmental variation. Trait differences between the well-established functional groups forbs and grasses have been shown for traits associated with resource uptake and use, such as root architecture and tissue nitrogen concentrations (Grime et al. 1997; Craine et al. 2001; Reich et al. 2003). In our study focussing on aboveground shoot traits, grass and forb species only differed in a few traits, while we found that species of varying growth statures (tall vs. small) differed in most studied traits. These results are in contrast to our previous common garden experiment with separately grown plant individuals, where we detected only few differences in the trait values of small- and tall-statured species (Siebenkäs et al. 2015) in the same traits as studied in the field experiment. These deviating results imply that interactions with other plants in the mixtures as well as in the monocultures may have large effects on the expression of functional traits.

As expected (question 1), grasses and forbs as well as species groups representing different growth statures (small vs. tall) did not differ in the direction of trait variation in response to varying light or nutrient availability with the exception of fertilizer effects on flowering phenology. We also hypothesized that the magnitude of trait variation in response to increased availability of nutrients is greater in grasses than in forbs because their root characteristics (e.g. larger specific root length) promote nutrient acquisition (Reich et al. 1998; Siebenkäs et al. 2015). Additionally, it has been shown that grasses are more responsive to nutrient addition in their biomass production compared to forbs (Bowman et al. 1993; Reich et al. 2001). Indeed, the increase in LNC in response to fertilization was stronger in grasses than in forbs, but we did not find differences between grasses and forbs in the response to fertilization in other traits. Grasses, however, showed a greater plasticity in leaf greenness, LNC and shoot height in response to shading than forbs, which is in line with our previous results from separately grown plant individuals (Siebenkäs et al. 2015), and suggests an inherently greater capability of grass species to respond plastically to low light conditions.

We also expected (question 1) that small-statured species show a greater trait variation under shading than tall-statured species. Given the unfavourable positioning of small-statured species in competition for light deep in the canopy, a greater ability to increase light-harvesting in low-light environments via the formation of leaves with larger SLA, increased chlorophyll concentrations and less nitrogen per leaf area (e.g. Werger et al. 2002; Niinemets 2007) or the use of temporal niches with low canopy densities (Roscher et al. 2011a) may explain their coexistence with taller species. Indeed, in our experiment, the increase in SLA and g_s in the shade was greater in small- than in tall-statured species. On the contrary, a greater increase of tissue nitrogen concentrations (LNC, SNC) in small- than in tall-statured species under fertilization indicated that small-statured species were carbon-limited and could not use the additional nutrients for growth (Bloom et al. 1985).

We also predicted (question 1) that increasing species richness has additional effects on trait variation if the environment experienced by individual plants changes with increasing plant diversity. For example, more diverse plant communities have been shown to form a denser and taller canopy with a greater leaf area index (Spehn et al. 2000; Marquard et al. 2009). Former studies on trait variation of grasses (Gubsch et al. 2011; Roscher et al. 2011b) and forbs (Daßler et al. 2008; Lipowsky et al. 2015) along experimental plant diversity gradients in the Jena Experiment (Roscher et al. 2004) included legumes and trait variation was to some extent attributable to the presence of legumes. Increased SLA and the formation of taller shoots in plant communities of higher species richness have been related to greater efforts for light acquisition in more diverse plant communities (Roscher et al. 2011b; Lipowsky et al. 2015). Our study, excluding legumes, showed that species formed leaves with larger SLA (Fig. 2a), but did not increase height growth (Fig. 2f) in plant communities with more species. Obviously, the adjustment in leaf morphology was not sufficient to cope with changes in growth conditions at higher species richness. Instead, the increasing SNC (Fig. 2k), decreasing SCC (Fig. 2e) and the lower shoot biomass (Fig. 2l) suggested that growth was carbon-limited at increasing species richness.

Do the traits of dominant species vary dependent on resource availability and plant diversity?

When assessing community characteristics, the focus is often directed towards dominant species due to their larger contribution to biomass. The greater competitive ability of dominant species is often assumed to be related to traits such as a taller growth, higher values for SLA and LNC and lower values for root nitrogen concentrations (Grime 1998; Mariotte 2014), which are typical characteristics of fast-growing species with a rapid acquisition of resources. A tall growth was also positively related to dominance in terms of aboveground biomass production in our experiment (Fig. 3f). In line with our expectations that the traits of dominant species are similar irrespective of resource availability (question 2) dominant species also had greater values for leaf greenness and shoot carbon concentrations in all treatments (Fig. 3h). Conversely, subordinate species tolerated shading in the lower canopy strata by forming leaves with greater SLA (Fig. 3a) and allocating more biomass to foliage instead of stems (Fig. 3d) compared to dominant species. Campbell et al. (1991) suggested that subordinates and dominants differ in their trade-offs between scale and precision in strategies of resource acquisition, whereby dominants monopolize the capture of above- and belowground resources by the development of extensive leaf canopies and root systems (= "high scale"). On the contrary, subordinate species are more dependent on the precise location of their leaves and roots in locally undepleted resource patches (= "high precision"). We expected that trait differences between dominants and subordinates should be more accentuated under conditions increasing competition for aboveground resources. Indeed, differences between dominants and subordinates in SLA increased under shaded conditions suggesting a stronger responsiveness of subordinates to low-light conditions. Furthermore, we found that fertilization led to greater differences in LMF and shoot nitrogen concentrations between dominants and subordinates. While the greater allocation to leaves suggests that subordinates increased their investment in light capture, the accumulation of nitrogen in shoots implies that their growth was carbon-limited due to competitive disadvantages in light acquisition. Our results suggest that the tall-growing species of our pools were more likely to become dominant, but our experiment also included mixtures with only small- or only tall-statured species. Under fertilization, dominants became more different in height and SNC from subordinates when small- and tall-statured species grew in combination than in mixtures of species of the same growth stature (Table S2). Apart from this CMT_W/CMT_S ratios were largely independent of growth stature composition. Thus, our findings are also valid in mixtures with less pronounced differences in height growth of the involved species.

Do differential effects of resource availability on trait variation of different species affect community-level trait dissimilarity?

There is still considerable uncertainty regarding the patterns of community-level trait dissimilarity dependent on resource availability, which may be modulated by varying species abundance proportions or trait variation. As we were particularly interested in the effects of trait variation, we assessed trait dissimilarity without considering species abundances. Our results are in accordance with previous findings showing that the effects of varying resource supply on trait dissimilarity depend on the considered traits (Spasojevic and Suding 2012; Price et al. 2014). Contrary to our expectations (question 3), shading did not generally affect trait dissimilarity and increased species richness had minor effects on trait dissimilarity. In line with our expectations, fertilization increased community-level dissimilarity in LNC and SNC, while we did not find fertilizer effects on dissimilarity in plant height or SLA as observed by Price et al. (2014) in a mesocosm experiment with grassland species.

Larger trait dissimilarity has been proposed to increase the potential for niche differentiation (Levine and HilleRisLambers 2009). However, it has also been suggested that a higher trait similarity among species, particularly in productive environments, promotes species coexistence through equalizing processes (Grime 2006). Our analyses of trait plasticity in response to resource availability (question 1) and trait differences between dominants and subordinates (question 2) have shown that small and subordinate species show a greater divergence in tissue nitrogen concentrations from tall and dominant species under fertilization, which is likely due to carbon-limitation at nutrient excess. Therefore, the observed greater dissimilarity in traits related to nitrogen acquisition under fertilization is probably not related to niche differentiation promoting species coexistence, but more likely an indicator of competitive inequalities. Our study was restricted to short-term effects of external resource supply in the first year of treatment applications and species abundance proportions did not vary strongly between treatments. The varying magnitude of trait variation dependent on functional group or growth stature identity suggests that species differed in their ability to adjust to varying resource availability, which is likely to affect the outcome of species interactions in the longer-term.

Acknowledgements

This work was supported by the German Research Foundation (RO2397/3, RO2397/4). We thank B. Sawall and several student helpers, especially M. Starck, R. Schubert and B. Schmidt, for their assistance during harvest and sample processing, A. Thondorf for chemical analyses, and the technicians of the field station Bad Lauchstädt for help with establishment and maintenance of the biodiversity experiment.

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Supplementary material

Table S1: Soil chemical properties of the experimental site

Soil properties	Depth (cm)	Range	Mean ± SD
Nitrogen concentration (mg g^{-1})	0-15	2.03 - 2.35	2.18 (± 0.11)
	15-30	2.01 - 2.14	2.08 (± 0.05)
Organic carbon concentration (mg g^{-1})	0-15	23.1 - 25.8	24.5 (± 0.8)
	15-30	22.6 - 23.8	23.3 (± 0.4)
CN ratio	0-15	10.6 - 11.7	11.0 (± 0.3)
	15-30	10.6 - 11.1	10.9 (± 0.2)
Carbonate concentration (%)	0-15	0.40 - 0.60	0.50 (± 0.06)
	15-30	0.47 - 0.70	0.60 (± 0.09)
pH	0-15	6.68 - 7.22	6.93 (± 0.15)
	15-30	6.85 - 7.46	7.12 (± 0.20)
Phosphorus concentration (mg kg^{-1})	0-15	36.7 - 42.3	39.3 (± 2.5)
	15-30	24.3 - 45.0	36.4 (± 7.2)
Potassium concentration (mg kg^{-1})	0-15	102.0 - 247.0	151.7 (± 50.8)
	15-30	71.5 -168.0	107.1 (± 37.8)

Soil samples (0-30 cm depth, segmented to depth increments of 0-15 cm and 15-30 cm depth) were taken at three locations in each block and pooled block-wise before establishment of the experiment. Phosphorus concentrations were obtained from extracts with double lactate, potassium concentrations were determined from calcium acetate lactate extracts. Total carbon and nitrogen concentrations were measured with an elemental analyser (Vario EL Element Analyzer, Elementar, Hanau, Germany). Carbonate concentrations were determined volumetrically according to Scheibler, and pH values were measured after suspending the soil with 0.01M $CaCl_2$. Soil organic carbon concentrations were obtained by subtracting inorganic carbon concentrations from total carbon concentrations. Shown are the range and means (±1SD) across blocks (N = 8).

Table S2: Summary of linear mixed-effects models for CMT_w/CMT_s ratios (ratio of community-weighted trait means over simple community trait means) of functional traits for the two- and four-species mixtures

	SLA		LeafG		g_s		LMF		SCC		Height		LNC		SNC	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Fertilizer	1.159	0.282	0.231	0.631	0.129	0.719	**4.417**	**0.036**	3.291	0.070	1.156	0.282	0.318	0.573	**5.537**	**0.019**
Shade	**4.651**	**0.031**	0.057	0.811	2.196	0.138	0.521	0.470	0.992	0.319	0.105	0.746	0.772	0.380	0.547	0.460
SR	0.182	0.670	0.090	0.764	0.059	0.808	0.493	0.483	0.607	0.436	1.145	0.285	0.000	0.985	1.579	0.209
FGR	0.150	0.699	0.616	0.433	1.250	0.264	2.669	0.102	0.171	0.680	1.693	0.193	2.424	0.119	3.263	0.071
GSR	0.445	0.505	0.076	0.783	0.117	0.732	1.191	0.275	0.017	0.895	0.163	0.687	0.485	0.486	0.569	0.451
Fertilizer x Shade	0.729	0.393	1.891	0.169	0.605	0.437	1.185	0.276	1.106	0.293	0.177	0.674	0.508	0.476	3.444	0.063
Fertilizer x SR	0.000	0.999	1.923	0.166	0.091	0.763	0.073	0.787	0.115	0.734	0.383	0.536	0.633	0.426	0.158	0.691
Shade x SR	2.895	0.089	0.486	0.486	0.840	0.359	0.560	0.454	0.431	0.511	0.300	0.584	0.092	0.761	0.116	0.733
Fertilizer x FGR	0.007	0.934	2.223	0.136	0.087	0.769	0.145	0.703	0.954	0.329	0.023	0.878	0.954	0.329	0.449	0.503
Shade x FGR	0.341	0.559	0.392	0.531	0.005	0.943	0.198	0.656	1.472	0.225	0.865	0.352	2.123	0.145	0.065	0.799
Fertilizer x GSR	0.004	0.947	0.168	0.681	2.573	0.109	**9.635**	**0.002**	0.523	0.470	**5.434**	**0.020**	0.968	0.325	**5.431**	**0.020**
Shade x GSR	0.107	0.743	0.148	0.700	1.123	0.289	0.024	0.878	0.730	0.393	0.087	0.768	2.507	0.113	2.037	0.154
Season	**11.877**	**0.001**	0.015	0.903	2.608	0.106	0.372	0.542	3.010	0.083	**9.522**	**0.002**	0.222	0.637	**5.531**	**0.019**

Models were fitted by stepwise inclusion of fixed effects. Likelihood ratio tests (χ^2) were used to assess model improvement and the statistical significance of the explanatory terms (P-values). Abbreviations are: SR = species richness, FGR = functional group richness, GSR = growth stature richness. For abbreviations and description of traits see Table 2.

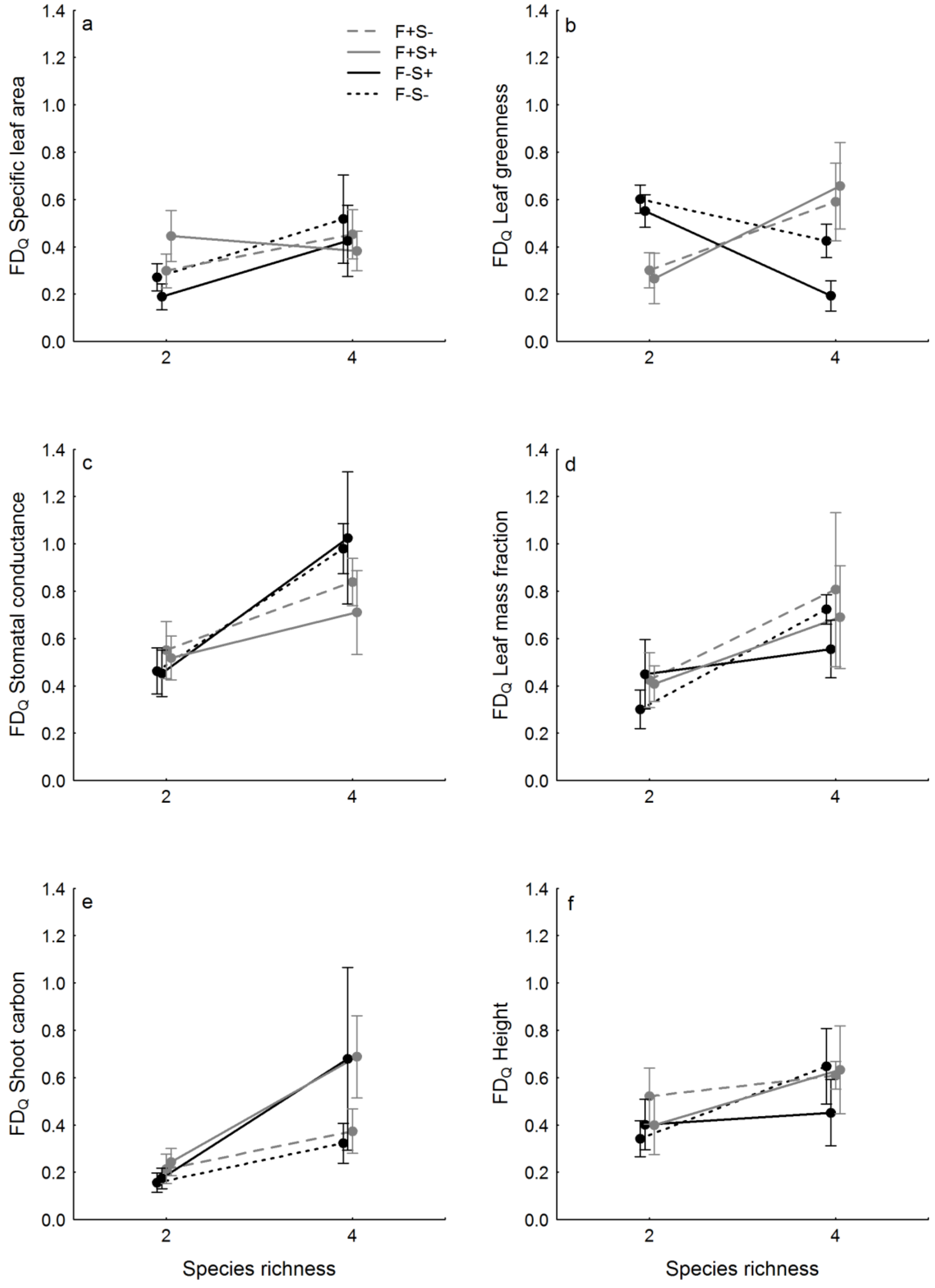

Figure S1: Community-level dissimilarity (FDQ) in (a) specific leaf area, (b) leaf greenness, (c) stomatal conductance, (d) leaf mass fraction, (e) shoot carbon concentration and (f) shoot length in two-species and four-species mixtures grown at different combinations of light and nutrient availability. Values are averaged across seasons (spring, summer) and shown as means (±1SE) per treatment and species-richness level. Abbreviations of treatments are: F-S- = no fertilization, no shading, F-S+ = no fertilization, shading, F+S- = fertilization, no shading, and F+S+ = fertilization, shading.

CHAPTER 4

FUNCTIONAL COMPOSITION RATHER THAN SPECIES RICHNESS DETERMINES ROOT CHARACTERISTICS OF EXPERIMENTAL GRASSLANDS GROWN AT DIFFERENT LIGHT AND NUTRIENT AVAILABILITY

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Plant and Soil (invited for major revision)

Abstract

Background and aims: Results from many biodiversity experiments have established evidence for positive effects of diversity on aboveground plant productivity. However, less is known about the relationships between plant diversity and belowground plant community characteristics and their consistency at altered environmental conditions.

Methods: Monocultures, two- and four-species mixtures of eight perennial temperate grassland species representing two functional groups (grasses or forbs) and differing in growth stature (tall or small) were grown in a field experiment at crossed levels of light and fertilization. Belowground standing biomass and root morphological traits were studied in the second year of treatment applications.

Results: Neither increased species richness nor fertilization affected belowground characteristics. However, shading decreased root standing biomass and affected root morphological characteristics. The vertical distribution of standing root biomass and root length density (RLD) over the depth profile and root morphological traits differed in communities of varying functional composition irrespective of resource availability, but differences were partly increased when shading was combined with fertilization.

Conclusions: Independent of resource availability, plant species richness does not increase vertical root segregation, but the potential for complementary use of belowground resources increases when species with different rooting patterns and root morphological traits are combined in mixtures.

Key words: functional groups, growth statures, shade, nutrients, root morphological traits, standing root biomass

Introduction

Experimental biodiversity research has repeatedly provided evidence that plant diversity positively affects a multitude of ecosystem processes (Hooper et al. 2005; Cardinale et al. 2012; Tilman et al. 2014). In particular, several grassland biodiversity experiments have demonstrated positive effects of plant diversity on aboveground productivity, which have mostly been attributed to species complementarity in resource use (Spehn et al. 2005; Roscher et al. 2005; Cardinale et al. 2007). Although it is well known that up to 80% of total plant biomass in temperate grasslands is attributed to belowground biomass (Jackson et al. 1996), the relationship between diversity and belowground biomass has been considered in fewer studies and has shown more variable results. For example, in short-term grassland biodiversity experiments, no effects of increased species richness on belowground biomass have been found at single sites of the BIODEPTH project (e.g. Gastine et al. 2003; Spehn et al. 2005), but positive effects have been demonstrated in other short-term biodiversity experiments (Spehn et al. 2005; von Felten and Schmid 2008; Mommer et al. 2010; Bessler et al. 2012). More recently, the importance of root characteristics, apart from root standing biomass, for ecosystem processes such as nutrient and carbon cycling has increasingly been emphasized in the context of diversity-ecosystem functioning research (Bardgett et al. 2014; Cong et al. 2014; Fornara et al. 2009). The primary function of roots is the uptake of resources essential for plant growth such as nutrients and water. In particular, specific root length (SRL, root length per unit of root biomass (cm g^{-1} dry mass); Ostonen et al. 2007) and root length density (RLD, cm cm^{-3} soil; Atkinson 2000) are important root characteristics related to exchange processes between root and soil like water and nutrient uptake, rhizodeposition and respiration (Bardgett et al. 2014).

