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DOES COMPLEMENTARY RESOURCE USE EXPLAIN BIODIVERSITY-ECOSYSTEM FUNCTIONING RELATIONSHIPS IN GRASSLAND?

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presented by

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Table of contents

Summary

Increasing awareness of species extinction of the Earth's biota has led to a rise in research analyzing the relationship between biodiversity and ecosystem functions. In many studies, ecosystem properties such as primary productivity were often positively affected by high plant diversity. However, a complete understanding of the underlying mechanisms is still lacking. The theory of niche complementarity often proposed to explain positive effects of high plant diversity on ecosystem functioning assumes that plant species differ in their spatial or temporal resource acquisition. Increased complementary resource acquisition might reduce competition among species, leads to a more complete resource use, and eventually results in increased biomass production in high compared to low diverse mixtures. Although key resources important for plant growth, it has been rarely assessed whether complementarity in water and light acquisition among plant species increases with increasing plant diversity.

This thesis comprises three studies aimed at increasing the knowledge on complementary resource use. All studies presented were carried out in the framework of the Jena Experiment, a large grassland biodiversity experiment located in Jena, Germany, with artificially assembled plant communities varying in species and functional group richness of 60 grassland species classified in grasses, legumes, small herbs and tall herbs.

Chapter 1 describes a study in which spatial and temporal complementary water use was investigated with a tracer experiment. Soil water at two different depths was enriched with two different stable water isotopes in 40 communities of varying species and functional group richness. The experiment was repeated three times during 2011 to assess the temporal variations in water uptake. The main water uptake was from the top soil in each community, regardless of species and functional group richness or functional group identity at each of the three measuring campaigns. Thus, these results did not suggest increased complementary water use with increasing plant diversity as explanation for positive effects of high plant diversity on ecosystem properties in temperate grasslands.

The second study (*Chapter 2*) examined the temporal development of light attenuation within the canopy and whether the adjustment of morphological and physiological leaf traits to changing light conditions increases complementary light use. Measurements of vertical light profiles in the canopy of 40 communities, varying in species and functional

group richness, were repeated five times during the 2011 growing season. These measurements showed that light attenuation was highly variable throughout the growing season and increased along the species richness gradient at peak biomass times, but not at the beginning of the growing season nor during regrowth. Leaf traits related to light acquisition were measured in the same communities. Leaf trait expressions varied temporally, but were not affected by species or functional group richness, except for one of the measured traits. Functional groups displayed differences in leaf trait expressions, which varied also temporally. However, the temporal patterns did not reflect the temporal patterns of light attenuation. The results suggest that functional groups differ in their resource use strategies, but do not support the hypothesis that adjustment of leaf traits to changing light conditions along the diversity gradient enhances complementary light use.

Since the effects of plant diversity on carbon fluxes have also been rarely assessed, in a third study (*Chapter 3*) ecosystem carbon and water fluxes of 12 plant communities, containing either four or 16 plant species inserted in individual closed chambers, were continuously measured. High diverse communities displayed higher carbon uptake, water and nitrogen use efficiency as well as apparent quantum yield. Path analyses, exploring the influence of vegetation characteristics and the functional diversity of leaf traits measured in the community, showed that the functional diversity of leaf nitrogen concentration was an important predictor of the ecosystem carbon fluxes. A higher functional diversity in leaf nitrogen concentration implied an optimized distribution of nitrogen within the canopy to increase carbon gain.

Zusammenfassung

Aufgrund der Erkenntnis, dass eine zunehmende Anzahl an Pflanzenarten vom Aussterben bedroht ist, wurden in den letzten 20 Jahren eine Vielzahl an Untersuchungen durchgeführt, um den Einfluss der Artenzahl eines Ökosystems auf verschiedene Ökosystemprozesse zu untersuchen. Häufig hat sich gezeigt, dass eine hohe Anzahl an Arten in einer Pflanzengemeinschaft positive Auswirkungen auf verschiedene Prozesse wie zum Beispiel die Biomasseproduktion hat. Trotz der Vielzahl an durchgeführten Studien konnte der zugrunde liegende Mechanismus dieser positiven Effekte noch nicht eindeutig geklärt werden. Es wird aber angenommen, dass sich verschiedene Arten in ihrer Ressourcenaufnahme komplementär ergänzen und diese Komplementarität mit steigender Artenzahl zunimmt. D.h., Arten unterscheiden sich zum Beispiel in ihrer Wurzeltiefe und nehmen somit Wasser oder Nährstoffe aus verschiedenen Bodentiefen auf. Diese räumliche Trennung der Ressourcenaufnahme reduziert Konkurrenzeffekte zwischen den Arten, erhöht die Ausnutzung der Ressource im Boden und kann somit zu erhöhter Biomasseproduktion beitragen. Die vorliegende Dissertation hatte zum Ziel, Wissenslücken bezüglich der Frage zu schliessen, ob eine gesteigerte komplementäre Ressourcennutzung verantwortlich für die positiven Effekte erhöhter Biodiversität ist. Dafür wurden drei Studien durchgeführt, die den Einfluss der Anzahl an Arten sowie an funktionellen Gruppen auf die Wasser- und Lichtnutzung als auch Kohlenstoffflüsse untersuchten. Alle Studien wurden im Rahmen eines grossen Biodiversitätsexperimentes in Jena (Deutschland) durchgeführt. Dieses Experiment besteht aus Pflanzengemeinschaften, die sich sowohl in ihrer Anzahl an Arten als auch in funktionellen Gruppen unterscheiden. Die Pflanzengemeinschaften wurden zufällig aus einem Pool von 60 in Zentraleuropa typischen Graslandarten aus vier funktionellen Gruppen (Gräser, Leguminosen, kleine Kräuter und grosse Kräuter) zusammengestellt.

Das erste Kapitel dieser Arbeit beschreibt einen Versuch, der durchgeführt wurde, um die komplementäre Wassernutzung in Abhängigkeit der pflanzlichen Diversität zu untersuchen. In einem Tracer-Experiments wurde das Bodenwasser in zwei verschiedenen Tiefen in 40 verschiedenen Pflanzengemeinschaften jeweils mit stabilen Wasserisotopen markiert, um damit die Bodentiefe der pflanzlichen Wasseraufnahme zu identifizieren. Der Versuch wurde dreimal im Laufe des Jahres 2011 wiederholt, um

zusätzlich zu untersuchen, ob sich die komplementäre Wassernutzung zeitlich verändert. Die Ergebnisse dieser Studie konnten zeigen, dass Pflanzen hauptsächlich Wasser aus oberen Bodenschichten aufnehmen. Die Hauptaufnahmetiefe von Wasser hat sich darüber hinaus weder in Abhängigkeit der Anzahl an Arten oder an funktionellen Gruppen in einem Plot verändert, noch wurden Unterschiede zwischen funktionellen Gruppen gefunden. Diese Ergebnisse zeigten, dass eine komplementäre Wassernutzung womöglich keine Rolle für die positiven Effekte einer erhöhten Artenzahl auf Ökosystemprozesse in Grasländern der gemässigten Zone spielt.

Die zweite Studie (Kapitel 2) untersuchte die Änderung der Lichtverfügbarkeit in einem Bestand im Laufe der Vegetationsperiode sowie in Abhängigkeit der Artenzahl. Zusätzlich wurde untersucht, ob sich Blattmerkmale entsprechend der veränderten Lichtbedingungen in einem Bestand anpassen, um so eine bessere bzw. komplementäre Lichtnutzung zu erreichen. Mit Messungen der Lichtintensität konnte gezeigt werden, dass sich die Lichtverfügbarkeit zeitlich stark ändert. Zusätzlich nahm die Lichtverfügbarkeit in Beständen mit hoher Artenzahl stärker ab als mit niedriger. Dieser Effekt wurde nur in Zeiten mit hoher Bestandsbiomasse, jedoch nicht am Anfang der Vegetationsperiode oder während des Wiederaufwuchses nach der Mahd gefunden. Die Ausprägung der gemessen Blattmerkmale war ebenso zeitlich variabel und änderte sich nicht mit zunehmender Artenzahl (mit Ausnahme eines Merkmals). Funktionelle Pflanzengruppen unterschieden sich stark in der Merkmalsausprägung. Einige Pflanzengruppen zeigten auch eine Änderung in einzelnen Blattmerkmalen entlang des Diversitätsgradienten in den untersuchten Beständen. Dies könnte auf eine Anpassung an eine zunehmende Lichtabschwächung mit steigender Artenzahl hindeuten. Darüber hinaus variierte die Merkmalsausprägung der funktionellen Gruppen im Laufe der Vegetationsperiode sehr stark, entsprach aber nicht der zeitlichen Variation der Lichtverfügbarkeit. Die Ergebnisse dieser Studie konnten nicht eindeutig belegen, dass die Anpassung von Blattmerkmalen an Lichtbedingungen, die sowohl zeitlich als auch entlang des Diversitätsgradienten variierten, dazu beiträgt, dass die Ressource Licht in Beständen mit erhöhter Artenzahl besser ausgenutzt wird.