Functional dissimilarity among species is regarded as a possible explanation for higher plant productivity in species mixtures (Dimitrakopoulos and Schmid 2004). Therefore, the presence of species varying in their belowground characteristics potentially increases the complementary use of soil resources at higher plant diversity (de Kroon et al. 2012). For example, grasses, legumes and non-legume forbs, which are often regarded as functional groups among grassland species, have been shown to differ in their root biomass (Gastine et al. 2003; Bessler et al. 2012; Mueller et al. 2013), rooting depth (Dimitrakopoulos and Schmid 2004; von Felten and Schmid 2008) and root morphological traits (Grime et al. 1997; Craine et al. 2001; Siebenkäs et al. 2015) thus holding the potential for complementary resource use (Parrish and Bazzaz 1976). Belowground complementarity in resource uptake and use could be reflected in different rooting patterns such as increased segregation in vertical root biomass distribution at higher plant diversity as opposed to monocultures (Mueller et al. 2013). However, this causal relationship has recently been questioned because despite the presence of belowground complementarity effects, no diversity effects on species vertical root biomass distribution had been found in experimental grasslands

(Mommer et al. 2010). Hence, complementarity might occur due to inherent differences of species in a mixture independent of species richness.

In temperate grasslands, nutrients and light are regarded as major resources limiting plant growth (Hautier et al. 2009). Characteristically, a few inherently tall-growing species obtain dominance and intercept a disproportional greater share of light (Werger et al. 2002), causing asymmetric competition with inherently small-growing species, which are consequently forced to grow in regimes of lower light availability (Weiner 1990). However, belowground competition has both been suggested to be size-asymmetric (Rajaniemi 2003) and has been found to be size-symmetric (Weiner et al. 1997).

Belowground complementarity has been discussed to depend on abiotic conditions such as soil fertility (Ravenek et al. 2014), but only a small number of experimental studies investigated the relationships between species diversity and belowground biomass at varying nutrient availability (Reich et al. 2001, Mommer et al. 2010, von Felten and Schmid 2008). Resource availability has also been shown to affect root morphological traits important for resource uptake, both in field and greenhouse studies. For example, differences in the availability of soil resources lead to plastic responses of root morphological traits, such as increased root diameter (RD) and decreased specific root length (SRL) with fertilization (Ryser and Lambers 1995; Siebenkäs et al. 2015) or increased SRL (Ryser and Eek 2000; Edwards et al. 2004; Siebenkäs et al. 2015) and decreased RD with shading (Wahl et al. 2001).

So far, vertical rooting patterns and root morphological traits have not been studied together in experimental studies simultaneously manipulating plant diversity and resource availability. Here, we present results of a field experiment with plant communities of varying species richness (monocultures, two- and four-species mixtures) composed of eight perennial temperate grassland species assigned to different functional groups (grasses and forbs) and varying in growth stature (tall and small). Plant communities were grown at different resource supply (light and nutrient availability). In the second year of treatment application, we studied standing root biomass and root morphological characteristics to answer the following questions:

(1) How does increasing species richness affect standing root biomass, root morphological traits and does it lead to differences in vertical root distribution?

(2) Are there any differences among communities varying in their functional group or growth stature composition in regard to standing root biomass, root morphological traits and vertical root distribution?

(3) What are the effects of light and nutrient availability on standing root biomass, root morphological traits, vertical root distribution and root-shoot-ratios and their relationships to plant diversity in terms of species richness and functional composition?

Materials and Methods

Experimental design

This study was conducted in experimental grasslands established in April 2011 at the experimental station of the Helmholtz Centre for Environmental Research (UFZ) in Bad Lauchstädt, Germany (51°23'38" N, 11°52'45" E, 118 m a.s.l.). Prior to the establishment of the experiment, agricultural crops were grown on the site. The area around Bad Lauchstädt has a mean annual temperature of 9.5 °C and an annual precipitation of 492 mm (1981-2010; weather data from intensive monitoring experiment in Bad Lauchstädt, working group C/N dynamics, UFZ, http://www.ufz.de/index.php?de=940). The soil is a czernozem (Altermann et al. 2005) with a texture of loamy sand (0-30 cm depth). Further soil chemical properties are summarized in Table S1.

Eight perennial species, four forb and four grass species, common in mown Central European temperate grasslands (Arrhenatherion community, Ellenberg 1988) were selected for the experiment and randomly separated into two species pools, each composed of a small-statured and a tall-statured forb and a small-statured and a tall-statured grass species (Table 1). The experiment comprised four replicates of monocultures of all species and all possible two-species combinations of each pool and eight replicates of the two possible four-species mixtures resulting in 96 plots of 2 x 2 m size. Plots were arranged in eight blocks, each consisting of an equal number of plots per species-richness level, whereby each species was represented in three plots per block.

Table 1: Species pools of the experiment containing studied species, their growth height (Jäger 2011), and assignment to functional groups (grasses or forbs) and different growth statures (small or tall)

Species	Family	Height (cm)	Functional group	Stature	Species pool
Anthoxanthum odoratum L.	Poaceae	20 - 50	grass	small	A
Lolium perenne L.	Poaceae	10 - 60	grass	small	B
Arrhenatherum elatius L.	Poaceae	60 - 120	grass	tall	A
Dactylis glomerata L.	Poaceae	50 - 120	grass	tall	B
Plantago lanceolata L.	Plantaginaceae	10 - 50	forb	small	A
Prunella vulgaris L.	Lamiaceae	5 - 30	forb	small	B
Centaurea jacea ssp. jacea L	Asteraceae	15 - 80	forb	tall	A
Knautia arvensis (L.) Coulter	Dipsacaceae	30 - 80	forb	tall	B

Seeds were acquired from a commercial supplier (Rieger-Hoffman GmbH, Blaufelden-Raboldshausen, Germany) choosing the regionally closest available provenance. The initial sowing density was 1000 viable seeds per m^2 (adjusted for germination rates determined in laboratory trials) equally distributed among species in mixtures. Plots were re-sown with a total density of 500 viable seeds per m^2 in October 2011 after the first mowing in September 2011 to achieve a more diverse age structure of populations.

After one year of growth, identical replicates per species composition (mixture identity) were assigned to the following experimental treatments manipulating nutrient and light availability:
(F-S-) no fertilization, no shading,
(F-S+) no fertilization, shading,
(F+S-) fertilization, no shading, and
(F+S+) fertilization, shading.

Four blocks were assigned to the shade treatment, which was realized by adjusting one layer of green shading cloth (polyethylene, aperture size 2 × 10 mm, Hermann Meyer KG, Rellingen, Germany) to wooden scaffolding of 2.10 m height ensuring that scaffoldings did not shade adjacent blocks. Shading cloths were installed in mid-April and removed in mid-September of each experiment year. Photosynthetically active radiation (PAR) in shaded blocks was reduced by 55% during daytime (based on continuous half-hourly measurements with SPK125, PAR Quantum Sensor; Skye Instruments Ltd, UK) opposed to blocks without shade treatment. Fertilizer for the nutrient addition treatment was applied as pellets (commercially available slow release NPK fertilizer 120:52:100 kg ha^{-1} yr^{-1}) in equal portions in spring (mid-March) and after first mowing (mid-June) in each experiment year. In regular intervals, species not being part of the original plot species combinations were weeded. All plots were mown twice a year (early June and September) and mown plant material was removed to mimic the usual management of extensive hay meadows in the region.

Standing root biomass and root morphology

After first mowing in June 2013, i.e. in the second year of treatment applications, three soil cores per plot were taken with a split-tube sampler (4.8 cm inner diameter; Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands) down to 40 cm depth. Samples were taken along transects with a minimum of 40 cm distance to the plot margin and between replicated samples per plot. Soil cores were segmented into 10 cm depth increments (0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm) and the corresponding layers were pooled per plot. In addition, soil depth from 40-60 cm was sampled with a smaller core diameter (1.8 cm; Pürckhauer Typ 100) and samples were pooled plot-wise.

Pooled samples were stored at -20°C until further processing. Then, samples were thawed and repeatedly rinsed with tap water over a 0.5 mm sieve. Remaining soil particles and organic debris were removed with tweezers. Cleaned root samples were scanned in a water filled tray on a flatbed scanner at 800 dpi. If samples exceeded tray capacity, representative subsamples were taken, scanned and kept separately. Afterwards, root samples were dried at 70° C for 48 h and weighed. Standing root biomass was calculated as g m^{-2}, root mass density as g m^{-3}. Root scans were analysed with the Winrhizo® Software (Regent Systems Inc., Quebec City, Canada) to calculate specific root length (SRL, root length per sample biomass, m g^{-1}), root length density (RLD, root length per soil volume, cm cm^{-3}) and root diameter (mm) per layer.

Evenness of standing root biomass distribution over the depth profile from 0 to 40 cm was calculated as Simpson's dominance index (Simpson 1949) following Ravenek et al. (2014)

$$E = \frac{D}{S} = \left(\frac{1}{\sum_{i=1}^{S} P_i^2}\right) / \mathrm{S} \qquad \text{(eqn. 1)},$$

where P_i is the proportion of biomass for each layer i, and S the number of layers.

Weighted mean depth (WMD) of vertical standing root biomass distribution was assessed as

$$WMD = \frac{\sum_{i=1}^{S} MD * BM_i}{BM} \qquad \text{(eqn. 2)},$$

where MD is the mean depth of each layer, BM_i is the root biomass of the respective layer and BM is total root biomass across all layers (Gibson et al. 1987). Evenness and WMD were calculated accordingly for RLD. Root diameter (RD) and specific root length (SRL) obtained per layer were weighted by root biomass of the respective layer to get mean values of root morphological traits per plot.

Aboveground biomass

Aboveground biomass was sampled by cutting plant material at 3 cm above soil surface in two randomly allocated rectangles (20 x 50 cm) prior to mowing in late May 2013. Aboveground biomass was sorted to sown species, detached dead plant material and weeds. Samples were weighed after drying for 48 h at 70°C and averaged per plot to calculate aboveground biomass per m^{-2}. Community-level root-shoot ratio (RSR) was attained by dividing root standing biomass by aboveground biomass for each plot.

Data analyses

All statistical analyses were conducted with the statistical software R 3.1.3 (R Core Team 2015). Linear mixed-effects models (package *lme4*, Bates et al. 2014) were applied to analyse the vertical distribution of root standing biomass and root length density (RLD) over the depth profile from 0 to 40 cm depth dependent on plant diversity and resource availability. Starting from a constant null model with block, plot nested in block and composition (= mixture identity) as random terms, the fixed effects were added in the following order: shade (with two factor levels: 0 = no shade, 1 = shade), fertilization (with two factor levels: 0 = no fertilization, 1 = fertilization), species richness (SR; as linear term), functional group composition (FG; three factor levels: G = pure grass mixtures, F = pure forb mixtures, GF = grass-forb mixtures), plant-stature combination (GS; three factor levels: S = pure small-species mixtures, T = pure tall-species mixtures, ST = small/tall-species mixtures), depth (four factor levels = 0-10, 10-20, 20-30, 30-40 depth) and all possible two-way and three-way interactions. In order to evaluate the statistical significance of model improvement by sequential adding of fixed effects, the maximum likelihood method and likelihood ratio tests were applied.

For analyses of plot-level data (i.e. total root biomass from 0 to 60 cm depth (BM_{0-60}), evenness and weighted mean depth of standing root biomass and RLD, means of SRL and RD as well as for root-shoot ratios), the random structure of the models consisted of the terms for block and composition (= mixture identity) and the above-mentioned sequence of fixed effects was fitted stepwise. All data was transformed to natural logarithms (except for WMD for RLD) to fulfil the assumptions of normal distribution. The *difflsmeans* function (package *lmerTest*, Kuznetsova et al. 2014) was applied to identify significant differences between communities of different functional composition (growth stature, functional groups) in post-hoc tests of the respective models with restricted maximum likelihood by calculating differences of least squares means and confidence intervals for these factors.

Results

Effects of resource availability, species richness and functional composition on standing root biomass and above- and belowground biomass allocation

Between 30% (F+S+) and 54% (F-S-) of total biomass were invested into root biomass (0-40 cm depth). Aboveground biomass was largest in fertilized communities grown at full light and did not differ among the other treatments (Fig. 1a). Effects of resource availability did not differ in dependency on which depths (0-40 or 0-60 cm) were considered in the analyses. On average, between 88 and 93% of standing root biomass in the examined profile down to 60 cm depth could be found in the upper 40 cm. Shading had negative effects on standing root biomass (Table 2, Fig. 1a), which was 57% lower under shading than under full light. The effect of fertilization depended on light conditions as it tended to increase root biomass in communities with ambient light and tended to decrease root biomass under shading (Table 2, Fig. 1a). Consequently, root-shoot ratios in biomass differed dependent on light and nutrient availability and the interaction between both resources (Table 2). On average, in unfertilized non-shaded communities more biomass was accumulated belowground than produced aboveground (root-shoot ratio > 1; Fig. 1b). The root-shoot ratio shifted in favour of aboveground biomass (root-shoot ratio < 1) when non-shaded communities were fertilized, due to a greater aboveground biomass production (Fig. 1b). In shaded communities, root-shoot ratios were also <1, especially in fertilized communities. Communities of different species richness or functional composition did not differ in standing root biomass or root-shoot ratios irrespective of resource manipulation. However, communities of small-statured species had higher root-shoot ratios than mixtures containing tall species in unshaded, unfertilized communities, but were similar in regard to biomass allocation into roots when both shaded and fertilized (significant interaction Shade x Fertilizer x GS, Table 2).

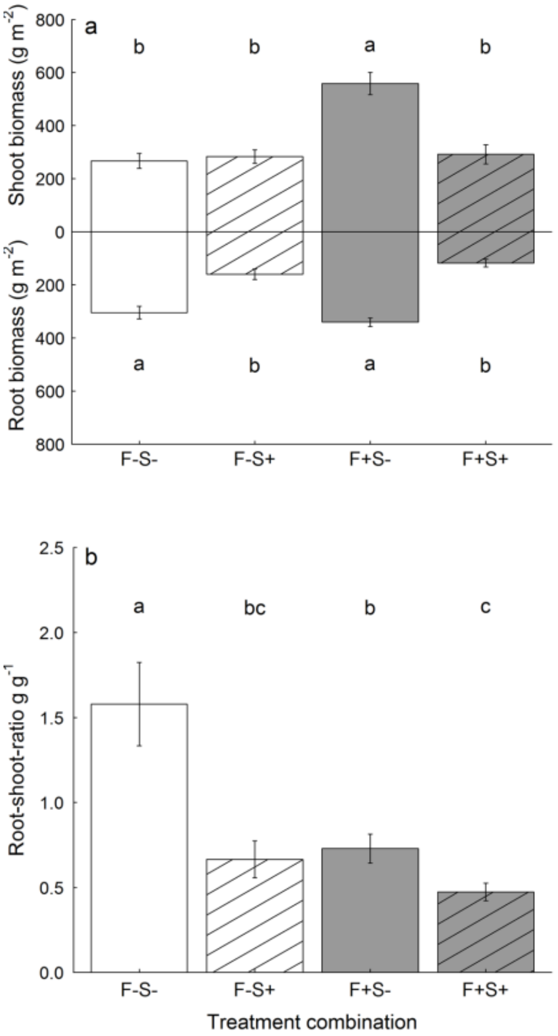

Figure 1: Above- and belowground community biomass (a) and root-shoot ratios as affected by resource availability, where F-S- = no fertilization, no shading, F-S+ = no fertilization, shading, F+S- = fertilization, no shading, and F+S+ = fertilization, shading. Values are shown as means ± 1 SE per resource treatment. Letters symbolize significant differences between resource treatments.

Effects of resource availability, species richness and functional composition on root length density and root morphological traits

Root length density (RLD) and average root diameters (RD) were smaller, whereas specific root length (SRL) was larger under shading than under full light (Table 2, Fig. 2a-c). Root length density and root morphological traits did not directly change in response to fertilization, but the effects of fertilization on SRL depended on shade (Table 2). In shaded fertilized plots, SRL was higher than in all other treatment combinations (Fig. 2b).

There was no detectable effect of increased species richness on RLD, SRL or RD (Table 2, Fig. 2d-f). However, functional composition affected root morphological traits. On average, RLD and SRL in pure grass communities were significantly higher ($p<0.01$) than in pure forb communities, whereas SRL in mixtures of grasses and forbs was between the respective mono-functional communities (Fig. 2g, h). Conversely, the average root diameter was larger in pure forb communities than in communities with grasses (Fig. 2i). Communities with small-statured species showed larger SRL than communities with tall-statured species, but RLD and average RD did not differ dependent on growth stature composition (Fig. 2j-m). The effects of resource availability on RLD and root morphological traits did not depend on species richness and functional composition in terms of growth statures or functional groups (Table 2).

Table 2: Summary of linear mixed effects models for standing root biomass in the profile from 0 to 40 cm depth (BM_{040}), root-shoot ratios, root length density (RLD), specific root length (SRL) and average root diameter (RD)

	BM_{040}		**Root-shoot ratio**		**RLD**		**SRL**		**RD**	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Shade	**19.975**	**<0.001**	**12.647**	**<0.001**	**17.812**	**<0.001**	**12.307**	**<0.001**	**4.843**	**0.028**
Fertilizer	0.028	0.866	**12.354**	**<0.001**	0.003	0.960	0.831	0.362	0.571	0.450
Shade x Fertilizer	**5.672**	**0.017**	**3.955**	**0.047**	1.472	0.225	**5.891**	**0.015**	3.123	0.077
SR	0.012	0.914	1.406	0.236	0.102	0.750	0.023	0.879	0.390	0.532
FG	0.136	0.934	1.003	0.606	**13.763**	**0.001**	**12.786**	**0.002**	**21.146**	**<0.001**
GS	0.167	0.920	2.998	0.223	2.316	0.314	**7.626**	**0.022**	3.449	0.178
SR x Shade	0.845	0.358	0.163	0.686	0.539	0.463	0.478	0.489	0.253	0.615
SR x Fertilizer	0.072	0.789	0.011	0.916	1.070	0.301	0.365	0.546	0.122	0.727
Shade x Fertilizer x SR	0.733	0.392	2.930	0.087	0.720	0.396	0.012	0.913	0.006	0.937
FG x Shade	1.655	0.437	3.600	0.165	1.491	0.475	0.882	0.643	0.475	0.789
FG x Fertilizer	0.602	0.740	1.686	0.430	0.520	0.771	0.126	0.939	0.573	0.751
Shade x Fertilizer x FG	0.622	0.733	0.848	0.654	1.397	0.497	1.291	0.524	0.122	0.941
GS x Shade	5.817	0.055	2.583	0.275	2.333	0.311	3.750	0.153	1.843	0.398
GS x Fertilizer	0.236	0.889	0.892	0.640	0.249	0.883	1.509	0.470	0.811	0.667
Shade x Fertilizer x GS	**6.139**	**0.046**	**10.362**	**0.006**	0.911	0.634	5.446	0.066	1.431	0.489

Models were fitted by stepwise inclusion of fixed effects. Likelihood ratio tests (χ^2) were applied to assess model improvement and the statistical significance of the explanatory terms (P values, significant values in bold). FG = functional group composition (3 factor levels: pure grass communities, grass-forb mixtures, or pure forb communities), GS = growth stature composition (3 factor levels: pure tall-statured species communities, mixtures of both growth statures or pure small-statured species communities), SR = species richness (linear).

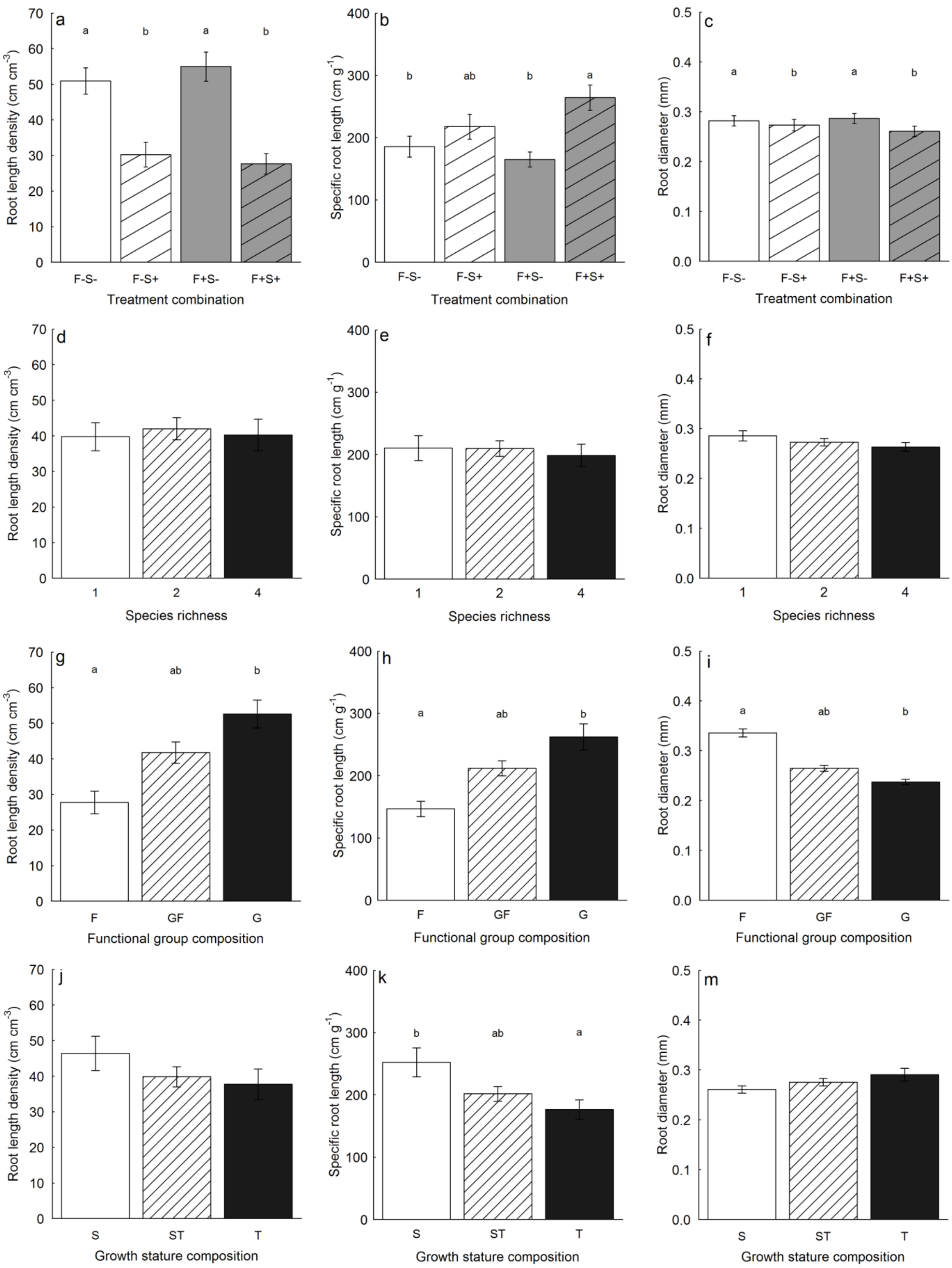

Figure 2: Root length density (a, d, g, j), specific root length (b, e, h, k), and root diameter (c, f, i, m) dependent on resource availability (a-c), where F-S- = no fertilization, no shading, F-S+ = no fertilization, shading, F+S- = fertilization, no shading, and: F+S+ = fertilization, dependent on species richness (d-f) for monocultures, two- or four-species mixtures, dependent on functional group composition (g-i), where F = only forbs, GF = forbs and grasses, and G = only grasses, and dependent on growth stature composition (j-m), where S = only small-statured species, ST = small- and tall-statured species, and T = only tall-statured species.

Shown are means ± 1 SE, letters symbolize significant differences between resource treatments, species richness, functional group and growth stature composition, respectively.

Effects of resource availability, species richness and functional composition on vertical root distribution

Standing root biomass (63%, mean ± sd: 146.08 ± 89.81 g m^3) and RLD (64%, 26.19 ± 16.1 cm cm^{-3}) were highest in the upper ten centimetres of the profile from 0 to 40 cm depth (Fig 3, S1). Accordingly, weighted mean depth (WMD) of standing root biomass and RLD were 11.2 ± 2.33 cm and 11.4 ± 1.81 cm, respectively (Fig. 4). Mean evenness of standing root biomass distribution and RLD in the 0-40 cm profile was similar with 0.56 ± 0.14 and 0.57 ± 0.12, respectively (Fig. S2). Resources, i.e. light and nutrient availability, or increased species richness did not affect the vertical distribution of standing root biomass and RLD (Fig. 3a-b, S1a-b, Table 3) or WMD and evenness of both variables (Fig. 4a-b, e-f, S2a-b, e-f, Table S2). However, the vertical distribution of standing root biomass and RLD depended on functional group and growth stature composition of the experimental communities (Table 3).

Table 3: Summary of linear mixed effects models for depth distribution of root length density (RLD) and standing root biomass in the profile from 0 to 40 cm depth (BM_{040})

	RLD		**BM_{040}**	
	χ^2	P	χ^2	P
Shade	**18.016**	**<0.001**	**21.465**	**<0.001**
Fertilizer	0.003	0.959	0.129	0.720
Shade x Fertilizer	0.391	0.532	3.225	0.073
SR	0.157	0.692	0.337	0.561
FG	**9.400**	**0.009**	0.476	0.788
GS	0.079	0.961	4.973	0.083
SR x Shade	0.438	0.508	0.271	0.603
SR x Fertilizer	0.062	0.804	0.074	0.786
Shade x Fertilizer x SR	0.153	0.696	0.241	0.623
FG x Shade	0.598	0.742	0.868	0.648
FG x Fertilizer	0.765	0.682	1.439	0.487
Shade x Fertilizer x FG	0.639	0.726	0.400	0.819
GS x Shade	1.439	0.487	5.215	0.074
GS x Fertilizer	0.253	0.881	0.535	0.765
Shade x Fertilizer x GS	0.205	0.903	2.509	0.285
Depth	**727.928**	**<0.001**	**586.921**	**<0.001**
Depth x Shade	5.203	0.158	3.306	0.347
Depth x Fertilizer	0.652	0.884	0.356	0.949
Depth x Shade x Fertilizer	2.273	0.518	2.092	0.554
Depth xSR	0.169	0.982	3.801	0.284
Depth xFG	**21.021**	**0.002**	**30.820**	**<0.001**
Depth xGS	**25.457**	**<0.001**	**57.814**	**<0.001**
Depth x SR x Shade	4.645	0.200	1.966	0.579
Depth x SR x Fertilizer	4.542	0.209	0.719	0.869
Depth x FG x Shade	8.411	0.209	11.997	0.062
Depth x FG x Fertilizer	**13.495**	**0.036**	8.190	0.225
Depth x GS x Shade	11.269	0.080	**20.933**	**0.002**
Depth x GS x Fertilizer	6.867	0.333	5.443	0.488

Models were fitted by stepwise inclusion of fixed effects. Likelihood ratio tests (χ^2) were applied to assess model improvement and the statistical significance of the explanatory terms (P values, significant values in bold). FG = functional group composition (3 factor levels: pure grass communities, grass-forb mixtures, or pure forb communities), GS = growth stature composition (3 factor levels: pure tall-statured species communities, mixtures of both growth statures, or pure small-statured species communities), SR = species richness (linear).

Communities composed of grass species exclusively, had a greater standing root biomass and RLD in the upper ten centimetres of the depth profile than mixtures of both functional groups and pure forb communities, while standing root biomass accumulated in deeper layers of the depth profile was larger in communities containing forb species than in pure grass communities (Fig. 3c, Fig. S1c, Table 3).

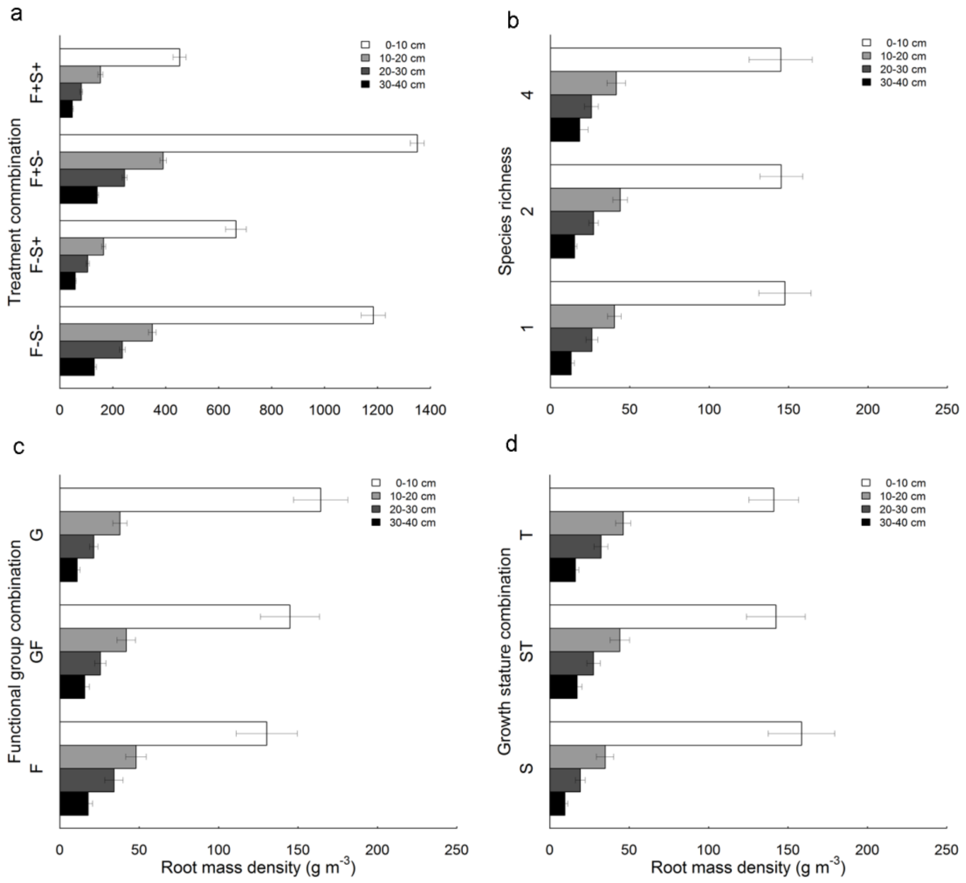

Figure 3: Root mass density (g m^{-3}) across the soil depth profile of 0 - 40 cm in 10 cm increments for (a) different resource treatment combinations (F-S- = no fertilization, no shading, F-S+ = no fertilization, shading, F+S- = fertilization, no shading, and F+S+ = fertilization, shading), (b) dependent on species richness (one, two or four species), (c) dependent on functional group composition (F = pure forb mixtures, GF = mixtures of both functional groups, G = pure grass mixtures) and (d) dependent on growth-stature composition (S = pure small-statured species mixtures, ST = mixtures of both growth statures, T = pure tall-statured species mixtures). Values are shown as means ± 1 SE.

Opposed to this, RLD in pure forb mixtures was generally lower in all layers compared to grass-forb and pure forb mixtures (Fig. S1c). Differences in the vertical distribution of RLD depending on functional group composition varied with fertilization (significant interaction "Depth x FG x Fertilizer", Table 3). A higher RLD in pure grass communities than in pure forb communities in 10-20 cm and 20-30 cm depth only occurred in fertilized communities.

Communities of only small-statured species showed significantly higher RLD in the upper 10 cm layer opposed to mixtures containing only tall-statured species (while mixtures of both growth statures were intermediate), while RLD in deeper layers of the depth profile did not differ depending on growth stature composition (Fig. S1d, Table 3). Vertical biomass distribution also differed depending on growth stature composition; with increasing depth, mixtures containing tall statured species had significantly more biomass than mixtures of only small statured species (Fig. 3d). This growth stature-dependent difference in the vertical distribution of root biomass was more pronounced in deeper layers in shaded communities (significant interaction "Depth x GS x Shade", Table 3).

Functional composition, both in terms of growth stature and functional groups, also led to differences in WMD and evenness of standing biomass and RLD (Table S2). WMD of root biomass was deeper and roots were more evenly distributed in the depth profile in pure forb communities than in pure grass communities, while WMD of mixed communities was in between (Fig. 4c, S2c). WMD in RLD did not vary with functional group composition (Fig. 4g), but evenness in RLD was also greater in pure forb communities than in pure grass communities (Fig. S2g). Differences in WMD and evenness of root biomass and RLD dependent on functional group composition did not change with resource availability.

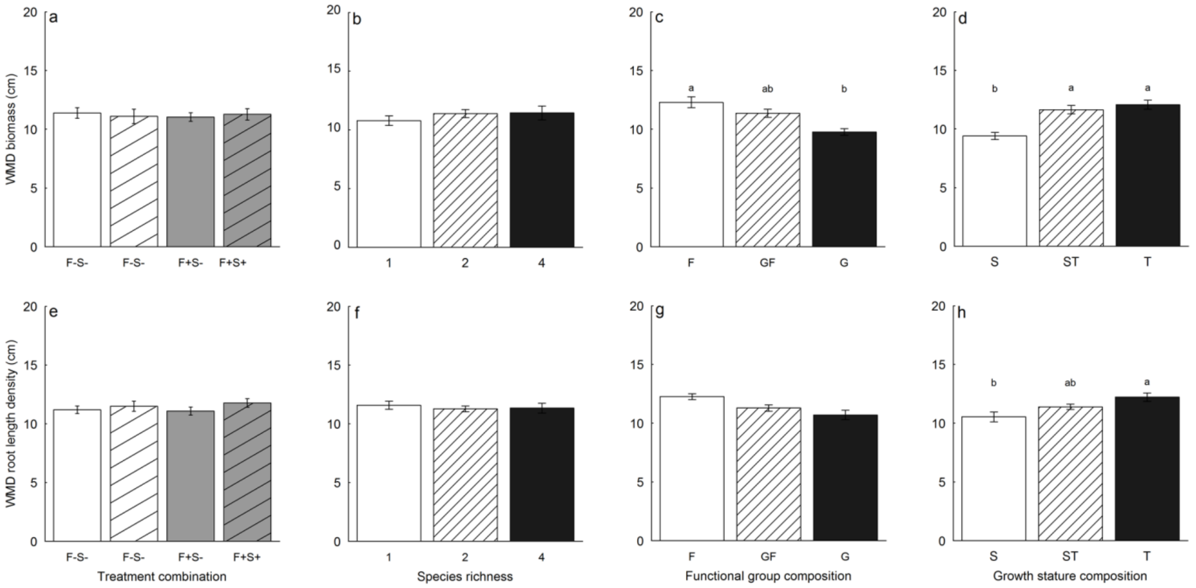

Figure 4: Weighted mean depth (WMD) of standing root biomass (a-d), and weighted mean depth (WMD) of root length density (e-h) dependent on resource availability (a, d), where F-S- = no fertilization, no shading, F-S+ = no fertilization, shading, F+S- = fertilization, no shading, and F+S+ = fertilization, shading, dependent on species richness for monocultures, two- or four-species mixtures (b, f), dependent on functional group composition (c, g), where F = only forbs, GF = forbs and grasses, and G = only grasses, and dependent on growth stature composition (d, h), where S = only small-statured species, ST = small- and tall-statured species, and T = only tall-statured species. Values are means ± 1 SE, letters symbolize significant differences between resource treatments and dependent on functional group or growth stature composition, respectively.

Communities comprising tall-statured species or both growth statures were characterised by a greater WMD and evenness in root biomass than communities with only small-statured species (Fig. 4d, Fig. S2d), while WMD and evenness in RLD solely differed between communities containing only small-statured or tall-statured species (Fig. 4h, S2h). Differences in WMD and evenness in standing root biomass attributable to species growth statures varied with shading (Table S2). Differences in WMD and evenness of root biomass between communities of only small-statured and communities including tall-statured species were more pronounced with shading, while there were only marginal differences in unshaded communities.

Discussion

Effects of species richness and functional composition on root morphology and standing root biomass

In our experiment, standing root biomass, RLD and other root morphological traits did not differ between communities of different species richness. Standing root biomass has been shown to increase with increasing species richness in several grassland biodiversity experiments depending on their duration and site. Results from other short-running experiments yielded for example no effect of increased species richness on root biomass after three years at the German BIODEPTH site (Gastine et al. 2003), but slightly positive effects of species richness on root biomass in the upper 20 cm soil depth were detected in analyses across all BIODEPTH sites (Spehn et al. 2005). However, positive effects of species richness on root biomass have been observed to become stronger in longer-running experiments (Mueller et al. 2013; Cong et al. 2014; Ravenek et al. 2014). The observed short-term effects (one year) of belowground overyielding in experimental monocultures and four species mixtures have been attributed to a single grass species, *Anthoxanthum odoratum* (Mommer et al. 2010). This grass species was also included in our experiment, but did not show extraordinary large effects compared to other grass species.

In contrast to other studies, where the presence of particular functional groups affected root biomass (Gastine et al. 2003; Bessler et al. 2012), standing root biomass did not depend on functional group or growth-stature composition in our experiment. However, the previously observed effects of functional group composition were mainly attributable to the low root biomass of legumes (Gastine et al. 2003; Bessler et al. 2012), which were deliberately not included in our study, while different groups of non-legume species also did not affect root biomass (Bessler et al. 2012).

In contrast to the non-significant effects of functional composition on root standing biomass, root morphological traits differed among communities composed of different functional groups or species with different inherent growth statures. This is in line with previous studies in monocultures or on separately grown plant individuals of grassland species showing that forbs are characterized by smaller SRL and RLD but larger average RD (Craine et al. 2001, Siebenkäs et al. 2015). However, none of the examined root morphological traits differed between communities of single functional groups (or growth statures in case of SRL) as opposed to mixtures thereof; but the communities of singular functional composition differed from each other. Assuming that species do not plastically adjust their root morphology in response to varying plant neighbourhood, our results on root standing biomass and root morphological traits suggest that grasses or forbs and tall- or small-statured species, respectively, did not heavily affect each other in their rooting patterns in the mixture. Indeed, we could show previously with the same species grown as individual plants that species ranking for root morphological traits remained unaffected by differences in resource availability, thus belowground plasticity did not differ between functional groups or growth statures opposed to inherent differences in their root morphological traits (Siebenkäs et al. 2015). Nevertheless, we cannot exclude that species adjusted their root morphological traits when grown in combination with other species in the mixtures. For example, there also exists evidence from other studies that species may achieve competitive superiority for nutrients by root growth stimulation in the presence of other species (Padilla et al. 2013).

Effects of species richness and functional composition on vertical root distribution

In addition to differences in root morphological and physiological characteristics, inherent differences in root depth distribution are regarded as prerequisite for root segregation in mixed plant communities, which could promote a more complete use of belowground resources (Parrish and Bazzaz 1976; Berendse 1982, 1983; Dimitrakopoulos and Schmid 2004). Root systems of different species have been shown to distribute more evenly in mixtures than in monocultures in grassland mesocosms (von Felten and Schmid 2008). However, similar to Ravenek et al. (2014) in the long-term Jena Experiment with a large species-richness gradient and Mommer et al. (2010) in mesocosms with up to four species, we also did not detect species richness effects on vertical root distribution. In contrast to this, mixtures exhibited different rooting patterns depending on their functional group or growth stature composition. A higher evenness in RLD and standing root biomass across the soil profile, however, did not depend on the number of functional groups or growth statures in a mixture, but on their identities. It has been shown that forb species generally exploit lower soil depths than grasses (Berendse 1982). Evenness and WMD were highest in communities with only tall species or only forbs and lowest in communities with only small-statured species or only grasses and intermediate in the respective mixtures of both (Figs. 4 and S2). The large effects of functional composition on root depth distribution are in line with results from Mueller et al. (2013), while other studies did not find effects of functional composition on root depth distribution (Ravenek et al. 2014). While in our study, mono-functional communities of forb species and tall species, respectively, were more thoroughly rooting across the soil profile. Communities of mixed composition showed a trend to differ from mono-functional small-statured or grass species communities that accumulated more biomass in the upper layers, thus exhibiting the potential for root segregation. Since belowground effects have been shown to increase with time (Ravenek et al. 2014), a stronger vertical segregation among species with varying rooting patterns might occur after longer time periods.

Effects of resource availability on belowground characteristics

Independently of species composition, effects of resource availability on root morphological traits have been shown in previous studies. Shading may increase SRL (Ryser and Eek 2000, Edwards et al. 2004) and decrease RD (Wahl et al. 2001), while effects of fertilization on SRL and RD are less consistent and apparently depend on species identity (Ryser and Lambers 1995; Leuschner et al. 2013; Siebenkäs et al. 2015). In our study, shade affected all examined root traits irrespective of species composition of the communities, while fertilization did not affect the studied root morphological characteristics.

Root-shoot ratios, however, varied depending on light and nutrient availability. Plant growth requires a functional equilibrium in the acquisition of different resources (Chapin et al. 1987). Shaded plants suffering from carbon limitation through reduced photosynthesis are known to invest a greater amount of resources into aboveground biomass compared to root biomass and consequently have lower root-shoot ratios (Ryser and Eek 2000), whereas nutrient-limited plants may be expected to increase allocation into belowground organs (Poorter et al. 2012). In our experiment, we factorially manipulated light and nutrient availability and neighbouring tall-growing plants might additionally influence light supply of smaller-statured plants in mixed communities. Indeed, light availability was apparently the major factor influencing belowground characteristics, while fertilization in unshaded communities increased aboveground biomass and thus lowered root-shoot ratios. The lower standing root biomass of plants growing in the shade was associated with higher SRL, which partly could compensate the reduced area for resource uptake due to a lower investment into belowground plant organs.

Although functional group or growth stature compositions of our experimental grasslands did not lead to differences in standing root biomass by themselves and resource availability alone had no impact on evenness or weighted mean depths, shading increased differences in the root depth distribution of different growth statures while differences in RLD between functional groups increased in lower depths when fertilizer was applied. These results suggest that functionally more diverse communities have a greater potential to adjust their rooting patterns to varying resource supply and increase the complementary use of resources.