In einer dritten Studie (Kapitel 3) wurde der Effekt der Artenzahl auf die Kohlenstoffaufnahme eines Bestandes untersucht. In 12 Lysimetern, die aus den Flächen in Jena ausgestochen worden waren und dessen Bestände sich aus vier oder 16 Arten

zusammensetzten, wurden Kohlenstoff- und Wasserflüsse in geschlossenen Kammern gemessen. Die Kohlenstoffaufnahme sowie die Wasser-, Stickstoff- und Lichtnutzungseffizienz war in Beständen mit 16 Arten höher als in Beständen mit vier Arten. Des Weiteren konnte gezeigt werden, dass die Variation bzw. die funktionelle Diversität in der Stickstoffkonzentration der Blätter eine mögliche Erklärung für eine erhöhte Kohlenstoffaufnahme und –nutzungseffizienz ist.

General introduction

The Earth's flora is a result of dynamic evolutionary processes and contains currently, among others, approximately 250.000 species of angiosperms, 24.000 species of mosses, 10.000 species of ferns and 800 gymnosperm species (Körner 2013). This *biodiversity* (Box 1) of plants deserves not only protection as a natural heritage, but also because it has many ecological and economical values important for human well-being, such as providing food, medicine and further *ecosystem goods and services*. However, more and more species are at risk of extinction due to human impacts on the environment (Cardinale et al. 2012). Thereby, land-use change, increasing nitrogen deposition or changing atmospheric CO_2 concentration were found to be major drivers of changes in biodiversity (Sala et al. 2000, Reich et al. 2001). Due to the awareness of increasing species extinction, biodiversity research has enormously increased within the last two decades, particularly to estimate the consequences of species loss on *ecosystem functi*oning (Schläpfer and Schmid 1999, Balvanera et al. 2006).

As summarized in several meta-analyses, increasing plant species richness very likely promotes ecosystem functioning (Balvanera et al. 2006, Isbell et al. 2011). For instance, high species richness was associated with increased biomass production, soil carbon storage or pollinator abundance (Quijas et al. 2010, Allan et al. 2013). Furthermore, high diverse mixtures displayed a higher stability, e.g. increased resistance to disturbance events (Tilman 1996, Yachi and Loreau 1999), or were found to be less susceptible for invasion of exotic species (Levine and D'Antonio 1999).

However, the underlying mechanisms for the positive relationships between plant species richness and ecosystem functioning are not well understood yet. Besides the sampling effect, which assumes that the chance for the presence of a species highly influencing a certain *ecosystem property* is increased in high diverse mixtures (Huston 1997), niche complementarity has been suggested to be an important mechanism. The concept of niche complementarity assumes that plant species growing together in a community partition the available resources, e.g. nitrogen or water. This leads to a more complete resource use and reduced competition among species, finally resulting in increased productivity at higher diversity levels (Loreau and Hector 2001). Niche complementarity can occur spatially, temporally as well as in terms of different chemical forms of a nutrient. For

Box 1 Glossary

Biodiversity

Biodiversity (biological diversity, biological richness) comprises in its broadest sense the variety of life, i.e., the genetic variation of organisms, the organismal variation in a community or within an ecosystem, and the variety of ecosystems on the planet. It is often used as surrogate for species richness, whilst in the present study, it is used for plant species richness (Harper and Hawksworth 1994, Hooper et al. 2005).

Ecosystem functioning

Ecosystem functioning is a superordinate concept for the properties, goods and services of an ecosystem (according to Hooper et al. (2005)).

Ecosystem properties

Ecosystem properties are the entity of structural and functional characteristics of an ecosystem. They comprise the pools and fluxes of materials such as carbon, nitrogen and organic matter and can also be considered as ecosystem processes such as productivity, nutrient cycling and decomposition.