Conclusions

In summary, functional composition in terms of functional groups (grasses vs. forbs) or species growth statures (small vs. tall) was a major factor determining rooting patterns in our experimental grasslands, especially since variation in vertical distribution of standing root biomass and RLD in response to resource availability was also dependent on functional composition. Thus, the combination of species with varying root characteristics and not the number of species in a community is important for positive relationships between plant diversity and complementary use of belowground resources.

Funding

This work was supported by the German Research foundation (DFG RO2397/3, RO2397/4).

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Supplementary material

Table S1: Soil chemical properties of the experimental location

Soil properties	Depth (cm)	Range	Mean	(±SE)
Carbonate concentration (%)	0-15	0.3 - 0.6	0.48	(±0.03)
	15-30	0.47 - 0.7	0.59	(±0.03)
C:N ratio	0-15	10.6 - 11.7	10.98	(±0.12)
	15-30	10.6 - 11.1	10.85	(±0.06)
Organic carbon concentration (mg g^{-1})	0-15	23 - 25	23.88	(±0.31)
	15-30	22 - 23	22.59	(±0.15)
pH	0-15	6.7 - 7	6.93	(±0.05)
	15-30	6.9 - 7.5	7.12	(±0.07)
Phosphorus concentration (mg kg^{-1})	0-15	36 - 42	39.33	(±0.88)
	15-30	24 - 44	36.35	(±2.55)
Potassium concentration (mg kg^{-1})	0-15	102 - 247	151.74	(±17.9)
	15-30	71 -168	107.12	(±13.36)

Before starting the experiment, soil was sampled at three locations within each block (0-30 cm depth, separated into layers of 0-15 cm and 15-30 cm depth) and pooled block-wise. Carbonate concentrations were attained according to Scheibler. Total carbon and nitrogen concentrations were measured with an elemental analyser (Vario EL Element Analyzer, Elementar, Hanau, Germany). Soil organic carbon concentrations were calculated by subtracting anorganic carbon concentrations from total carbon concentrations. The pH values were obtained after suspending the soil with 0.01M $CaCl_2$. Phosphorus concentrations were determined from extracts with double lactate, potassium concentrations were obtained from calcium acetate lactate extracts. Displayed are means across blocks (± 1SE).

Table S2: Summary of linear mixed effects models for weighted mean depth (WMD) and evenness for root length density (RLD) and biomass (BM)

	WMD BM		**WMD RLD**		**Evenness BM**		**Evenness RLD**	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Shade	0.195	0.659	1.672	0.196	0.003	0.958	1.970	0.160
Fertilizer	0.003	0.956	0.088	0.766	0.021	0.885	0.001	0.982
Shade x Fertilizer	0.627	0.428	0.429	0.512	1.080	0.299	0.920	0.337
SR	0.390	0.532	0.123	0.726	0.291	0.589	0.173	0.678
FG	**7.356**	**0.025**	5.657	0.059	**7.488**	**0.024**	**6.156**	**0.046**
GS	**19.660**	**<0.001**	**9.239**	**0.010**	**18.261**	**<0.001**	**10.238**	**0.006**
SR x Shade	1.978	0.160	0.150	0.699	1.335	0.248	0.554	0.457
SR x Fertilizer	0.036	0.849	1.124	0.289	0.129	0.719	0.819	0.366
Shade x Fertilizer x SR	0.060	0.806	0.116	0.734	0.012	0.913	0.054	0.817
FG x Shade	1.833	0.400	2.734	0.255	1.148	0.563	2.591	0.274
FG x Fertilizer	3.305	0.192	2.800	0.247	3.785	0.151	4.271	0.118
Shade x Fertilizer x FG	0.566	0.754	0.332	0.847	0.676	0.713	0.203	0.904
GS x Shade	**9.864**	**0.007**	3.576	0.167	**9.217**	**0.010**	3.817	0.148
GS x Fertilizer	2.572	0.276	3.331	0.189	1.975	0.373	2.446	0.294
Shade x Fertilizer x GS	1.364	0.506	0.701	0.704	0.905	0.636	0.786	0.675

Models were fitted by stepwise inclusion of fixed effects. Likelihood ratio tests (χ^2) were applied to assess model improvement and the statistical significance of the explanatory terms (P values, significant values in bold). FG = functional group (grass vs. forb), GS = Growth stature (tall vs. small), SR= species richness.

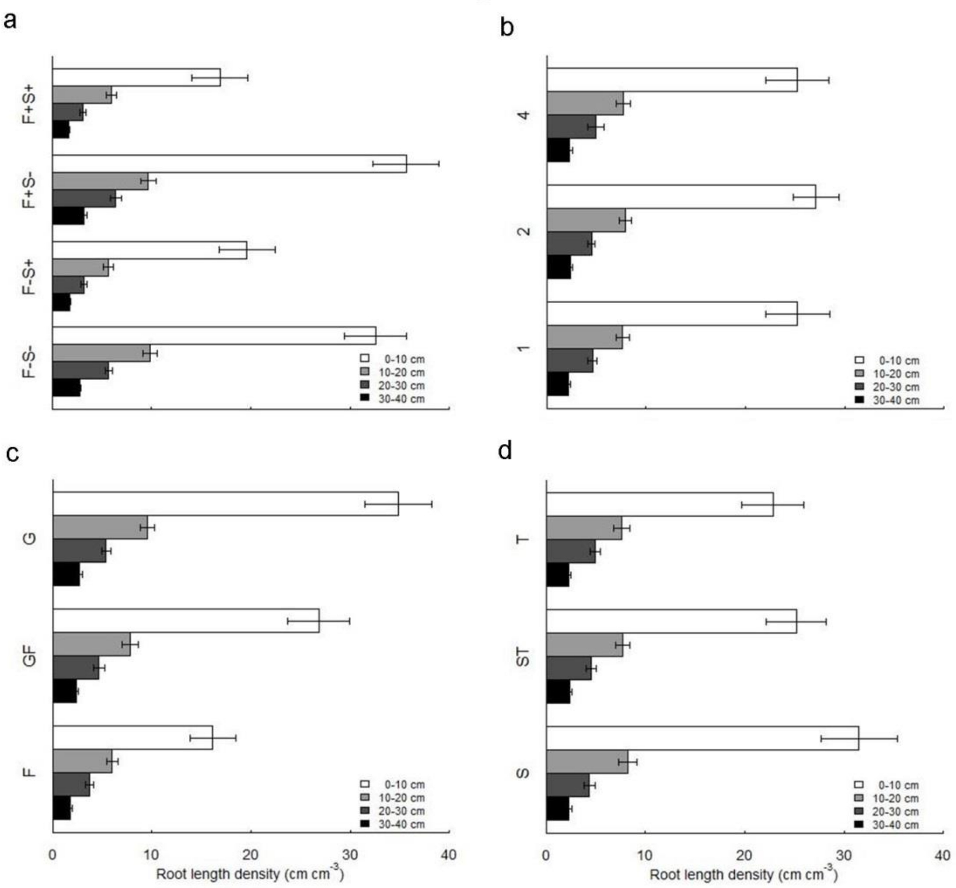

Figure S1: Root length density in cm m^{-3} across the soil depth profile of 0 - 40 cm in 10 cm increments for (a) different resource treatment combinations (F-S- = no fertilization, no shading, F-S+ = no fertilization, shading, F+S- = fertilization, no shading, and F+S+ = fertilization, shading), (b) dependent on species richness (one, two or four species), (c) dependent on functional group composition (F = pure forb mixtures, GF = mixtures of both functional groups, G = pure grass mixtures) and (d) dependent on growth-stature composition (S = pure small-statured species mixtures, ST = mixtures of both growth statures, T = pure tall-statured species mixtures). Values are shown as means ± 1 SE.

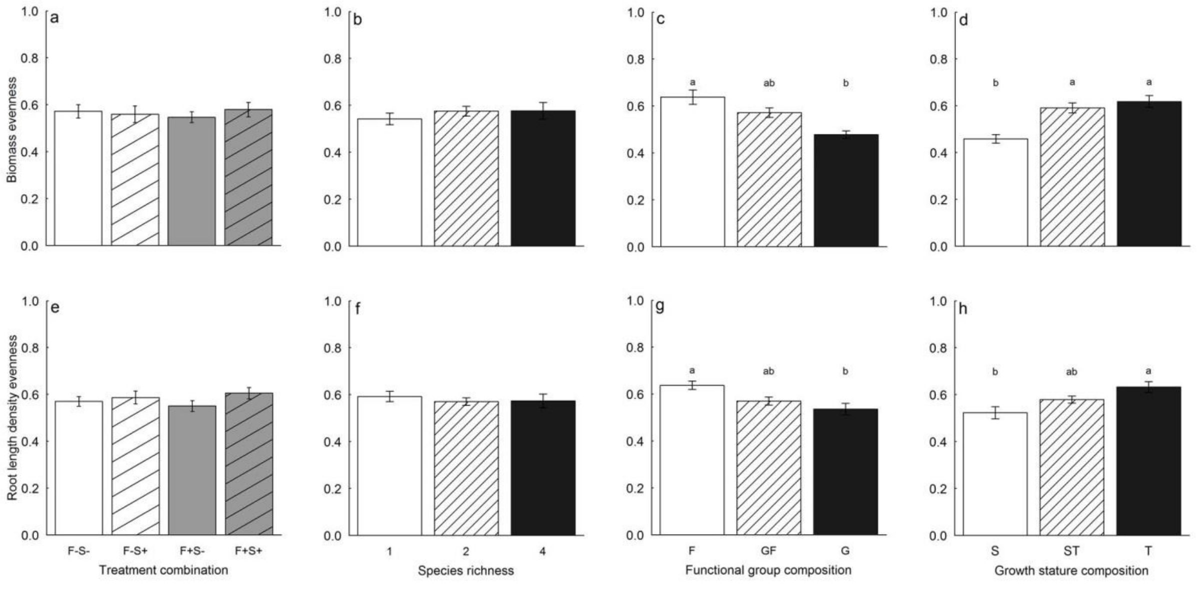

Figure S2: Evenness in community standing root biomass (a-d) and root length density (e-h) dependent on resource availability (a, d), where F-S- = no fertilization, no shading, F-S+ = no fertilization, shading, F+S- = fertilization, no shading, and F+S+ = fertilization, shading, dependent on species richness of one, two or four species mixtures (b, f), dependent on functional group composition (c, g), where F = only forbs, GF = forbs and grasses, and G = only grasses, and dependent on growth stature composition (d, h), where S = only small-statured species, ST = small- and tall-statured species, and T = only tall-statured species. Values are means ± 1 SE, letters symbolize significant differences between resource treatments and dependent on functional group or growth stature composition, respectively.

CHAPTER 5

RESOURCE AVAILABILITY ALTERS BIODIVERSITY EFFECTS IN EXPERIMENTAL GRASS-FORB MIXTURES

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PLoS one (invited for major revision)

Abstract

A number of experimental studies, mostly performed under particular environmental conditions, have shown positive diversity-productivity relationships. Although the complementary use of resources is discussed as an important mechanism explaining diversity effects, less is known of how resource availability controls the strength of diversity effects and how this response depends on functional composition of the plant communities. We studied aboveground biomass production in the second year of treatment applications in communities of eight perennial grassland species of two functional groups (grasses or forbs) and varying in growth stature (small or tall) in experimental monocultures, two- and four-species mixtures exposed to different combinations of light and nutrient availability. On average, shade led to a decrease in aboveground biomass production of 24% while fertilization increased biomass production by 36%. Mixtures were on average more productive than expected from their monocultures (relative yield total, RYT>1) and showed positive net diversity effects (+27% biomass increase). Both selection effects (+12%) and complementarity effects (+15%) positively contributed to net diversity effects. Shading did not affect diversity effects and overyielding. Fertilization decreased complementarity and net diversity effects, while selection effects did not change dependent on resource availability. Diversity effects did not increase with species richness and were independent of functional group or growth stature composition. Trait-based analyses showed that traits associated with the acquisition and use of nutrients best explained complementarity and selection effects, while traits associated with light acquisition did not explain the various extents of diversity effects. Our field experiment shows that positive diversity effects may occur in grass-forb mixtures irrespective of differences in light availability, but that the chance for the complementary resource use increases when nutrients are not available at excess.

Key words: biodiversity, complementarity effect, forbs, trait diversity, grasses, growth statures, overyielding, selection effect

Introduction

Experimental studies repeatedly showed that higher species or functional group richness increases primary productivity in grassland ecosystems and thus influences ecosystem processes (Hooper et al. 2005; Marquard et al. 2009; Cardinale et al. 2012). Positive diversity-productivity relationships and overyielding, i.e. a higher productivity of mixtures than expected from the average productivity of the component species in monocultures (Loreau 1998), are commonly explained by two mechanisms, which are not mutually exclusive. The *complementarity effect hypothesis* is based on the assumption that different species complement each other in the acquisition of resources, thereby decreasing interspecific competition and increasing total resource use in space and time, resulting in higher mixture productivity (Tilman et al. 1997). The *selection effect hypothesis* explains positive diversity-productivity relationships by the greater probability of more diverse communities to contain a particularly dominant and productive species (Aarssen 1997, Huston 1997). Both hypotheses propose distinct ecological mechanisms, but rely on the functional traits of the involved species: complementarity effects depend on the interactions between functionally different species, while selection effects require the presence of a species with particular functional characteristics (Fargione et al. 2007).

Functional groups are thought to capture the most relevant functional differences among species (Dyer et al. 2001). In grassland ecosystems, commonly distinguished functional groups are grasses, non-legume forbs and N_2 fixing legumes, which differ in their growth forms, root and leaf architecture and other morphological and physiological traits important for resource acquisition and use (Körner 1993; Craine et al. 2001; Roscher et al. 2004). Indeed, the combination of different functional groups in experimental grasslands has been shown to increase complementarity effects. These positive effects of functional groups were mostly due to legumes (Marquard et al. 2009; Wacker et al. 2009; Spehn et al. 2005), but positive complementarity effects have also been found in experimental grasslands excluding legumes (van Ruijven and Berendse 2003). *A priori* defined functional groups have been found to show large variation within groups (Tjoelker et al. 2005; Wright et al. 2006) and it is still uncertain which functional traits are of key importance for higher biomass production of mixtures. Hence, recent approaches stress the importance of continuous variables that quantify the trait composition of a community (Mouchet et al. 2010). As such, both community weighted mean traits (CWM), which reflect the dominance of trait values (Garnier et al. 2004) and indices of trait diversity, which quantify trait dissimilarity among species, have been proven as suitable predictors of aboveground biomass production in semi-natural and experimental grasslands (Díaz et al. 2007; Mokany et al. 2008; Schumacher and Roscher 2009; Roscher et al. 2012).

Light and nutrient availability are among the most important factors limiting plant productivity in temperate grasslands (Grime 1973). The size-asymmetry of competition for aboveground resources is generally accepted (Weiner 1990; Lepš 1999). The vertical stratification of plant canopies leads to a reduced light quantity and changes in light quality in lower strata, thus the potential to reach upper canopy strata with greater light availability is an important growth characteristic. Competition for soil resources is thought to be more size-symmetric (Wilson 1988) although it has been suggested that size-asymmetry in competition for belowground resources may occur when soil resources are heterogeneously distributed (Fransen et al. 2001, Rajaniemi 2003).

Apart from size-related differences in competitive abilities, environmental factors, i.e. the external supply of resources, are likely to control the nature and intensity of plant interactions. For example, fertilization may increase asymmetric competition for light by increasing productivity, when tall and fast-growing species reduce light supply for smaller species growing deep in the canopy (Hautier et al. 2009). Thus, positive selection effects are likely to increase under fertilization but it also has been discussed that positive complementarity effects may occur when increased soil fertility promotes light partitioning by accentuating species differences in height and growth forms (Fridley 2003). However, it is also possible that a greater diversity in root characteristics increases the chance for a complementary use of soil resources in unfertile conditions when competition for belowground resources prevails (Lanta and Lepš 2007). Fertilization has been shown to increase diversity effects and overyielding in several experiments (Reich et al. 2001; He et al. 2002; Fridley 2002, 2003). However, it also has been reported that the impact of soil fertility on diversity effects varies dependent on the involved species and their abundances (Lanta and Lepš 2007, Wacker et al. 2009), the amount of added fertilizer (Nyfeler et al. 2009, Wacker et al. 2009) and the manipulation of other resources such as light (Fridley 2003) or CO_2 (He et al. 2002). So far to our knowledge, only a single biodiversity experiment crossed the manipulation of nutrient availability by fertilization with light availability by shading. This experiment showed that overyielding was largest under increased soil fertility in full light, but the experimental species pool was restricted to annual forb species (Fridley 2003).

Here we present results of a field experiment based on a species pool of eight perennial grassland species of two different functional groups (four grasses and four forbs) both representing two inherently small-statured and two tall-statured species. We grew these species in monocultures, two-species and four-species mixtures at different light and nutrient availability. We analysed data on aboveground biomass production in the second year of treatment applications to address the following questions:

(1) How does resource availability alter the relative importance of complementarity and selection effects in explaining diversity effects in grass-forb mixtures, and what are the consequences for diversity effects and overyielding at different resource supply? We expected that complementarity effects decrease and selection effects increase with fertilization due to a shift from prevailing

competition for soil resources to greater importance of light competition. We also expected that the effects of fertilization on complementarity and selection effects would be lower under shading, when reduced light availability generally decreases productivity and nutrients are available at excess. Consequently, we hypothesized that diversity effects are greater without shading, while the expected shift from complementarity to stronger selection effects implies that the functional composition of the plant communities is important for varying diversity effects dependent on fertilization.

(2) Do the effects of resource availability on diversity effects depend on functional group or growth stature composition? We expected that positive selection effects are greater in mixtures of tall and small species due to asymmetric light competition and that these effects are accelerated under fertilization and less important under shading. Furthermore, we assumed that positive complementarity effects are higher in grass-forb mixtures compared to mixtures with species of a single functional group irrespective of resource availability.

(3) Are there general relationships between diversity effects and functional trait composition (community mean traits, trait diversity) and which traits are associated with increased complementarity and selection effects? We supposed that complementarity effects are positively related to greater diversity in traits related to the acquisition of above- and belowground resources, while selection effects rely on the dominance of particular trait values accentuating the asymmetry in the acquisition of resources.

Material and Methods

Experimental design

The field experiment was established in 2011 on a former agricultural crop site at the experimental station of the Helmholtz Centre for Environmental Research (UFZ) in Bad Lauchstädt, Germany (Saxony-Anhalt; 51°23'38" N, 11°52'45" E, 118 m a.s.l.). The soil is a chernozem developed from loess (Altermann et al. 2005); soil texture is loamy sand (0-30 cm depth, for additional soil properties see Table S1). The region is characterized by a mean of 492 mm for annual precipitation and 9.5°C for air temperature (1981-2010; weather data from intensive monitoring experiment in Bad Lauchstädt, working group C/N dynamics, UFZ, http://www.ufz.de/index.php?de=940).

Four grass and four forb species with a perennial life cycle and native to semi-natural temperate grasslands (Arrhenatherion community, Ellenberg 1988) were chosen for the experiment and randomly assigned to two species pools (Table 1). Each experimental species pool included an inherently small-statured and a tall-statured species of each functional group.

Table 1: Species pools of the experiment containing studied species, their growth height (Jäger 2011), and assignment to functional groups (grasses or forbs) and different growth statures (small or tall)

Species	Family	Height (cm)	Stature	Functional group	Species pool
Anthoxanthum odoratum L.	Poaceae	20 - 50	small	grass	A
Lolium perenne L.	Poaceae	10 - 60	small	grass	B
Arrhenatherum elatius L. P.Beauv. ex J.Presl & C.Presl	Poaceae	60 - 120	tall	grass	A
Dactylis glomerata L.	Poaceae	50 - 150	tall	grass	B
Plantago lanceolata L.	Plantaginaceae	10 - 50	small	forb	A
Prunella vulgaris L.	Lamiaceae	5 - 30	small	forb	B
Centaurea jacea ssp. jacea L.	Asteraceae	15 - 80	tall	forb	A
Knautia arvensis (L.) Coulter	Dipsacaceae	30 - 80	tall	forb	B

The experiment consisted of 96 plots of 2 x 2 m size, encompassing monocultures of each species and all possible two-species combinations that were replicated four times, while the four-species combinations of each species pool were established with eight replicates. Plots were arranged in eight blocks, each containing an equal number of plots per species-richness level (four monocultures, six two-species mixtures and two four-species mixtures) and ensuring that individual species occurred an equal amount of times (= three times) in each block. Seeds from the closest possible regional provenance were purchased from a commercial supplier (Rieger-Hoffman GmbH, Blaufelden-Raboldshausen, Germany). In mixtures, seeds were sown in equal proportions with a total initial sowing density of 1000 viable seeds per m^2 (adjusted for germination rates determined in laboratory tests) on 5 April 2011. After a first mowing in September 2011, all plots were re-sown on 4 October 2011 with 500 viable seeds per m^2 to imitate a more diverse age structure of plant populations.