Ecosystem services

Ecosystem services are ecosystem properties from which mankind benefit, for instance, provisioning of pure drinking water, climate regulation, pollination, flood regulation and recreation.

Ecosystem goods

Ecosystem goods are separated from ecosystem services as ecosystem properties with direct market values, such as food, construction material, fiber and medicines.

(Schaefer 2003, Hooper et al. 2005, Millenium Ecosystem Assessment 2005, Reiss et al. 2009)

Trait

A trait is any morphological, physiological or phenological feature measurable at the individual level (according to Violle et al. (2007))

Functional diversity

Functional diversity captures the variation of traits within a mixture by assessing the dissimilarity between species in a trait space and is measured with functional diversity indices (Petchey et al. 2004, Petchey and Gaston 2006).

Rao's Q is an often used measure of functional diversity. It is the sum of the pairwise distances between species in a trait matrix, weighted by the abundance of the species and calculated with the following equation:

$$FD_Q = \sum_{i=1}^{N} \sum_{j=1}^{N} d_{ij} p_i p_j,$$

where N is the number of species in the community, d_{ij} is the pairwise distance in trait values of species i and j, p_i and p_j is the proportion of species i and j in the community (Botta-Dukát 2005).

instance, spatial resource partitioning can be achieved due to different rooting depths, while temporal resource partitioning can be achieved by differences in phenology among species (Fargione and Tilman 2005). Complementary resource use in grasslands was tested many times with overyielding experiments that revealed higher aboveground productivity of mixtures than expected from the weighted average aboveground productivity of the containing individual species grown in monoculture (Hector et al. 2002, Roscher et al. 2005, van Ruijven and Berendse 2005). Furthermore, nutrient concentrations of different species mixtures were compared and used as indication for complementary resource use. For instance, nitrate (NO_3^-) and ammonium (NH_4^+) concentrations were found to decrease with increasing species richness, indicating more complete nitrogen use (Tilman et al. 1996, Oelmann et al. 2007). On the other hand, two studies testing complementary nitrogen use in grassland mixtures by using stable nitrogen isotopes did not find evidence for a diverging spatial or temporal separation of nitrogen use among the species in mixture with increasing diversity (Kahmen et al. 2006, von Felten et al. 2009). Concerning water use, Silvertown et al. (1999) showed that species in a diverse community have separate hydrological niches, measured by different soil water parameters such as soil moisture. Caldeira et al. (2001) used $\delta^{13}C$ values as indicators for complementary water use in a Mediterranean grassland. The $\delta^{13}C$ value of leaves reflects the stomatal behavior of a leaf. Neglecting the effect of photosynthetic capacity, decreasing $\delta^{13}C$ values relate to increasing stomatal conductance (Farquhar et al. 1989). In mixtures, species displayed lower $\delta^{13}C$ values than in monocultures, which can indicate a higher water availability due to complementary resource use. Furthermore, De Boeck et al. (2006) found grassland species to have higher water use efficiency (calculated by combining evapotranspiration and biomass measurements) in diverse communities compared to monocultures that might result in higher complementary water use. Increased light capture in more diverse plant communities was indicated by well adapted canopy architectures that enables the plants, namely their leaves, to fill out the space more effectively to intercept as much light as possible (Naeem et al. 1994, Mason et al. 2013). However, evidence for complementary resource use, especially of water and light, as an explanation for increased biomass production are still scarce (Schmid et al. 2002). Furthermore, the aforementioned studies on complementary water and light use have one aspect in common: they rely on indirect measurements. Thus, direct approaches

Box 2 Stable isotopes

Isotopes are different forms of an element, differing in their number of neutrons in the atomic nuclei, but not in their number of protons and electrons, which results in a different mass. Therefore, isotopes display different physical properties, but nearly identical chemical properties. Isotopes with a higher number of neutrons are described as heavy isotopes, while isotopes with less neutrons are called light isotopes. Stable isotopes do not decay radioactively over time. Stable isotopes differ in their natural abundance (as indicated in parentheses in the following). Hydrogen has two stable isotopes, i.e. ^{1}H (99.984 %) and ^{2}H (0.0156 %), oxygen has three stable isotopes, i.e. ^{16}O (99.759 %, ^{17}O (0.037 %) and ^{18}O (0.204 %). Combining these different stable isotopes of hydrogen and oxygen, nine isotopic configurations of water are possible (isotopologues), while the most common water molecules are: $^{1}H_2{}^{16}O$ (99.731 %), $^{1}H^{2}H^{16}O$ (0.0155 %) and $^{1}H_2{}^{18}O$ (0.2005 %), which also differ largely in their natural abundance.

The isotopic signature of a substance is expressed as the ratio of the heavy to the light isotope in relation to a standard material and often given in the δ-notation:

$$\delta^{X}E = \frac{R_{sample}}{R_{standard}} - 1,$$

where E is the element, X gives the mass of the heavier isotope, R_{sample} and $R_{standard}$ are the ratios of the heavy to the light isotope in the sample and the standard, respectively. Since the δ-values are very small, they are commonly expressed in ‰. Water standards used for measurements of the hydrogen and oxygen signatures are V-SMOW (Vienna Standard Mean Ocean Water), SLAP (Standard Light Antarctic Precipitation) and GISP (Greenland Ice Sheet Precipitation). When comparing samples, those with lower δ-values are considered depleted in regard to the heavy isotope, while those with higher δ-values are enriched.

The differences in natural abundance make stable isotopes a useful tool to follow and trace element cycling and to explain ecological processes. For instance, stable water isotopes were widely used to infer plant's water sources. Different soil depths have different isotopic compositions as a result of increased evaporation of the lighter isotopes at shallow soil depths compared to the heavier isotopes, leading to an isotopic profile with depth. Since there is no isotope fractionation (i.e., partitioning of the light and heavy isotopes) during water uptake by plants, the isotopic signal of the plant's xylem water reflects the depth of water uptake. However, when working with stable water isotopes at natural abundance levels, one is much dependent on a clear isotopic profile of soil water, which is not always given. Another approach is to enrich a substance, e.g., the soil water, with heavy isotopes to reveal more unequivocal results and to clearly determine water uptake depth.

References: Dawson et al. (2002), Gat (2010), Coplen (2011)

testing resource use are needed, for instance, by applying stable water isotopes (Box 2) or by relating direct measurements of light availability within the canopy to plant traits associated with light use (e.g. chlorophyll concentration).

An emerging trend within biodiversity research is to assess the relationship of *functional diversity* and ecosystem functioning (Díaz and Cabido 2001, Cadotte 2011). Functional diversity is a measure that quantifies resource use complementarity by calculating the dissimilarity of species in a mixture regarding *traits* associated with resource use (Petchey et al. 2004). A larger functional diversity or greater dissimilarity among plant species should indicate higher variation in resource acquisition (Roscher et al. 2013). Several studies found functional diversity to be the better predictor of ecosystem functioning than species richness (Cadotte et al. 2011, Flynn et al. 2011). However, in order to relate functional diversity and ecosystem functioning, it is not only crucial to decide which traits are important for a certain ecosystem function, but also to know how these traits respond to variable environmental conditions (Petchey and Gaston 2006).

Thesis outline

This thesis aims to increase the knowledge on mechanisms, in particular on complementary water and light use, explaining positive effects of high biodiversity on ecosystem functioning.

All work presented has been carried out in the framework of a large grassland biodiversity experiment in Jena, Germany (Roscher et al. 2004), because only studies in a biodiversity experiment have the potential to test the relationships between plant species richness and ecosystem functioning under constant abiotic conditions, which are otherwise potentially confounding biodiversity effects in observational studies (Schmid and Hector 2004). Furthermore, grasslands are a well suited study system as they are a widespread ecosystem and provide important ecosystem goods and services, e.g., forage production (Balvanera et al. 2006). The Jena Experiment was established in 2002 and focuses on relationships between plant diversity and several aspects of ecosystem functioning, ranging from biomass production, plant-fauna interactions to element cycling. On 82 plots, mixtures with one, two, four, eight, 16 and 60 plant species were established. The mixtures were randomly assembled out of a pool of 60 species, naturally

common in grasslands of Central Europe (*Molinio-Arrenatheretea* plant community). In parallel, these plots cover a gradient of one to four plant functional groups (i.e., grasses, legumes, small herbs and tall herbs).

Three studies were carried out and are described in the following chapters.