In the second experimental year, one replicate per species composition (or two replicates in case of the four-species mixtures) was assigned to the following "environments" manipulating nutrient and light availability: (F-S-) no fertilization, no shading, (F-S+) no fertilization, shading, (F+S-) fertilization, no shading, and (F+S+) fertilization, shading. In four blocks, wooden scaffoldings were installed reaching from ground level to 2.10 m height. In mid-April, one layer of green shading cloth (polyethylene, aperture size 2 × 10 mm, Hermann Meyer KG, Rellingen, Germany) was attached to these scaffoldings and fastened to the ground on all sides until removal in mid-September of each experiment year to simulate shading. Photosynthetically active radiation (PAR) in shaded blocks was reduced by 55% during daytime compared to blocks without shade treatment (based on continuous half-hourly measurements with SPK125, PAR Quantum Sensor; Skye Instruments Ltd, UK). The arrangement of blocks in the field ensured that shade scaffoldings did not interfere with light availability in surrounding blocks. In each block, an equal number of plots per species-richness level was selected for the nutrient addition treatment. Fertilizer was administered in granular form (commercially available slow release NPK fertilizer 120:52:100 kg ha^{-1} yr^{-1}) and was applied in two equal portions in spring (mid-March) and after first mowing (mid-June) in each experiment year. The amount of added nutrients is equivalent to usual fertilizer intensities in agriculturally managed semi-natural grasslands in Europe (Olff et al. 1990).

All plots were mown in early June and mid-September and mown plant material was removed to imitate the regionally common management of extensive hay meadows. Species not being part of the original plot species combinations were removed in regular intervals.

Data collection

In the second treatment year, aboveground biomass was harvested at estimated peak biomass prior to the mowing in spring (27-29 May 2013) and summer (26-29 August 2013). Plant material was cut 3 cm above ground level in two randomly allocated rectangles (0.2 x 0.50 m) excluding the outer 0.40 m to the plot margin. All samples were sorted to sown species; detached dead plant material and weeds were separated from the samples. After drying for 48h at 70°C, samples were weighed and community- and species-level annual aboveground biomass production (g m^{-2}) was attained by summing up both harvests.

Data analyses

Calculations of measures of overyielding and diversity effects

Overyielding in relative terms (de Wit & van der Bergh 1965) was quantified as relative yield total (RYT), The relative yield (RY_i) of a species i is the quotient of a species' mixture biomass (Y_{iO}) and its monoculture biomass (BM_i). The RYT of a mixture is the sum of the relative yields of each component species i (RY_i):

$$RYT = \sum_{i=1}^{S} RY_i \qquad \text{(eqn.1)}$$

A RYT > 1 indicates that the proportional increase in the biomass of particular species is greater than the possible proportional decrease in the biomass of other species, i.e. a mixture outperforms the average of its component monocultures. It is directly associated to non-transgressive overyielding

$$D_{mean} = (Y_O - \overline{BM})/\overline{BM} \qquad \text{(eqn. 2),}$$

where Y_o is the observed biomass of a given mixture and $\overline{BM}$ is the average monoculture biomass of all species in this mixture (D_{mean} = RYT-1; Loreau 1998). Transgressive overyielding, i.e. a higher productivity of a mixture than of its most productive monoculture, was calculated by comparing the biomass of a given mixture to the maximum monoculture biomass of the species in that mixture (Loreau 1998):

$$D_{max} = (Y_O - \max(BM_i))/\max(BM_i) \qquad \text{(eqn. 3).}$$

The relative yields (RY_i) of individual species corrected for sowing proportions (i.e. multiplied by species number S) were used to compare the performance of species and their contribution to overyielding. Species perform better in mixture than in monoculture if values are (RY_i *S) > 1, their performance in mixture does not differ from monoculture if (RY_i * S) = 1, and values of (RY_i * S) < 1 indicate that species perform worse in mixture than in monoculture.

The additive partitioning method (Loreau and Hector 2001) was applied to assess diversity effects on aboveground biomass production in absolute terms. The net diversity effects (NE) is the difference between observed (Y_O) and expected (Y_E) biomass in mixtures and also the sum of two additive components, the complementarity effect (CE) and the selection effect (SE). The complementarity effect is positive (negative) if species biomass in a mixture is on average higher (lower) than the average of species biomass in monocultures and is calculated as

$$CE = \overline{\Delta RY}\,\overline{BM}\mathrm{S} \qquad \text{(eqn. 4)},$$

where $\overline{\Delta RY}$ is the average ΔRY_i (= difference between the observed relative yield and the expected relative yield) and $\overline{BM}$ is the average monoculture biomass of all species growing in this mixture. The selection effect (SE) represents the extent to which species with a high biomass in monoculture contribute to a mixture at the expense of other species quantified as the covariance of species' relative yield differences (ΔRY_i) and species' biomass in monocultures (BM_i):

$$SE = S\;cov\;(BM_i, \Delta RY_i) \qquad \text{(eqn. 5)}.$$

In all calculations, the observed species biomass in the monoculture of a particular “environment”, i.e. resource treatment combination, was used to derive the expected biomass in the mixtures of the same species × resource treatment combination.

To account for the absolute differences in productivity dependent on resource availability, relative diversity effects were obtained by dividing the absolute values of NE, CE, SE by the average monoculture biomass production per resource treatment combination (= NE_{Rel}, CE_{Rel} and SE_{Rel} respectively).

Functional trait composition of the mixtures

To evaluate if the complementary and selection effects could be explained by the functional trait composition of the mixtures, we selected pairs of above- and belowground traits, which are known to be related to resource uptake above- and belowground (specific leaf area = SLA, specific root length = SRL), to plant stature (maximum stretched shoot length = H_{max}, weighted mean depth of standing root biomass = WMD) and nitrogen acquisition and use (leaf nitrogen concentration = LNC, root nitrogen concentration = RNC). To account for environment-induced trait variation, data obtained in each of the studied “environments”, i.e. resource treatment combinations, were used for the characterisation of functional trait composition.

Species-specific traits for root characteristics were taken from monocultures, while values for aboveground traits were averaged for each species across all communities per "environment", see Supporting Information (File S1) for details on trait measurements.

Rao's quadratic entropy diversity index Rao's Q (Rao 1982), which quantifies trait diversity as the sum of pairwise distances between species weighted by their relative abundance, was assessed separately for each of the chosen six traits using the R package *FD* (Laliberté and Legendre 2010)

$$FD_Q = \sum_{i=1}^{S}\sum_{j=1}^{S} p_i p_j d_{ij} \qquad \text{(eqn. 6)},$$

where S is the number of species in the community, p_i and p_j are the relative abundances of species i and j , and d_{ij} is the trait distance between species i and j in the community.

Community weighted means of trait values were computed as

$$CWM = \sum_{i=1}^{S} p_i t_i \qquad \text{(eqn. 7)},$$

where S is the number of species in the community, p_i is the relative abundance of the i-th species in the community and t_i is the trait value of species i (Garnier et al. 2004). Species proportions in aboveground biomass were used as relative abundances for the calculation of both measures.

Statistical analyses

All statistical analyses were performed with the statistical software R 3.1.3 (R Core Team 2015). Linear mixed-effects models (package *lme4*; Bates et al. 2014) were applied to analyse the effects of the experimental factors on the measured variables. For community-level analyses the random structure of the models included independent terms for block and community composition (= mixture identity). Starting from a constant null model, the fixed effects were added in the following order: shade (two factor levels: no shade, shade), fertilization (two factor levels: no fertilization, fertilization), species richness (SR; log-linear term), functional group composition (FG; three factor levels: pure grass communities, pure forb communities, grass-forb communities), growth stature composition (GS; three factor levels: pure small species communities, pure tall species communities, small/tall-species communities) followed by all possible two-way and three-way interactions. In order to evaluate the statistical significance of model improvement by sequential adding of fixed effects, the maximum likelihood method and likelihood ratio tests were applied. A likewise approach was applied to species-level data (relative yields, species biomass). Here, the structure of random terms consisted of block, plot nested in block, mixture identity and species identity. Additionally, terms for the functional composition of the mixtures were replaced by terms for functional group identity (FG-ID) and growth stature identity (GS-ID) and the respective interactions. Species biomass and relative yields were multiplied by the number of species in the mixtures before statistical analyses to account for decreasing sowing proportions with increasing species richness. The *difflsmeans* function (package *lmerTest*; Kuznetsova et al. 2014) was applied to identify significant differences between communities of different functional composition (growth stature and functional group composition) in community-level analyses and among species in species-level analyses in post-hoc tests of the respective models analysing the differences between least squares.

In order to assess which functional trait characteristics best accounted for variation in CE_{Rel} and SE_{Rel}, multimodel inference (package *MuMIn*; Barton 2015) was applied entering all measures of trait composition as predictor variables into a global model with block as random term. The most appropriate combination of predictor variables was automatically selected by comparing Akaike information criteria (AIC) of models with all possible combinations of fixed effects, where the number of fixed effects was restricted to a maximum of three to avoid overfitting. To account for model selection uncertainty, 95%-confidence intervals for coefficients associated with predictors included in models with very similar AIC were estimated by averaging over models with delta < 4 (the contribution of each potential model to the averaged parameter estimates is proportional to the Akaike model weight). The relative importance of predictors was calculated as the sum of Akaike model weights of models where the respective predictors appear (Burnham&Anderson 2002). To fulfil the assumptions of normal distribution, data were transformed to their natural logarithms if necessary.

Results

Community and species biomass production

Shading had marginally negative effects on aboveground biomass production, while fertilization increased aboveground community biomass (Table 2). Positive effects of fertilization on community biomass production were abated under shading (Fig. 1). Unshaded fertilized communities produced significantly more biomass (mean ± SD: 915 ± 239 g m^{-2}) than shaded fertilized communities (511 ± 233 g m^{-2}), and unfertilized communities with shading (490 ± 169 g m^{-2}) and without shading (443 ± 202 g m^{-2}).

Increasing species richness only marginally increased aboveground community biomass. While aboveground biomass production did not change dependent on functional group composition, communities comprising only small-statured species produced less biomass than mixtures of only tall-statured species, while mixtures of both growth statures showed intermediate productivity.

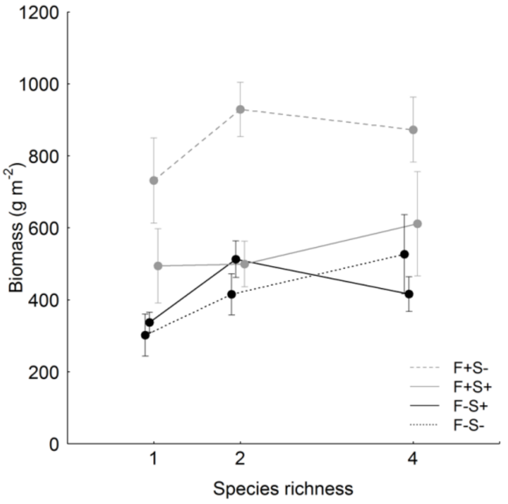

Figure 1: Effects of resource availability on aboveground biomass production in communities with different species richness. Values are means (± 1 SE) per resource treatment and species-richness level. Values are staggered along the x-axis for enhanced clarity. Treatments manipulating resource availability are abbreviated with: F-S- = no fertilization, no shading, F-S+ = no fertilization, shading, F+S- = fertilization, no shading, and F+S+ = fertilization, shading.

Table 2: Summary of linear mixed effects models for community aboveground biomass (BM_{com}), relative yield totals (RYT) and relative biodiversity effects

	BM_{com}		**D_{max}**		**RYT**		**NE_{Rel}**		**CE_{Rel}**		**SE_{Rel}**	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Shade	3.800	0.051	0.010	0.922	1.330	0.249	0.64	0.424	1.031	0.31	0.047	0.828
Fertilizer	**27.831**	**<0.001**	**7.955**	**0.005**	**7.470**	**0.006**	**5.731**	**0.017**	**7.627**	**0.006**	<0.001	0.998
Shade x Fertilizer	**25.009**	**<0.001**	**5.023**	**0.025**	1.400	0.237	0.447	0.504	0.287	0.592	0.281	0.596
SR	3.564	0.059	1.590	0.207	0.036	0.850	0.172	0.679	0.187	0.665	0.819	0.365
FG	0.843	0.656	1.165	0.558	0.192	0.909	0.279	0.87	0.562	0.755	0.050	0.975
GS	**12.476**	**0.002**	0.070	0.966	1.156	0.561	0.449	0.799	2.941	0.230	3.580	0.167
SR x Shade	0.940	0.332	0.002	0.961	0.083	0.773	0.402	0.526	0.087	0.768	0.963	0.326
SR x Fertilizer	1.055	0.304	0.112	0.738	0.200	0.655	0.014	0.904	0.353	0.552	0.938	0.333
Shade x Fertilizer x SR	0.549	0.459	2.319	0.128	1.934	0.164	3.053	0.081	1.671	0.196	2.913	0.088
FG x Shade	2.280	0.320	2.730	0.255	5.871	0.053	2.468	0.291	5.240	0.073	1.353	0.508
FG x Fertilizer	3.702	0.157	1.479	0.477	0.356	0.837	1.155	0.561	0.440	0.802	1.612	0.447
Shade x Fertilizer x FG	0.547	0.761	0.022	0.989	0.369	0.831	0.011	0.995	0.207	0.902	2.393	0.302
GS x Shade	2.455	0.293	2.628	0.269	1.362	0.506	1.078	0.583	1.724	0.422	1.080	0.583
GS x Fertilizer	0.715	0.699	3.708	0.157	2.340	0.310	3.783	0.151	3.505	0.173	3.024	0.220
Shade x Fertilizer x GS	**11.268**	**0.004**	4.125	0.127	4.921	0.085	5.844	0.054	**7.732**	**0.021**	2.266	0.322

Models were fitted by stepwise inclusion of fixed effects. Likelihood ratio tests (χ^2) were applied to assess model improvement and the statistical significance of the explanatory terms (P values, significant values in bold). Abbreviations are: FG = functional group composition (3 factor levels: pure grass communities, grass-forb mixtures, or pure forb communities), GS = growth stature composition (3 factor levels: pure tall-statured species communities, mixtures of both growth statures or pure small-statured species communities), SR = species richness (log-linear), NE_{Rel} = relative net diversity effect, CE_{Rel} = relative complementarity effect and SE_{Rel} = relative selection effect.

The combination of fertilization and shading affected community biomass production differentially, depending on growth stature composition. Communities comprising tall species, especially mixtures with only tall species, were more productive in fertilized plots and were less negatively influenced by shading than communities with only small species and mixtures of tall and small species. Shading led to a lower biomass production of individual species, whereas species-level aboveground biomass in fertilized communities was significantly higher than without fertilization (Table 3). Shade reduced the positive effect of nutrient addition (significant interaction Shade × Fertilizer, Table 3). The biomass of grasses and forbs did not differ significantly, but tall-statured species produced more biomass than small-statured species (Fig.

2a). The effects of resource availability on species-level biomass production varied between functional groups and growth statures (Table 3).

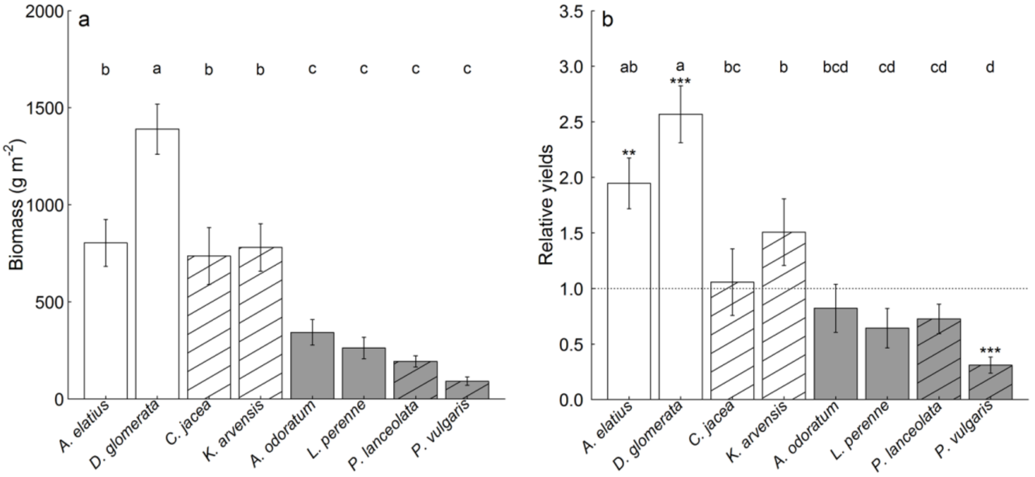

Figure 2: Species-level (a) biomass production, and (b) relative yields (RYs). Shown are means (± 1 SE) across communities of different species richness and grown at varying resource availability. The threshold for greater biomass production of a species in the mixtures than expected from its monoculture (RYT > 1) is indicated with a dotted line in (b). Results of overall tests for RY ≠ 1 for each species are indicated with * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$. Different letters indicate significant differences among species in their biomass and RYs. Hatched bars = forbs, open bars = grasses; filled bars = small-statured species, unfilled bars = tall-statured species.

Whereas grasses produced more biomass with fertilization in full light than without fertilization, the effects of fertilization on biomass production of grasses were minor under shading. Fertilization did not increase biomass production of forbs, but forbs were less productive under shading (Fig. S1a). Aboveground biomass of small-statured species did not differ among resource treatments. Tall-statured species produced more biomass in full light with fertilization than without, while this positive effect of fertilization on biomass production of tall-statured species did not occur under shading (Fig. S1b).

Table 3: Summary of linear mixed effects models for aboveground biomass across all communities (BM$_{spec}$) and relative yields of single species in mixtures (RY)

	BM$_{spec}$		**RY**	
	χ^2	P	χ^2	P
Shade	**4.245**	**0.039**	**4.431**	**0.035**
Fertilizer	**8.901**	**0.003**	**7.699**	**0.006**
Shade x Fertilizer	**6.937**	**0.008**	1.837	0.175
SR	1.769	0.184	0.416	0.519
FG-ID	0.796	0.372	1.553	0.213
GS-ID	**14.397**	**<0.001**	**9.049**	**0.003**
FG-ID x GS-ID	0.669	0.414	**5.506**	**0.019**
SR x Shade	0.019	0.889	0.151	0.698
SR x Fertilizer	1.541	0.214	0.021	0.885
Shade x Fertilizer x SR	0.535	0.464	0.464	0.496
FG-ID x Shade	**6.882**	**0.009**	**16.684**	**0.000**
FG-ID x Fertilizer	**8.110**	**0.004**	**6.058**	**0.014**
Shade x Fertilizer x FG-ID	2.483	0.115	1.476	0.224
GS-ID x Shade	**11.736**	**0.001**	**4.099**	**0.043**
GS-ID x Fertilizer	**4.875**	**0.027**	0.164	0.686
Shade x Fertilizer x GS-ID	**5.845**	**0.016**	2.697	0.101

Models were fitted by stepwise inclusion of fixed effects. Likelihood ratio tests (χ^2) were applied to assess model improvement and the statistical significance of the explanatory terms (P values, significant values in bold). Abbreviations are: FG-ID = functional group identity (2 factor levels: forbs or grasses, GS-ID = growth stature identity (2 factor levels: tall-statured or small-statured species) and SR = species richness (log-linear).

Species relative yields

Species relative yields (RYs) were higher without fertilization and in full light than with fertilization or under shading, yet not different between two-species and four-species mixtures (Table 3). Functional groups did not differ in RYs. The RYs of small-statured species were smaller than those of tall-statured species, but the differences between the RYs of tall- and small-statured species varied between functional groups (significant interaction FG-ID × GS-ID, Table 3). Tall grasses (*A. elatius*, *D. glomerata*) had significantly higher biomass in mixtures than expected from their monocultures (RY > 1), while the RYs of forbs and small grasses either did not differ from expected values or were even smaller than expected in the case of *P. vulgaris* (Fig. 2b). The effects of resource availability on RYs also differed between functional groups (Table 3). Species relative yields of forbs were lower in shaded compared to unshaded communities, whereas grass species attained higher relative yields under shading compared to full light conditions (Table 3, Fig. S1c). While fertilization did not affect RYs of grass species, fertilization lowered RYs of forb species. Differences in the RYs between tall- and small-statured species were smaller in shaded communities (Table 3, Fig. S1d).

Non-transgressive and transgressive overyielding

In total, 67% of the mixtures (N=64) showed non-transgressive overyielding (i.e. RYT > 1). Fertilization had negative effects on relative yield totals (Table 2, Fig. 3a). On average, the RYTs were > 1 without fertilization while the RYTs were not significantly different from 1 across fertilized communities. Irrespective of resource availability, RYTs did not differ depending on species richness, functional group or growth stature composition (Table 2).