Chapter 1 addresses the question if positive effects of high plant species richness on ecosystem functioning can be explained by increased spatial or temporal complementary water use in high diverse mixtures compared to low diverse mixtures. Plant water uptake in 40 grassland mixtures of the Jena Experiment differing in their plant species number was directly tested with a tracer experiment. Therefore, the soil water in each mixture was enriched with two different stable water isotopes in two different soil depths. The experiment was repeated three times during the year to assess the temporal variations in water uptake.

Chapter 2 focuses on light use and the question whether the adjustment of leaf traits to changing light conditions with increasing plant species richness is a mechanistic explanation for increased light exploitation or complementary light use. Several leaf traits related to light acquisition as well light intensity along a vertical profile in the canopy were measured within 40 grassland mixtures of the Jena Experiment, covering a plant species richness gradient. The measurements were replicated five times during the growing season to investigate temporal differences in light use.

Chapter 3 describes a study comparing ecosystem carbon fluxes and parameters of carbon uptake efficiency of low and high diverse plant mixtures and identifying potential drivers for the observed patterns. Monoliths containing either four or 16 plant species were excavated in the Jena Experiment and inserted in individual closed chambers of the Ecotron facility in Montpellier, France. Carbon and water fluxes were continuously measured and their most important predictors were identified, using the functional diversity of the mixtures based on measurements of several plant traits.

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Chapter 1

No evidence of complementary water use along a plant species richness gradient

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Abstract

Niche complementarity in resource use has been proposed as a key mechanism to explain the positive effects of increasing plant species richness on ecosystem processes, in particular on primary productivity. Since hardly any information is available for niche complementarity in water use, we tested the effects of plant diversity on spatial and temporal complementarity in water uptake in experimental grasslands by using stable water isotopes. We hypothesized that water uptake from deeper soil depths increases in more diverse compared to low diverse plant species mixtures.

We labeled soil water in 8 cm (with ^{18}O) and 28 cm depth (with ^{2}H) three times during the 2011 growing season in 40 grassland communities of varying species richness (2, 4, 8 and 16 species) and functional group number and composition (legumes, grasses, tall herbs, small herbs). Stable isotope analyses of xylem and soil water allowed identifying the preferential depth of water uptake.

Higher enrichment in ^{18}O of xylem water than in ^{2}H suggested that the main water uptake was in the upper soil layer. Furthermore, our results revealed no differences in root water uptake among communities with different species richness, different number of functional groups or with time. Thus, our results do not support the hypothesis of increased complementarity in water use in more diverse than in less diverse communities of temperate grassland species.

Introduction

Many results from experimental biodiversity research support the hypothesis that increased plant species richness has positive effects on several aspects of ecosystem functioning (Schläpfer and Schmid 1999, Balvanera et al. 2006, Isbell et al. 2011, Allan et al. 2013), such as plant biomass production aboveground (Hector et al. 1999, Loreau et al. 2001, Tilman et al. 2001, Marquard et al. 2009), whereas the underlying mechanisms for these positive effects are not yet fully understood (Hooper et al. 2005). One frequently proposed explanation is niche complementarity (Tilman et al. 1997b, Loreau and Hector 2001), assuming that partitioning of resources such as light, nutrients or water reduces competitive interactions among the species of a mixture. Consequently, resource exploitation at the community level is more complete, resulting in greater productivity

compared to a monoculture or low diverse mixture. Partitioning of belowground resources might be achieved spatially via different root distribution patterns or temporally because of differences in phenology among species (Berendse 1982, Fargione and Tilman 2005). However, experimental evidence, particularly for the resource water, is still sparse.

Furthermore, the hypothesis of complementary resource use was mainly tested indirectly, for instance by comparing aboveground biomass production in mixtures with values expected from monocultures (Hector et al. 2002, Roscher et al. 2005, van Ruijven and Berendse 2005) or by interpreting a more complete filling of available biotope space, i.e., soil depth and volume, indicated by increased vertical root biomass distribution with increasing species richness as greater complementarity (Dimitrakopoulos and Schmid 2004, von Felten and Schmid 2008, Mueller et al. 2013). In addition, complementary water use was suggested based on increased evapotranspiration rates in plant mixtures with increasing species richness (Verheyen et al. 2008) or based on lower $\delta^{13}C$ values in mixtures compared to monocultures (Caldeira et al. 2001). Although water is an important resource for plant performance, there is, to our knowledge, a lack of direct measurements to assess water partitioning in mixtures and to test complementarity in water use with increasing species richness under field conditions.

Stable water isotopes have often been applied to directly estimate the water source used by plants (e.g., water of different soil depths or even fog) and was used in many studies aiming to explain coexistence of plants in different natural ecosystems (e.g., Ehleringer et al. 1991, Ehleringer and Dawson 1992, Gordon and Rice 1992, Grieu et al. 2001, Nippert and Knapp 2007, Hoekstra et al. 2014). Potential water sources of co-occurring species were identified by comparing the natural abundance of oxygen or hydrogen isotopes in xylem water and soil water of different depths. As no isotopic fractionation occurs during water uptake, the isotopic signal of the xylem water reflects the signal of a plant's water source (White et al. 1985, Dawson et al. 2002). In herbaceous plants, it has been shown that the isotopic signal of xylem water in the root crown was the best indicator of the water source (Barnard et al. 2006). However, natural abundance analyses rely on a pronounced isotopic profile of soil water, which is often not given under field conditions (Allison et al. 1983). More unequivocal results can be obtained by enriching the soil water at different depths with different stable water isotopes (Ogle et al. 2004, Kulmatiski et al. 2010).

Thus, we carried out a labeling experiment in the Jena Experiment (Roscher et al. 2004) and applied water enriched in stable water isotopes (oxygen and hydrogen) at two different depths three times during the growing season 2011. The Jena Experiment is a large grassland biodiversity experiment with communities of varying species richness and functional group number, based on a pool of 60 temperate grassland species which greatly differ in their functional characteristics (grasses, legumes, tall herbs, small herbs). Based on the niche complementarity theory, we expected (i) increased uptake of water from different soil layers with increasing species richness or functional group number, and (ii) functional characteristics, i.e., functional group identity, to explain spatial and seasonal variations in water uptake patterns.

Material and Methods

Study site

The Jena Experiment is a large grassland biodiversity experiment located in the floodplain of the Saale river near the city of Jena (Germany, 50°55'N, 11°35'E, 130 m a.s.l.), which was established in 2002 on a former arable field. There was no specific permission required to work on "The Jena Experiment". The soil is a Eutric Fluvisol developed from up to 2 m thick fluvial sediments. Mean annual precipitation is 587 mm, mean annual temperature is 9.3°C (Kluge and Müller-Westermeier 2000). The Jena Experiment consists of 82 plots with different plant species number (1, 2, 4, 8, 16 and 60 species) and functional group richness (1, 2, 3 and 4 functional groups), from a species pool of 60 species assigned to four plant functional groups (grasses, legumes, small herbs and tall herbs). This study did not involve endangered or protected species. The experimental plots are arranged in four blocks to account for a gradient in soil texture, ranging from sandy loam to silly clay with increasing distance from the river. All plots are regularly weeded three times per year (April, June and September) and mown two times per year (June, September) to mimic the management of extensive hay meadows.

Tracer application and field sampling

The tracer experiment was conducted at the start of the growing season (April) and during the regrowth after the first and the second mowing (June and September) 2011. The experiment was carried out on a subset of 40 plots, covering a species richness gradient

with 2, 4, 8 and 16 plant species mixtures (ten replicates per species richness level, list of mixtures in Supporting Information Table S1). These plots were equally distributed among the experimental blocks. At each plot, three subplots were established (44 cm x 56 cm), each for one of the three labeling campaigns of the tracer experiment.

About five days before starting the tracer application, plant and soil samples were collected 10 cm next to the study plots to identify the natural abundance of ^{18}O and ^{2}H (later referred to as background samples). Using a soil auger of 1 cm diameter (Eijkelkamp, The Netherlands), soil background samples were taken at one plot per species richness level in each of the four blocks in 0-10, 10-20, 20-30 and 30-40 cm soil depth, resulting in a total of 64 samples per campaign. Root crowns, the connection between above- and belowground tissues, of single plants were collected and immediately placed into 12 ml glass vials (Labco Limited, UK), sealed with a cap and parafilm, and frozen until cryogenic water extraction was carried out. In total, 49 root crown background samples, homogenously distributed along the species richness gradient and representing species of each functional group in each species richness level, were collected prior each campaign.