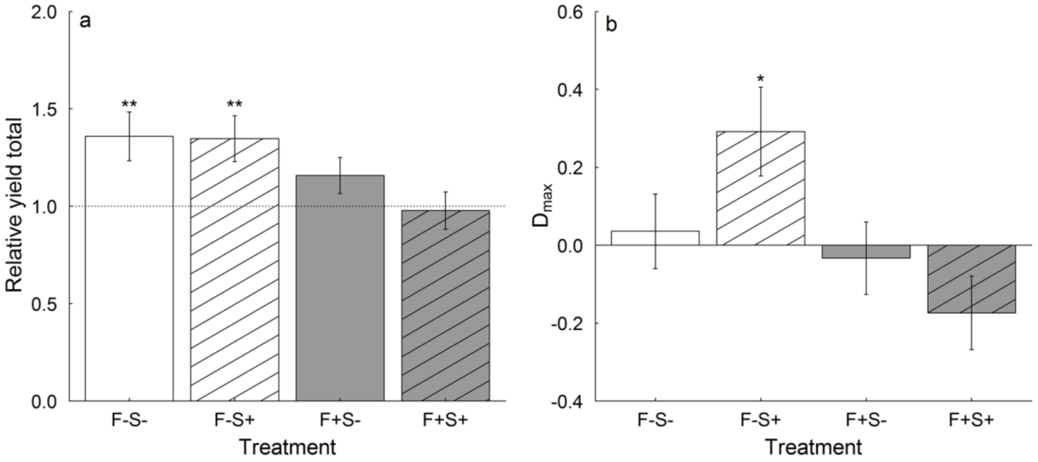

Figure 3: Relative yield totals (RYT) (a), and D_{max} (b). Shown are means (± 1 SE) across two- and four-species mixtures per resource treatment. Treatments manipulating resource availability are abbreviated with: F-S- = no fertilization, no shading, F-S+ = no fertilization, shading, F+S- = fertilization, no shading, and F+S+ = fertilization, shading (unfilled bars = no shading, filled bars = shading, open bars = no fertilization, hatched bars = fertilization). Results of tests for overall means of RYT ≠ 1 and D_{max} ≠ 0, respectively, for each resource treatment are indicated for different levels of significance with * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$.

Only 44% of mixtures displayed transgressive overyielding ($D_{max} > 0$). Transgressive overyielding also varied with resource availability (Table 2). On average, mixture productivity exceeded the most productive monoculture in the shade without fertilization ($D_{max} > 0$), while this was not the case in the other resource treatment combinations. D_{max} did neither depend on species richness, nor functional group- and growth-stature composition.

Net diversity, complementarity and selection effects

Resource availability, sown species richness, functional group or growth stature composition did not significantly affect NE (Table S2). (test for overall mean > 0; $p < 0.001$; Fig. 4a) and amounted to 367 +128 (± 213) g m^{-2}, which was attributable to similar levels of positive complementarity effects (69 ± 193 g $m-^{2}$) and positive selection effects (58 ± 112 g m^{-2}) (Fig. 4c, e). Due to the higher total biomass production of fertilized communities, the biomass gain in mixtures compared to the monocultures was 17% under fertilization and reached 45% without fertilization. Consequently, the relative net effects (NE_{Rel}) correcting for absolute differences in productivity-levels dependent on resource availability were larger without fertilization (Fig. 4b, Table 2). The greater NE_{Rel} without fertilization were attributable to larger positive relative complementarity effects (CE_{Rel}) in unfertilized compared to fertilized mixtures (Fig. 4d). In contrast, relative selection effects (SE_{Rel}) did not change dependent on resource availability (Fig 4f, Table 2). The NE_{Rel} and SE_{Rel} did not differ between two- and four-species mixtures or dependent on functional group and growth stature composition. The effect of fertilization and shading on CE_{Rel} varied depending on growth stature composition: in unfertilized unshaded communities of only tall species, complementarity effects were larger than in communities with other growth stature combinations, but with shading and fertilization, these differences decreased.

Relationships between functional trait composition and complementarity and selection effects

Relative complementarity effects (CE_{Rel}) decreased with increasing community means in root nitrogen concentrations (negative correlation with CWM_{RNC}) and increasing diversity in leaf nitrogen concentrations and specific leaf area (negative correlation with FD_{LNC} and FD_{SLA}, Fig. 5). Combined analyses of all predictor variables showed that CWM_{RNC}, CWM_{SRL} and FD_{LNC} (all negative effects) in combination best explained variation in CE_{Rel}, whereby the relative importance of CWM_{RNC} and FD_{LNC} was greater than that of CWM_{SRL} (Table S3 and S4). Relative selection effects (SE_{rel}) increased with decreasing diversity in leaf nitrogen concentrations and specific root length (negative correlation with FD_{LNC} and FD_{SRL}).

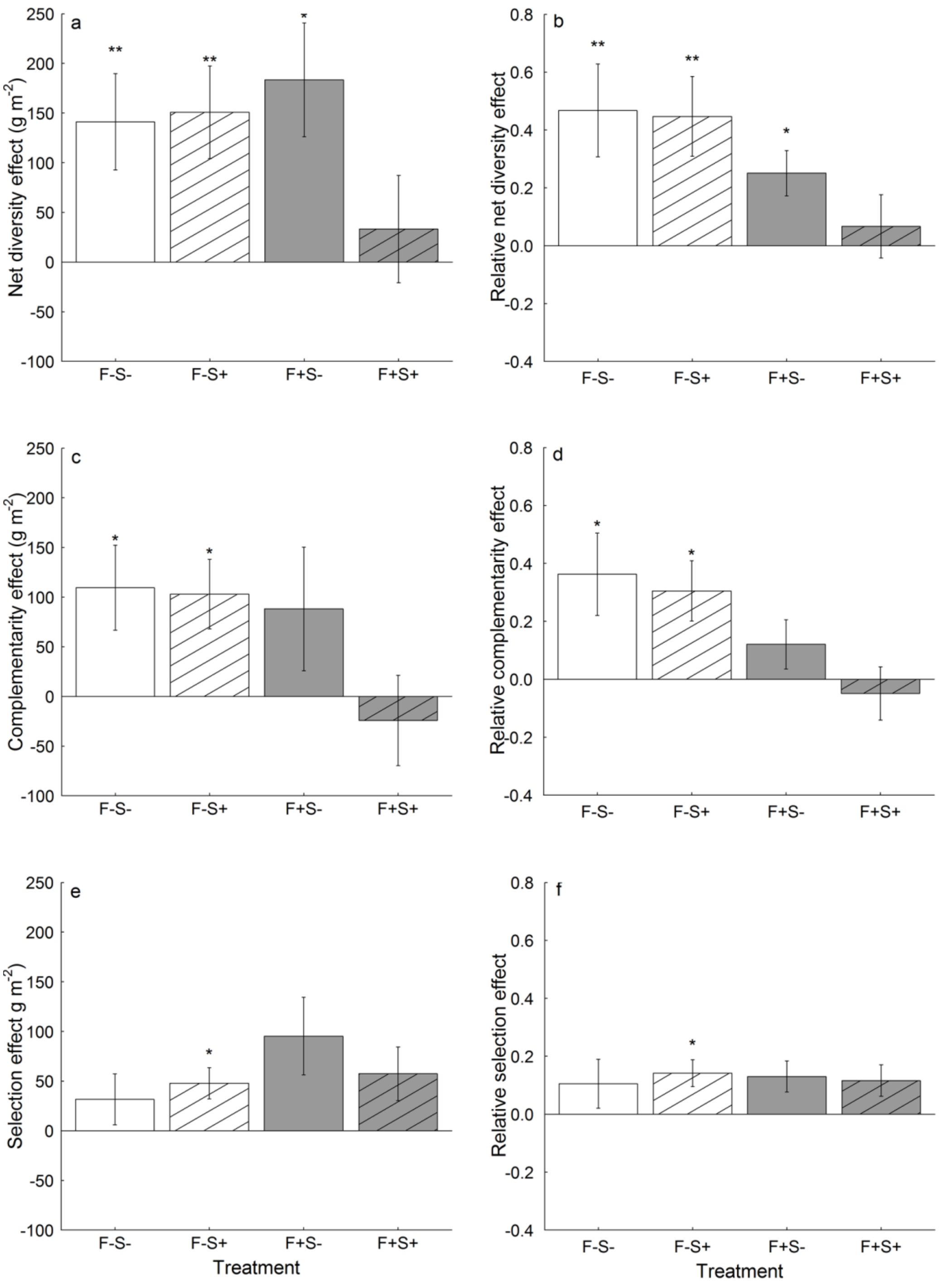

Figure 4: Net diversity effects (NE) (a), relative net diversity effects (NE$_{Rel}$) (b), complementarity effects (CE) (c), relative complementarity effects (CE$_{Rel}$) (d), selection effects (SE) (e) and relative selection effects (SE$_{Rel}$) (f). Shown are means (± 1 SE) across two- and four-species mixtures per resource treatment. Treatments manipulating resource availability are abbreviated with: F-S- = no fertilization, no shading, F-S+ = no fertilization, shading, F+S- = fertilization, no shading, and F+S+ = fertilization, shading (unfilled bars = no shading, filled bars = shading, open bars = no fertilization, hatched bars = fertilization). Results of tests for overall means of diversity effects ≠ 0 for each resource treatment are indicated for different levels of significance with * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$.

In the combined analyses with all predictors, FD$_{LNC}$ was the best predictor variable for SE$_{Rel}$ while FD$_{SRL}$ and CWM$_{WMD}$ (i.e. community means in root mean depth) were of lower explanatory power (Table S4).

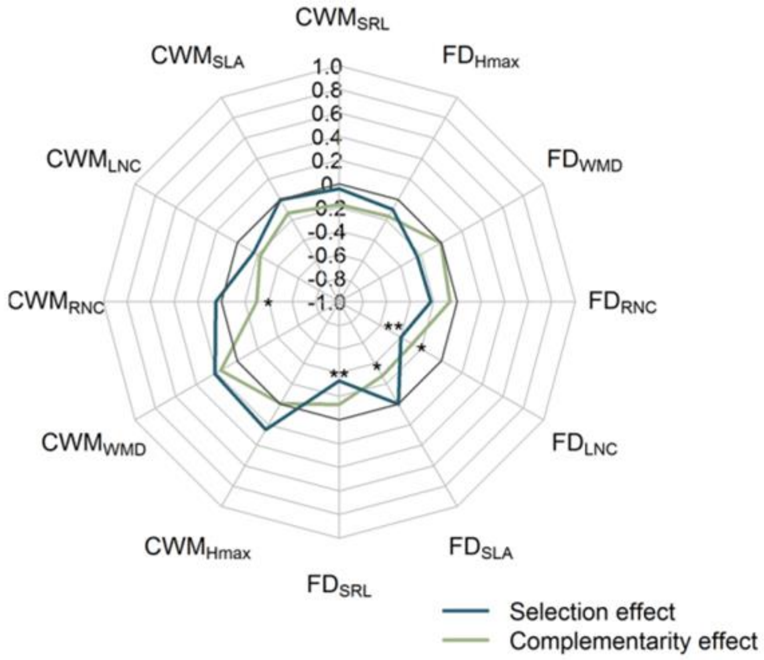

Figure 5: Pearson correlations between relative complementarity effects (CE_{Rel}) and relative selection effects (SE_{Rel}) and trait diversity (FDQ) and community mean traits (CWM) for single traits across different resource treatments. The centroid in each plot indicates perfect negative correlations (r = -1), while the outer margin indicates perfect positive correlation (r = +1). Significant correlations are marked with * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$. Abbreviations for traits are: Hmax = shoot length, WMD = weighted mean depth of root biomass distribution, RNC = root nitrogen concentration, LNC = leaf nitrogen concentration, SLA = specific leaf area, SRL = specific root length.

Discussion

Positive diversity-productivity relationships have been observed in many grassland diversity experiments (e.g. Spehn et al. 2005; Fargione et al. 2007; Marquard et al. 2009). Legume presence has been repeatedly reported as main factor increasing biomass production via facilitation (HilleRisLambers et al. 2004; Marquard et al. 2009), but increased mixture productivity has also been observed in biodiversity experiments excluding legumes (van Ruijven and Berendse 2003). This is in line with our results showing that mixtures were on average more productive than expected from monocultures (RYT > 1). However, increasing species richness had only marginally positive effects on aboveground biomass production, diversity effects did not increase from the two- to the four-species mixtures and their extent varied with the availability of soil resources.

How does resource availability alter overyielding and diversity effects in grass-forb mixtures?

Although total productivity levels increased with fertilization in our experiment, the relative biomass increase in the mixtures compared to the monocultures was greater without fertilization as indicated by larger RYT (Fig. 3a), NE_{Rel} (Fig. 4b) and CE_{Rel} (Fig. 4d). These results concur with our expectations that relative complementarity effects decrease with increasing nutrient availability due to released competition for soil resources. In contrast to our results, fertilization did not affect CE in another experiment, while Lanta and Lepš (2007) also observed lower CE under fertilization. It has been suggested that diversity effects due to the complementary use of soil resources might only be visible if a certain level of nutrient availability is achieved because there exist a limited number of possible strategies for resource capture and use at low nutrient availability (He et al. 2002). Conversely, it is also possible that a complementary use of soil resources becomes dispensable, when nutrients are available at excess. For example, reduced complementarity effects have been found at very high levels of fertilization (≥ 400 kg N ha^{-1} yr^{-1}; Lanta and Lepš 2007; Nyfeler et al. 2009). In contrast, the amount of added fertilizer in our experiment was more similar to the study by Wacker et al. (2009; 80, 160 and 240 kg N ha^{-1} yr^{-1}), who did not find effects of fertilization on CE, but the chernozem at our experimental site represents a nutrient rich substrate, where fertilization could result in nutrient excess.

Contrary to our expectations, selection effects (SE) did not increase with fertilization, while other experiments have shown positive effects of fertilization on SE (Lanta and Lepš 2007, Wacker et al. 2009). Increased aboveground biomass production due to fertilization is likely to enhance competition for light by increasing plant height and/or density and possibly result in larger selection effects due to size-asymmetric competitive advantages of tall growing, highly productive species (Lepš 1999). Unexpectedly, we found that the selection effects (SE and SE_{Rel}) were positive across all mixtures, when unfertilized communities were shaded (Fig. 4e-f). As D_{max} correlated positively with SE_{Rel} ($r = 0.314$, $p = 0.015$; $N = 64$) in our experiment, we also found that most mixtures in this treatment showed transgressive overyielding. One possible explanation for the frequent transgressive overyielding in this treatment is that the difference between the biomass of the most productive and the average monoculture was small (14% compared to appr. 40% in the other treatments), which increases the probability that mixtures with non-transgressive overyielding also achieve transgressive overyielding (Nyfeler et al. 2009). The reason for the smaller yield differences among the monocultures in this treatment might be that carbon limitation through shading limited nutrient uptake, while in the mixtures complementarity in nutrient acquisition between different species was still large enough to cause transgressive overyielding.

Do the effects of resource availability on diversity effects depend on functional groups or growth stature composition?

In contrast to our expectations, we did not detect a dependency of complementarity and selection effects on functional group or growth stature composition although the RYs of individual species varied dependent on growth stature and functional group identity in different resource treatments (Table 3). It has been shown in several biodiversity experiments that complementarity effects depend on functional group (Spehn et al. 2005; Lanta and Lepš 2007; Marquard et al. 2009) or growth stature composition (Marquard et al. 2009). Mostly, the effects of functional group composition were attributable to the inclusion of N_2 fixing legumes, which are well known to facilitate the growth of neighbouring non-legumes. In our experiment, in particular the tall-statured grasses *A. elatius* and *D. glomerata* reached RY > 1, i.e. their performance in the mixtures was larger than expected from their monocultures. These species also reached the highest biomass production in absolute terms. The same two tall-statured grass species have been shown previously as highly productive both in monocultures and mixtures (Roscher et al. 2007, 2011).

In contrast to the tall-statured grass species, the tall-statured and highly productive forbs *K. arvensis* and *C. jacea* did not achieve RY > 1 in the mixtures. Similar results were obtained for *C. jacea* in another biodiversity experiments without legumes (van Ruijven and Berendse 2003).

Although the RYs of the small-statured species were on average lower than the RYs of the tall-statured species, their biomass production in the mixtures was on average not lower than expected from their monocultures. Consequently, the presence of the overyielding tall-statured grass species was not related to greater selection effects, which would require that their biomass gain is at expense of other less productive species. Obviously, the small-statured species included in our experimental species pool were able to compensate for greater canopy shade and reduced light supply in the presence of tall-statured species. Thus, we also could not confirm our expectations of higher selection effects in mixtures of different growth statures due to asymmetric light competition.

Are there any general relationships between diversity effects and functional trait composition?

Despite the lack of any relationships between complementarity or selection effects and the designed functional group or growth stature composition, we found significant relationships with functional trait composition. Both selection and complementarity effects were best explained by traits related to the acquisition and use of belowground resources.

Small community-weighted means of root nitrogen concentration (CWM_{RNC}) and specific root length (CWM_{SRL}) were important predictors related to large CE_{Rel}. High root nitrogen concentrations and specific root length are indicators for high root respiration (Reich et al. 1998; Tjoelker et al. 2005). Root respiration is supposed to indicate a greater investment into root growth, nutrient uptake and transport, while reducing root carbon storages for the release of energy. Hence, mixtures with high CWM_{RNC} and CWM_{SRL} had supposedly high root respiration rates and larger costs for nutrient uptake, which could explain the smaller extent of complementarity effects. Furthermore, larger CE_{Rel} were associated with a smaller diversity in leaf nitrogen concentrations (FD_{LNC}). Leaf nitrogen concentrations are closely related to photosynthetic capacity (Gaudet and Keddy 1988). Small diversity in LNC led to large complementarity effects, when species showed similar values of LNC and thus had similar prerequisites for photosynthetic capacity and carbon assimilation. Our results are in contrast to findings from another biodiversity experiment (Jena Experiment) showing positive effects of FD_{LNC} on complementarity effects (Roscher et al. 2012). The Jena Experiment includes legumes which had higher leaf nitrogen concentrations than grasses and forbs; thus facilitating effects of legumes caused the positive effects of FD_{LNC} on complementarity effects in the Jena Experiment. Interestingly, small FD_{LNC} were also the most important predictor for higher SE_{Rel} in our study. Small FD_{LNC} were not only possible if a mixture consists of species with similar leaf nitrogen concentrations, but low trait diversity could also be caused by the dominance of particular species. Species, which grow taller and produce more photosynthetically active tissue with high nitrogen concentrations are likely to have a competitive advantage in light acquisition and may cause strong selection effects (Gaudet and Keddy 1988; Lepš 1999; Roscher et al. 2012). A likewise pattern could underlie the observed importance of low FD_{SRL} for large selection effects. Specific root lengths (SRLs) provide information on the belowground resource uptake capabilities and economic aspects of the root systems. A highly abundant species with large SRL values may show high nutrient uptake and productivity if light is not a limiting factor, and thus lead to high SE_{Rel}. Large CWM_{WMD} incorporated in models explaining high SE_{Rel} best, may indicate asymmetry in competition for belowground resources in these mixtures. Consequently, functional trait-based analysis implies that the dominance of tall-growing and deep-rooting species most likely causes positive selection effects irrespective of the traits of the subordinate species in the mixtures or external resource supply.

Conclusions

Our experiment with grass-forb mixtures showed that the complementary use of belowground resources was the strongest determinant of diversity effects, which became more pronounced without fertilization although we started our experiment at nutrient-rich conditions and added a moderate amount of nutrients. Surprisingly, we did not find effects of fertilizer addition on selection effects. These results do not imply that selection effects due to asymmetric light competition did not play a role in our experiment, but at least in the short-term, small-statured species were able to compensate for low-light conditions in mixtures with tall species and did not underyield. Lanta and Lepš (2007) observed that the effects of fertilization on complementarity and selection effects varied strongly in a three-year study. Hence, it is likely that our results obtained in the second year of treatment applications might change throughout time and that selection effects increase with regular fertilization, while complementarity effects become stronger without fertilization and the continuous export of nutrients through mowing.

Acknowledgements

We greatly acknowledge the technical staff of the experimental field station Bad Lauchstädt (UFZ), who helped with the establishment and maintenance of the biodiversity experiment. We thank B. Sawall and An. Siebenkäs for help during biomass harvest and sample processing.

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Supplementary material

Appendix S1: Functional trait measurements

Single shoots of each species were sampled for trait measurements in late May and August 2012, i.e. in the first year of treatment applications, in each plot (for details see Siebenkäs et al. 2015, unpublished results). When only one life stage was available (vegetative or reproductive), five shoots per species were sampled, and when both life stages occurred, four vegetative and four reproductive shoots were selected. Shoots were cut close to ground level, and stored in individual plastic bags in a cooler. In the laboratory, maximum stretched shoot length (H_{max}, cm) of all individuals was measured. The area of a maximum of five fully developed leaves (leaf blades in case of grasses) was determined with a leaf area meter (LI-3100 Area Meter, Li-COR, Lincoln, USA) and specific leaf area (SLA, leaf area per dry weight, m^2 kg^{-1}) was calculated by dividing leaf area by leaf dry mass after drying at 70°C for 48 h.

Following a first mowing in June 2013, i.e. in the second year of treatment applications, three soil cores per plot were sampled using a split-tube sampler (4.8 cm inner diameter; Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands) to a depth of 40 cm in each monoculture. Cores were taken along transects with a minimum of 40 cm distance both to the plot margin and between the samples within the plot. Each soil core was separated into 10 cm depth increments (0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm) and the corresponding layers were pooled plot-wise. Until further processing, samples were stored at -20° C. After thawing, samples were immediately rinsed with tap water over a 0.5 mm sieve. Organic debris and remaining soil particles were removed with tweezers. The clean roots were then scanned in a water filled tray on a flatbed scanner at 800 dpi. If sample volume exceeded tray capacity, representative subsamples were taken and kept separately. Dry mass of all samples was determined after drying at 70°C for 48 h. The Winrhizo® Software (Regent systems Inc., Quebec City, Canada) was used on the scans of root samples to calculate specific root length (SRL, root length per sample biomass, m g^{-1}) for each plot and depth increment. SRL obtained per layer was weighted by root biomass of the respective layer to attain mean values over the whole depth profile. Weighted mean depth (WMD, cm) of vertical root biomass distribution was assessed as

$$WMD = \frac{\sum_{i=1}^{S} MD * BM_i}{BM} \qquad \text{(eqn. 1),}$$

where MD is the mean depth of each layer, BM_i is the root biomass of the respective layer and BM is total root biomass across all layers (Gibson et al. 1987).

For subsequent chemical analyses, leaf samples pooled per species and plot (separately for each life stage and harvest) and root samples pooled per plot were ground to fine powder with a ball mill (Mixer Mill MM2000, Retsch, Haan, Germany). Nitrogen concentrations of leaf (LNC, mg N

g_{leaf}^{-1}) and root (RNC, mg N g_{root}^{-1}) samples were measured with an elemental analyser (Vario EL Element Analyzer, Elementar, Hanau, Germany).

Table S1: Soil chemical properties of the experimental location

Soil properties	**Depth (cm)**	**Range**	**Mean ± SD**
Nitrogen concentration (mg g^{-1})	0-15	2.03 - 2.35	2.18 (± 0.11)
	15-30	2.01 - 2.14	2.08 (± 0.05)
Organic carbon concentration (mg g^{-1})	0-15	23.10 - 25.80	24.50 (± 0.8)
	15-30	22.60 - 23.80	23.30 (± 0.4)
CN ratio	0-15	10.60 - 11.70	11.00 (± 0.3)
	15-30	10.60 - 11.10	10.90 (± 0.2)
Carbonate concentration (%)	0-15	0.40 - 0.60	0.50 (± 0.06)
	15-30	0.47 - 0.70	0.60 (± 0.09)
pH	0-15	6.68 - 7.22	6.93 (± 0.15)
	15-30	6.85 - 7.46	7.12 (± 0.20)
Phosphorus concentration (mg kg^{-1})	0-15	36.70 - 42.30	39.30 (± 2.5)
	15-30	24.30- 45.00	36.40 (± 7.2)
Potassium concentration (mg kg^{-1})	0-15	102.00 - 247.00	151.70 (± 50.8)
	15-30	71.50 -168.00	107.10 (± 37.8)

Before starting the experiment, soil was sampled at three locations within each block (0-30 cm depth, separated into layers of 0-15 cm and 15-30 cm depth) and pooled block-wise. Carbonate concentrations were attained according to Scheibler. Total carbon and nitrogen concentrations were measured with an elemental analyser (Vario EL Element Analyzer, Elementar, Hanau, Germany). Soil organic carbon concentrations were calculated by subtracting anorganic carbon concentrations from total carbon concentrations. The pH values were obtained after suspending the soil with 0.01M $CaCl_2$. Phosphorus concentrations were determined from extracts with double lactate, potassium concentrations were obtained from calcium acetate lactate extracts. Displayed are means across blocks (± 1 SD).

Table S2: Summary of linear mixed effects models for net effects (NE), complementarity effects (CE) and selection effects (SE) according to the additive partitioning method (Loreau and Hector 2001)

	NE		**CE**		**SE**	
Source of variation	χ^2	P	χ^2	P	χ^2	P
Shade	1.789	0.181	1.554	0.213	0.202	0.653
Fertilizer	0.606	0.436	2.513	0.113	3.124	0.077
Shadexfertilizer	2.726	0.099	1.316	0.251	1.075	0.300
SR	0.099	0.753	0.182	0.669	0.470	0.493
FG	0.893	0.640	1.315	0.518	0.096	0.953
GS	0.412	0.814	1.246	0.536	4.168	0.124
SRxshade	0.029	0.864	0.005	0.942	0.267	0.606
SRxfertilizer	0.052	0.820	0.140	0.708	0.226	0.634
ShadexfertilizerxSR	2.915	0.088	1.570	0.210	1.120	0.290
FGxshade	5.128	0.077	5.476	0.065	0.961	0.618
FGxfertilizer	1.604	0.448	1.450	0.484	1.874	0.392
ShadexfertilizerxFG	1.428	0.490	0.331	0.848	0.793	0.673
GSxshade	1.378	0.502	0.646	0.724	2.097	0.350
GSxfertilizer	5.250	0.072	1.914	0.384	2.241	0.326
ShadexfertilizerxGS	**6.800**	**0.033**	5.801	0.055	3.777	0.151

Models were fitted by stepwise inclusion of fixed effects to a constant null model with block and mixture identity as random effects. Likelihood ratio tests (χ^2) were applied to assess model improvement and the statistical significance of the explanatory terms (P values, significant values for $P < 0.05$ in bold). Abbreviations are: FG = functional group composition (3 factor levels: pure grass communities, grass-forb mixtures, or pure forb communities), GS = growth stature composition (3 factor levels: pure tall-statured species communities, mixtures of both growth statures or pure small-statured species communities), SR = species richness

Table S3: Summary of the coefficient estimates of the five best models for all measures of functional trait composition in global models with CE$_{Rel}$ or SE$_{Rel}$ as response variables

CE$_{Rel}$	**Intercept**	**CWM$_{LNC}$**	**CWM$_{RNC}$**	**CWM$_{SLA}$**	**CWM$_{SRL}$**	**CWM$_{WMD}$**	**FD$_{Hmax}$**	**FD$_{LNC}$**	**FD$_{SRL}$**	**df**	**logLik**	**AIC**	**delta**	**weight**
	2.039	-	**-0.483**	-	**-0.205**	-	-	**-0.085**	-	6	-32.314	76.600	0.000	0.058
	0.716	-	**-0.643**	-	-	0.059	-	**-0.065**	-	6	-32.417	76.800	0.210	0.053
	0.808	-	**-0.745**	-	-	0.078	-0.071	-	-	6	-32.654	77.300	0.680	0.042
	1.201	-	**-0.566**	-	-	-	-	**-0.071**	-	5	-33.748	77.500	0.870	0.038
	2.519	-	**-0.578**	-	**-0.236**	-	-0.090	-	-	6	-33.209	78.400	1.790	0.024
SE$_{Rel}$														
	1.091	**-0.529**	-	0.024	-	-	-	**-0.052**	-	6	7.634	-3.267	0.000	0.038
	-0.459	-	-	-	-	**0.033**	-	**-0.040**	**-0.043**	6	7.477	-2.953	0.314	0.033
	-0.403	-	-	-	-	**0.029**	-	**-0.054**	-	5	6.372	-2.745	0.523	0.030
	-0.085	-	-	-	-	-	-	**-0.057**	-	4	5.246	-2.493	0.775	0.026
	-0.093	-	-	-	-	-	-	**-0.045**	**-0.035**	5	5.985	-1.969	1.298	0.020

Block was entered as random effect. Models were automatically selected with the *R* package *MuMIn* (Barton, 2015) by comparing Akaike information criteria (AIC) as differences (delta) for models consisting of possible combinations of a maximum of three fixed effects. The table was restricted to measures of functional trait composition that were included in the respective five best models (reflected by their weight). Abbreviations are: df= degrees of freedom, logLik=log likelihood, CWM = community weighted mean traits and FD = functional diversity, Hmax = plant height, WMD = weighted mean depth of root biomass distribution, RNC = root nitrogen concentration, LNC = leaf nitrogen concentration, SLA = specific leaf area, SRL = specific root length.

Table S4: Standardised parameter estimates for the models of the effects of functional trait composition (CWM = community weighted mean traits and FD = functional diversity) on relative complementarity effects (CE$_{Rel}$) and relative selection effects (SE$_{Rel}$) including estimate, standard error (se), 95% confidence interval (CI) and relative variable importance after full model averaging. Abbreviations for traits are: Hmax = plant height, WMD = weighted mean depth of root biomass distribution, RNC = root nitrogen concentration, LNC = leaf nitrogen concentration, SLA = specific leaf area, SRL = specific root length.

	Functional trait composition	**estimate**	**se**	**95% CI**		**Relative variable importance**
				lower	**upper**	
CE$_{Rel}$	Community weighted mean traits					
	CWM$_{SRL}$	-0.061	0.123	-0.496	0.056	**0.28**
	CWM$_{SLA}$	0.001	0.008	-0.042	0.053	0.10
	CWM$_{LNC}$	-0.083	0.233	-1.156	0.283	0.19
	CWM$_{RNC}$	-0.391	0.355	-1.120	-0.083	**0.65**
	CWM$_{WMD}$	0.015	0.034	-0.022	0.147	0.24
	CWM$_{Hmax}$	0.000	0.001	-0.008	0.004	0.09
	Functional diversity					
	FD$_{SRL}$	-0.003	0.020	-0.147	0.089	0.09
	FD$_{SLA}$	-0.014	0.038	-0.188	0.040	0.18
	FD$_{LNC}$	-0.042	0.046	-0.148	-0.007	**0.54**
	FD$_{RNC}$	-0.001	0.010	-0.077	0.062	0.09
	FD$_{WMD}$	0.002	0.014	-0.065	0.104	0.09
	FD$_{Hmax}$	-0.012	0.032	-0.152	0.030	0.20
SE$_{Rel}$	Community weighted mean traits					
	CWM$_{SRL}$	-0.012	0.039	-0.214	0.066	0.16
	CWM$_{SLA}$	0.002	0.007	-0.020	0.043	0.14
	CWM$_{LNC}$	-0.066	0.168	-0.765	0.211	0.24
	CWM$_{RNC}$	0.010	0.054	-0.179	0.365	0.11
	CWM$_{WMD}$	0.009	0.018	-0.009	0.072	**0.27**
	CWM$_{Hmax}$	0.000	0.001	-0.001	0.005	0.19
	Functional diversity					
	FD$_{SRL}$	-0.015	0.029	-0.115	0.012	**0.30**
	FD$_{SLA}$	0.001	0.010	-0.042	0.071	0.10
	FD$_{LNC}$	-0.043	0.027	-0.091	-0.015	**0.82**
	FD$_{RNC}$	-0.003	0.009	-0.052	0.017	0.15
	FD$_{WMD}$	-0.001	0.008	-0.055	0.029	0.11
	FD$_{Hmax}$	0.002	0.010	-0.036	0.065	0.11

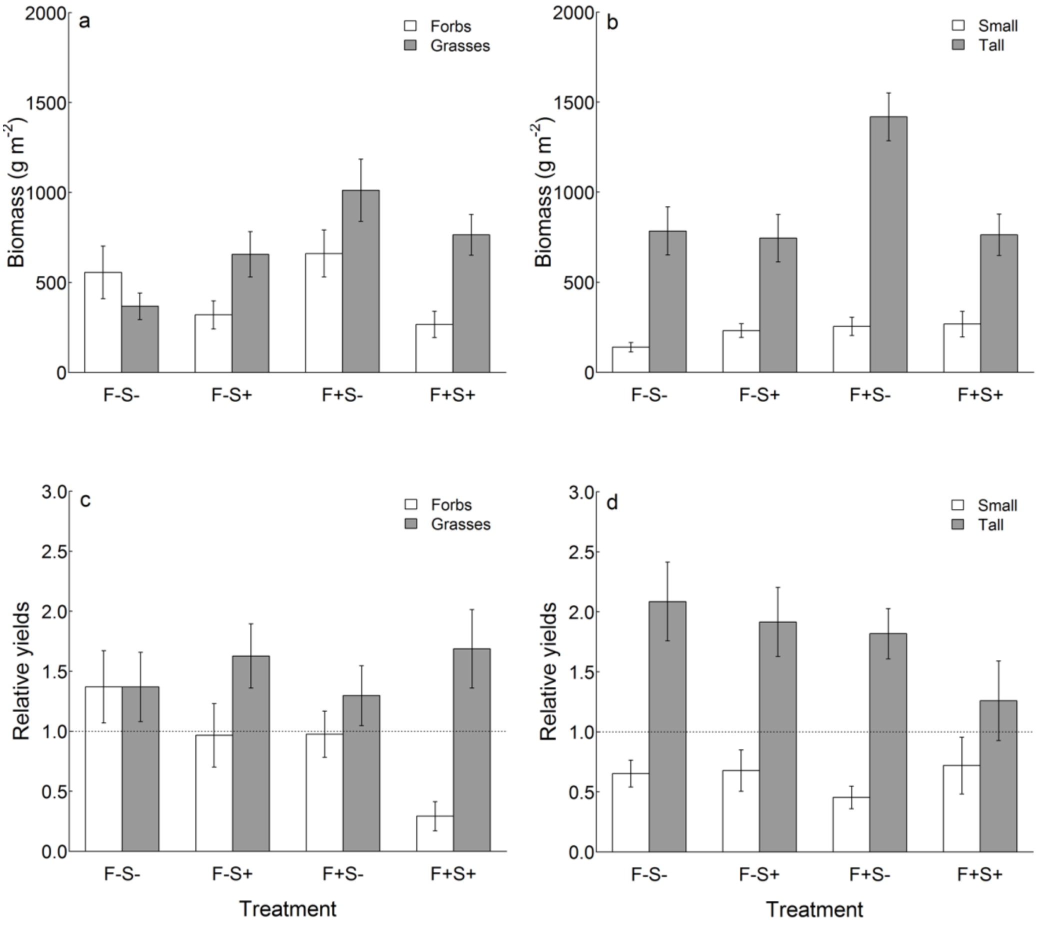


Figure S1: Species biomass production corrected by sowing proportions (i.e. multiplied by the number of species in a mixture) depending on (a) functional group identity and (b) growth stature identity and species relative yields depending on (c) functional group identity and (d) growth stature identity in different resource treatments: F-S- = no fertilization, no shading, F-S+ = no fertilization, shading, F+S- = fertilization, no shading, and F+S+ = fertilization, shading. Values are means (± 1 se) averaged per resource treatment for monocultures, two- and four-species mixtures. Dotted lines represent threshold for larger yields of mixtures than expected from respective species' monocultures.

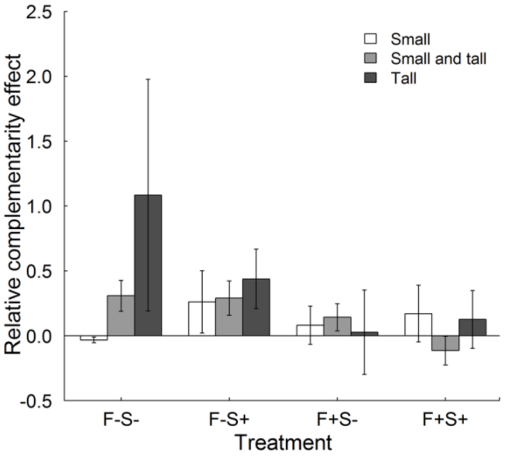


Figure S2: Relative complementarity effects (CE_{rel}) depending on growth stature composition of mixtures and resource availability (F-S- = no fertilization, no shading, F-S+ = no fertilization, shading, F+S- = fertilization, no shading, and F+S+ = fertilization, shading). Values are means (± 1 se) per resource treatment averaged across two- and four-species mixtures.

CHAPTER 6

SYNTHESIS

SYNTHESIS

Despite a large body of research on effects of resource availability on trait variation and diversity-dependent overyielding in plant communities, several aspects have not been addressed so far. This study contributes to the mechanistic understanding of how the effects of variation in resource availability on the magnitude of trait variation in aboveground and belowground traits of different species increase their dissimilarity in traits important for resource acquisition. Furthermore, it sheds light on how changes in resource availability lead to smaller diversity effects and ecosystem functions (as biomass production). This study is novel in imposing several simultaneous treatments, measuring their effect on a large number of traits of individual plants and in a diversity experiment, especially on belowground characteristics, hitherto unstudied in this context, and in elucidating the role that functional trait variation plays for diversity effects and overyielding.

Specifically, this thesis focused on the trait variation of grass and forb species of differing inherent growth statures and dominance in response to differences in light and nutrient supply and species richness to assess how this trait variation induces changes in whole community characteristics, such as community mean trait values, functional dissimilarity, and biomass production. In the experiments, I showed differences between functional groups and small and tall species both as inherent trait value differences and in the magnitude of variation due to resource availability, while species richness effects were generally small and there were mostly no differences in the direction of trait variation.

The experiments underline that the functional composition of communities is more important than species richness *per se* to promote biodiversity effects and insure higher productivity of mixtures opposed to monocultures in grasslands without legumes.

General discussion

How do nutrient and light availability affect the magnitude and the direction of functional trait variation in above- and belowground traits of species belonging to different functional groups (grasses or forbs) and being of different growth statures (tall or small)? Is functional trait variation to nutrient and light availability affected by the species richness of communities?

In the last decade, the significance of functional traits as predictors of ecological functions of communities and their response to environmental variation has been stressed repeatedly (McGill et al. 2006; Fridley et al. 2007; Roscher et al. 2012). An important mechanism of promoting species co-occurrence in diverse communities is thought to be the complementary resource use resulting from trait variation (Ashton et al. 2010). In turn, complementary resource use may enhance biomass production of more diverse communities opposed to monocultures (Tilman et al. 1997). A different magnitude if not even different directions of the trait variation depending on functional group or growth stature identity induced by different levels of resource availability, would serve as basis for the occurrence of complementarity effects or selection effects due to dominant more competitive species.

In chapters 2 and 3 I showed above- and belowground trait variation induced by manipulating light and nutrient availability. The main factors determining trait variation in the greenhouse experiment were either functional group identity (traits related to nutrient acquisition) or shade (SLA and traits related to biomass allocation), although fertilizer effects could be shown on the variation of most traits except for tissue carbon concentrations and few leaf traits (Ch. 2). However, in the field experiment nutrient effects were small (Ch. 3). This can have several causes. Soil at the experimental site is classified in a rather nutrient rich category (a chernozem). Therefore, additional fertilizer might not have shown effects because species were already supplied with sufficient soil nutrients. The same substrate was used for the greenhouse experiment. However, the greenhouse experiment was only conducted for four months, after which many plants showed thorough root distribution throughout the pots and were likely approaching a point of nutrient limitation. This indicates why the detectability of positive fertilization effects was increased. In the field experiment, trait differences due to nutrient effects may take longer to emerge than only one year of treatment application since it would take longer to reach nutrient limitation, especially since belowground biomass and thus nutrient uptake opportunities increase at a slower rate (Mommer et al. 2010; Ravenek et al. 2014). Despite growing in communities instead of single pots, plants did not experience negative belowground competition effects from lower nutrient availability in unfertilized communities.

The other main distinction to the greenhouse experiment was that aboveground trait differences within functional groups, i.e. between the growth statures, were larger than the differences between the functional groups in the field (Ch.3). This was especially apparent when light was a critical factor. Apparently, asymmetric competition additional to experimentally reduced light availability between tall-statured and small-statured species appeared to be the main factor determining trait variation in the field. Although small species were more plastic in adjusting to low light conditions in respect to their leaf morphology and physiology, there was no benefit in it compared to tall-statured species, as reflected in high nitrogen accumulation in stems due to carbon limitation. Tall-statured species were also subjected to the restrictions of reduced light availability in the shading treatment but growing taller than the small statured ones provided them with sufficient light to gain dominance (Ch. 3). This is consistent with previous studies showing similar effects of shading and species richness on the variation of traits associated with light acquisition in small subordinate species (Daßler et al. 2008; Gubsch et al. 2011).

Studies on trait variation in response to differences in resource availability that differentiate between functional groups are surprisingly scarce albeit the accepted notion that functional groups differ from each other. A study that analysed leaf trait variation of grasses and forbs in response to fertilization determined whether trait based ranking was conserved (which it was) but did not test directly for differences in trait variation between the functional groups. However, the authors concluded that leaf dry matter content values of grasses might serve as a suitable indicator for habitat properties because they were less variable according to the ranking (Al Haj Khaled et al. 2005). In a study of root characteristics (diameter, tissue density, specific root length, and root nitrogen concentration) of six grassland forbs and grasses, observed trait variation to nutrient addition was mostly found for one or two of the examined forbs, but was inconsistent between traits (Leuschner et al. 2013). However, neither of these studies examined such a large number of traits of different functional groups in response to manipulation of several resources as done in this thesis.

The greenhouse experiment clearly showed differences in trait values as well as the extent of trait variation between grasses and forbs depending on the trait function (Ch. 2). As could be expected from established knowledge, biomass allocation to above- or belowground organs showed high variation to changes in light and nutrient availability (Poorter and Nagel 2000; Valladares and Niinemets 2008), as well as leaf traits, height and shoot constitution (Ryser and Lambers 1995; Ryser and Eek 2000; Evans and Poorter 2001). However, the magnitude of variation in root morphological traits was similar for grasses and forbs. This shows the overall importance of the ability to adjust to differences in nutrient availability by changing morphological root traits to insure belowground resource acquisition under limiting conditions, despite inherent differences between functional groups.

Furthermore, I assumed that the magnitude of aboveground trait variation in response to fertilization is greater in grasses than in forbs, because their inherent root characteristics (e.g. larger specific root length) promote nutrient acquisition (Reich et al. 1998; Ch.2) and grasses were shown to react with higher increases in aboveground biomass production to nutrient addition (Bowman et al. 1993). One would thus expect grasses to be more exploitative, i.e. show faster nutrient acquisition and growth rate (Chapin 1980; Reich et al. 2003). In my experiments grass species showed conservative characteristics which still resulted in a better performance (Chapters 2, 3 and 5). Overall, grasses invested less in tissue “quality” (e.g. higher carbon concentrations as reflected in leaf dry matter content or tissue density), but in shoot length, root length density and specific root length. This promoted light and nutrient acquisition through advanced space use, supposedly also increased capture of diffuse light due to the leaf angles characteristic of grasses, resulting in higher biomass production (Chapters 2 and 3). Grass species showed also dominant characteristics (Chapter 3) and the tall-statured grasses were the most productive species irrespective of resource availability and species richness (Chapter 5).

Summary: In the greenhouse as well as the field experiments, I could show inherent trait differences between grasses and forbs and tall-statured and small statured species as well as differences in the extent of trait variation as affected by fertilization and shading. The main differences between the two experiments lie in the smaller fertilization effect on trait variation in the field and the greater differences between small-statured and tall-statured species due to asymmetric competition for light in field communities than between grasses and forbs.

Is trait dissimilarity increased by interactive effects of species richness and resource availability and can this be attributed to the functional composition of communities (functional groups, growth statures)?

Environmental conditions select for species with to some extent similar trait values, while plant interactions, mostly as competition, limit species from having too similar trait values (MacArthur and Levins 1967; Weiher and Keddy 1995). Trait dissimilarity is generally assumed to promote complementary use of resources in multi-species communities (Levine and HilleRisLambers 2009). However, predicting the combined effects of fertilization and shading on trait dissimilarity in communities with different functional group and growth stature composition, which thus differ in aboveground and belowground competition, is difficult.

In this thesis, resource availability not only affected trait variation in species *per se* but also led to increased dissimilarity between functional groups and growth statures. However, these effects were not visible across all traits but on all considered levels from individuals to communities. Trait dissimilarity in terms of increased trait differences between functional groups or growth statures was observed for traits related to light acquisition in lower-light conditions both for individuals of grasses and small-statured species (Ch. 2 and 3). On the community-level, shading increased differences in the root depth distribution of different growth statures while fertilization led to larger differences in root length density between functional groups in lower depth (Ch.4). Thus, functionally more diverse communities showed a greater potential to adjust their rooting patterns to varying resource supply and increase the complementary use of resources (Ch. 4). While other studies did not find effects of functional composition on root depth distribution (Ravenek et al. 2014), the effects of functional composition on root depth distribution in this thesis are in line with results from Mueller et al. (2013). However, both studies did not analyse the effects of resource availability on root biomass and root length density distribution.

Dissimilarity between dominant and subordinate species was increased by shading due to larger plasticity of subordinate species in specific leaf area, a trait promoting light acquisition (Ch. 3). This is in line with other studies showing the greater ability of small statured plants to adjust to low light conditions (Roscher et al. 2011a, b). Fertilization increased differences in shoot nitrogen concentrations and biomass allocation into leaves between dominants and subordinates as subordinate species invested more biomass into leaves and accumulated more nitrogen in stems. However, it is not always beneficial to show higher trait variation because it may require a high resource investment and result in limitation of other factors e.g. resistance to herbivores (DeWitt et al. 1998; Valladares et al. 2007). This was also the case in my experiment, despite larger trait variation and the ability to acquire more nitrogen to promote photosynthetic tissue development, subordinate species could not benefit from fertilization as they accumulated surplus nitrogen in stems and suffered from carbon limitation because of limited light availability (Bloom et al. 1985). For community-level trait dissimilarity in tissue nitrogen concentrations, it was however increased nutrient availability, which led to an increase in dissimilarity, while shading had no effect (Ch. 3). Dissimilarity in traits related to light acquisition and stature remained unaffected. Li et al. (2015) also found no fertilizer effects on dissimilarity in specific leaf area and height of many alpine meadow species, concluding that productivity is more dependent on the traits of dominant species than species richness and that functional dissimilarity may remain stable while species richness declines. My study shows that while this applies to some of the investigated traits, it is not consistent for all traits. For example, community trait values for specific leaf area in my experiment were determined by subordinate species, independent of resource availability, opposed to the findings in the other study (Li et al. 2015). This stresses the importance of the choice which specific traits are analysed and of the distinctive features of the investigated species. In several traits, increased richness of functional groups or growth statures resulted in increased dissimilarity (e.g. trait dissimilarity in shoot carbon and nitrogen concentrations due to different growth stature richness and in stomatal conductance due to functional group richness, Ch. 3). Most importantly, differential trait variation of functional groups and growth statures induced by fertilization led to the increased dissimilarity in tissue nitrogen concentrations. The implications of this increased dissimilarity due to different community composition will be discussed in the next section.

Summary: I detected potential for complementary resource use due to environmentally induced functional dissimilarity, especially in mixtures of different growth statures that might lead to greater productivity of plant mixtures opposed to monocultures.

Which impacts do the combined effects of plant diversity and resource availability have on ecosystem functioning, such as above- and belowground biomass production, and diversity effects, and which role does trait dissimilarity play in this context?

Positive effects of diversity on biomass production have been observed both aboveground (e.g. Fornara and Tilman 2008; Marquard et al. 2009) and belowground (Mueller et al. 2013; Cong et al. 2014; Ravenek et al. 2014).

In my experiments, after two years of treatment application, fertilization resulted in a reduction of standing root biomass in shaded communities. Under these combined conditions, plants have easy access to soil nutrients, which can be invested into aboveground biomass to improve the area for light acquisition until carbon limitation restricts further growth both belowground and aboveground. Additionally, the shading treatment, apart from reducing available carbon for investment into root biomass, had additional effects on soil characteristics like soil moisture (mean increase 25%) within blocks. This in turn may affect soil microbial processes and negatively impact root development because sufficient water availability at top soil layers does not require deeper rooting and oxygen reduction due to increased water in soil intercellular space may decrease root respiration. As mentioned earlier for small fertilizer effects on trait variability in the field, it may also take longer time to detect independent nutrient effects on root biomass production when light is not a limiting factor. In the Jena Experiment, the same trend could be observed for species richness effects on aboveground and belowground biomass: aboveground responses were visible several years ahead of belowground responses (Ravenek et al. 2014).

Opposed to the findings for belowground biomass, the impact of resource availability on diversity effects was mainly due to fertilization, which decreased positive complementarity and net diversity effects (Ch. 5). However, selection effects were only positive in unfertilized plots. Mixtures in my experiment were on average more productive than monocultures; almost half of the mixtures showed even transgressive overyielding (Ch.5).

Overyielding and diversity effects did not increase with increasing species richness from two to four species and were not directly dependent on functional group or growth stature compositions of the mixtures. However, other experiments have shown - albeit partly time inconsistent - effects of species and functional group richness on diversity effects, but were mainly due to the facilitative interactions with legumes (Fargione et al. 2007; Lanta and Lepš 2007; Marquard et al. 2009).

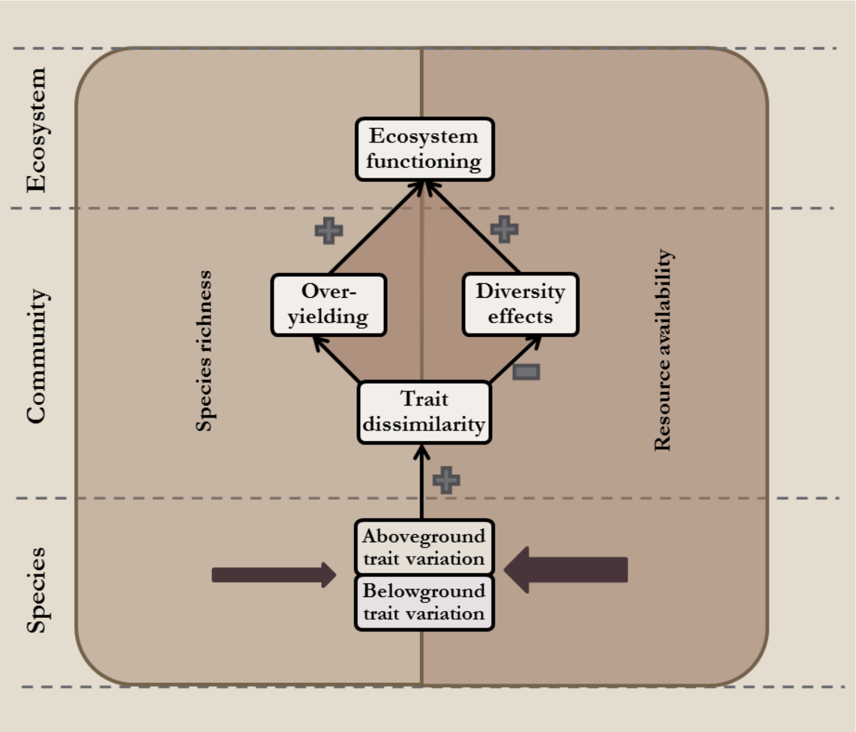
Another possibility of exploring the importance of trait differences due to functional group or growth stature identity for diversity effects is through analysing the relationship between community functional trait composition as community mean traits and functional dissimilarity indices and diversity effects (Roscher et al. 2012; Ch. 5). Since community weighted mean traits reflect traits of dominant species, it would be expected that these trait values promoted selection effects, while increasing functional dissimilarity would lay the basis for complementary effects by implying the possibility of complementary resource use either aboveground or belowground, or both. However, my results did not fully fulfil these expectations (Ch. 5). Instead, complementarity effects were larger when species were more similar in their ability to fix carbon due to higher leaf nitrogen concentrations and supposedly lower root respiration through lower specific root length and root nitrogen concentrations (Ch. 5). Meanwhile, higher selection effects were possibly promoted by dominant species with deep roots and large aboveground resource uptake area with high nitrogen concentrations (Ch. 5).

In short, selection and complementarity effects were best explained by traits related to resource acquisition and use of soil nutrients (Ch. 5). Trait dissimilarity in two of the abovementioned traits (leaf nitrogen concentration, weighted mean depth) was increased by changes in resource availability (Ch. 4). This has important implications. If resource availability leads to larger trait dissimilarity, this may have negative consequences for diversity effects. For example, if fertilization leads to both increased functional dissimilarity and larger mean trait values of leaf nitrogen concentrations, this in turn would lead to a decrease in both complementarity and selection effects. Selection effects would likewise be reduced by an increase in dissimilarity in root weighted mean depth distribution due to shading.

A negative impact of fertilization on complementarity effects could be shown by Lanta and Lepš (2007), but not by Wacker et al. (2009). Chapter 4 discusses in detail possible causes for this observation. Additionally, I propose here a further explanation. Trait divergence in leaf nitrogen concentrations (and possibly also root nitrogen concentrations) due to increased nutrient availability, i.e. a greater gap between species with higher and species with lower concentrations or lower abundance of species with high trait values in the community, results in less effective resource use and decreased productivity, which leads to lower overall community biomass production.

The observations with regard to root depth distribution give an indication in a similar direction of interpretation. In this case, shading, i.e. a reduction in resource availability, resulted in larger dissimilarity between growth statures (Ch. 4), probably due to asymmetric belowground competition promoting selection effects of dominant tall-growing species. However, these results have to be regarded with more carefulness. Former results on vertical root segregation are mixed including not showing any different patterns between functional groups (Ravenek et al. 2014) or actually segregating vertically (Berendse 1982). For the trait-based analyses of correlations with diversity effects in this study, only data from monocultures were used to insure a better comparability to aboveground data with regard to species affiliation. This was because it was impossible to separate roots from root cores down to species level with the given methods (while other working groups by now have developed DNA-based separation methods, see Mommer et al. 2010). Therefore, it could be argued that interactions with other species could have affected trait variation leading to dissimilarities in rooting patterns affecting diversity effects in ways that I could not observe. However, the comparison between mono-functional and bi-functional mixtures implies that species followed somewhat inherent rooting patterns irrespective of the identity of other mixture species.

Summary: Root biomass production was not affected by species richness, but the combined effects of shading and fertilization led to lower standing root biomass. While positive diversity effects and overyielding occurred, there were no differences between two-species and four-species mixtures. Net diversity and complementarity effects were affected by nutrient availability but variation in selection effects had other causes. My analyses demonstrated that traits associated with the acquisition and use of nutrients best explained complementarity and selection effects and could be partly explained by differences in trait dissimilarity due to the varying extent of observed trait variation. However, traits associated with light acquisition could not explain differences in diversity effects. In conclusion, positive diversity effects may occur in mixtures of grasses and forbs irrespective of light availability, but a lower availability of belowground resources increases the possibility of complementary resource use.



Box 3: Brief schematic summary of the effects observed in the studies of this thesis including positive (plus) or negative (minus) relationships, arrow width symbolizing stronger effects of resources

Conclusions and Outlook

How does resource availability modulate diversity effects on ecosystem functioning?

Experimental studies repeatedly showed that not only higher species richness but also functional group richness increases biomass production in grassland ecosystems (Hooper et al. 2005;

Marquard et al. 2009) and that positive effects are inconsistent depending on resource availability (Lanta and Lepš 2007; Wacker et al. 2009). This thesis gives implications through which mechanisms this may occur in grasslands without legumes, whose specific effects are already better studied. Moreover, I show that the magnitude of trait variation among species representing different growth statures, functional groups and differing in dominance may affect their functional dissimilarity under different resource supply, which in turn shapes diversity effects on ecosystem functioning (Box 3). Additionally, opposed to the common assumption that trait values of dominant species have most influence on ecosystem processes, diversity effects were influenced stronger by the large trait variation of subordinate species. In traits that are connected to resource acquisition, a smaller dissimilarity between species with large trait values was more beneficial for complementary resource use than large dissimilarity, which would result in competitive inequalities.

What are the future implications?

Interestingly, in the field experiment, fertilization effects were visible aboveground, whereas most other factors mainly varied due to the impact of shading. I presented some explanation for this. Most importantly, I expect that effects of fertilization and species or functional richness will increase with time, but there are other factors to be taken into account as well. Overyielding has been suggested to be mainly driven by complementary belowground resource use (Cardinale et al. 2007). While facilitative interactions with legumes are well-documented, an alternative explanation for positive effects of species richness has been presented by negative plant-soil feedback mechanisms in monocultures (Bever et al. 1997). This effect is the result of plant roots interacting with their adjacent soil environment, leading to changes in the soil biota (number of pathogens, fungi, herbivores, nematodes), which result in increasingly unfavourable conditions for the plant (Bever et al. 2010). Since plant-soil biota relationship is species-specific (Philippot et al. 2013), species are less negatively affected by other species' soil biota compared to their own (Bever et al. 1997). Thus, a higher number of individuals of the same species may lead to a more hostile environment than communities including different species, thus promoting community productivity (de Kroon et al. 2012). Studies increasingly try to investigate these mechanisms, but due to the cumbersome work related to it, the research field is experiencing a major increase only recently as studies suggest that they are highly important (Maron et al. 2011; Schnitzer et al. 2011; Hendriks et al. 2013). This thesis focused solely on the plant-plant interactions in explaining observed effects, so it would be a future challenge to include analyses of plant-soil feedback mechanisms in the framework of explaining the observed effects of trait dissimilarity on diversity effects and ecosystem functioning.

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APPENDIX

ACKNOWLEDGEMENTS

I particularly want to thank:

- PD Dr. Christiane Roscher for employing me and presenting me with the opportunity of working in this project and the review. Moreover, for her guidance, supervision, inspiration, motivation, perseverance and overall role model function, showing that it is possible to teach, supervise, write papers, proposals, analyse data, and still do tons of field work. Personally. And still have personal time and go on vacations. You are like superwoman.
- Prof. Dr. Helge Bruelheide for the review and his support in my scientific education.
- Prof. Dr. Jan Lepš for agreening to write the external review.
- Birgit for all her assistance during field and lab work, motivation, encouragement, great fun and conversations in the field in the hard times when the bucket started to talk back...
- Den supertollen unverzichtbaren Bad Lauchstädter Frauen & Männern (besonders Olaf, Gerd und Sven) für ihre vielen Ideen und Tips und Hilfe beim Ernten, Sortieren, Mähen, Jäten, Gießen, Eintopfen, Abwaschen und die viele Geduld jedesmal, wenn ich mich territorial arg ausgebreitet habe bzw. dem Wurzelbohrkerne nehmen bei 30 Grad im Schatten, der Perfektionierung der Raushebelvorrichtung und vielen witzigen Dialogen.
- All my Hiwis: Madi, Gaby, Thilo, Benni, Robin, Steffi, Katharina, Thorsten, Alex, Axel, Mandy, Annika, Manuel, Toni, Ramona & Kathrin for all their help and patience with measuring, writing, counting, sorting, washing roots, great conversations and musical entertainment.
- Erik & Gunnar because they set me on this path and I really enjoyed my former times there. Erik for always giving me back my faith (in science) whenever I lost it.
- All my BZF colleagues that are always polite and in a good mood and especially Ellen, who is really our heart and soul.
- Tamar for standing by me in the hard times, for editing and trying to force me to write shorter sentences (obviously failing) and her instant native speaker support for every quick "what sounds more correct/better" question.
- Tommy for being my first friend here and always having an open ear and a fun refreshing perspective on everything.
- The BZF PhDs: the "Party office" for welcoming me and making me feel at home, especially Small P for all the useful tips in R, Lotte and Anja for giving me advice on my introduction and discussion.
- Aga for fruitful discussions and fun on our common adventures. And complaining. Lots of complaining.

- Alexia & Anna for always making me smile and complaining with or to me. Η ζωή είναι δύσκολη.
- Andreas for the moral support in the past years, especially the last weeks and the really enjoyable travels we did and all other great conversations and your optimism, the daily art, graphical advice...
- Nati & Antje, if only there were more people like you in the world, it would be a happy warm place full of love and sunshine. I do not know where you two take it from, you are an inspiration in your optimism and positivity and your ability to always smile and even make the worst day brighter. Thank you for always having an open ear, words of support and "old granny advice".
- A heartfelt thanks to my family for supporting me in all these years and understanding my absence. Especially to my mum, my role model, for helping me in my harvests and everything else.
- The Internationals, for all the amaaaazing conversations and perspectives, dances...; Dracula for fixing my car, bike, computer, being a great friend and comfort; Pepela, for everything you taught me, good or bad, all your experiments, the field trips; you made me grow and see a lot of things in a different light. Tack för allt. Life is complicated. Tomorrow is another day.
- My friends: Christoph, who told me to apply, Dom for the great albeit few times we had during his Master's, Thilo for always being there and working as my Hiwi. Mic because he is always a calm rock in my chaotic life.
- My poster-Doc! For everything, for existing, for being who you are, for being with me, for believing in me, for encouraging me,....for all your love and support.

Andererseits ist echte Wissenschaft oft mit sehr unangenehmer, widerlicher Arbeit verbunden.

~August Bebel~

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Siebenkäs A, Schumacher J, Roscher C (invited for revision) Trait plasticity in response to resource availability and biodiversity modulates functional dissimilarity among species in experimental grasslands. J Plant Ecol

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Other publications by the author

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EIGENSTÄNDIGKEITSERKLÄRUNG

Hiermit erkläre ich, dass die Arbeit mit dem Titel "How resource availability modulates biodiversity effects on ecosystem functioning" bisher weder bei der Naturwissenschaftlichen Fakultät I Biowissenschaften der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion vorgelegt wurde.

Darüber hinaus erkläre ich, dass ich die vorliegende Arbeit eigenständig und ohne fremde Hilfe verfasst sowie keine anderen als die im Text angegebenen Quellen und Hilfsmittel verwendet habe. Textstellen, welche aus verwendeten Werken wörtlich oder inhaltlich übernommen wurden, wurden von mir als solche kenntlich gemacht.

Ich erkläre weiterhin, dass ich mich bisher noch nie um einen Doktorgrad beworben habe.

Halle, den

…………………………………………..

Alrun Siebenkäs