For the tracer experiment, labeled water ($^{1}H_2{}^{18}O$, Sigma-Aldrich, Germany, and $^{2}H_2O$, Euriso-top, France) was injected at the same subplot (44 cm x 56 cm), but in different soil depths ($^{1}H_2{}^{18}O$ at 8 cm and $^{2}H_2O$ at 28 cm depth). To achieve a homogenous distribution of the tracer within the subplots, injection points were arranged on a grid of seven horizontal lines, which had a distance of 8.7 cm. The injection points for the two depths were alternating along the lines with a distance of 10 cm. This resulted in 32 injection points for the upper and 31 injection points for the lower soil depth (Application scheme in Figure S1 in Supporting Information). Holes of 8 mm diameter were drilled down to the two target depths of 8 and 28 cm with a handheld automated drill during five days prior to labeling, stabilized with wooden sticks.

The tracer solutions were created to achieve an enrichment of 400 ‰ for ^{18}O (upper soil depth) and 800 ‰ for ^{2}H (lower soil depth), based on the average soil water content of all plots measured a few days prior to labeling. Thus, the following tracer solutions were created and added to the soil water: 8’700 ‰ $\delta^{18}O$ and 26’500 ‰ $\delta^{2}H$ in April (18 to 19 April 2011), 12’100 ‰ $\delta^{18}O$ and 33’000 ‰ $\delta^{2}H$ in June (27 to 28 June 2011) and 15’500 ‰ $\delta^{18}O$ and 39’000 ‰ $\delta^{2}H$ in September (27 to 28 September 2011). The tracer solutions

were applied at 20 subplots per day between 8 am and 4 pm. The respective tracer solution was applied at 3 cm (^{18}O-enriched water) or 23 cm depth (^{2}H-enriched water) within 30 min per depth, using a 3 mm diameter four-sideport needle connected by a silicon tube to a bottle top dispenser (Sartorius, Germany) put on a 1 L glass bottle. As the solutions infiltrated into the soil rather slowly and to prevent the overflow of the solution out of the drilled holes in the upper depth during the injections, the injection depth differed from the drilled depth. Each hole received 2 ml of the respective tracer solution, resulting in a total of 64 ml for the upper depth and 62 ml for the lower depth per subplot. A funnel was placed around the injection hole to prevent contamination of the vegetation with the tracer solution during tracer application.

Exactly 48 h after finishing the labeling of each subplot (20 to 21 April, 29 to 30 June and 29 to 30 September 2011), root crowns of three to five individual plants of each species present per plot were collected, cleaned and pooled by plant species and subplot. Three soil samples were taken at each subplot with a soil auger of 1 cm diameter (Eijkelkamp, The Netherlands) in nine soil depths (0-3, 3-6, 6-10, 10-15, 15-20, 20-23, 23-26, 26-30 and 30-40 cm). One soil replicate was taken very close to an injection point for the upper soil depth, one very close to an injection point for the lower depth, and one in between injection points. Soil samples in each depth were pooled, resulting in nine soil samples per subplot, covering the top 40 cm. All plant and soil samples were immediately placed into 12 ml glass vials (Labco Limited, UK), sealed with a cap and parafilm, kept cool in a cooling box and transported to a freezer within two hours. Samples were kept frozen until cryogenic water extraction. In total, 360 soil samples were taken and analyzed at each labeling campaign. In addition 197 plant samples were taken in April, 192 in June, and 193 in September. Due to the low water content of some plant samples, only 148, 136 and 145 samples were analyzed for each campaign, respectively.

Laboratory analyses

Xylem water in root crowns and soil water were extracted for isotopic analyses using a cryogenic water extraction line (Barnard et al. 2006) and measured with a TC/EA high-temperature conversion/elemental analyzer coupled with a DeltaplusXP isotope ratio mass spectrometer via a ConFlo III interface (Thermo-Finnigan, Bremen, Germany; see Werner et al. (1999) for further information). Oxygen and hydrogen isotopic composition of the water samples are given in δ notation measured as ($R_{Sample}/R_{Standard}$) − 1, and

expressed in ‰. R is the ratio of ^{18}O to ^{16}O or ^{2}H to ^{1}H of the sample or the standard. Our standard was a working control standard, regularly calibrated against international standards (V-SMOW, SLAP, GISP). The overall precision of the measurements was ± 0.09 ‰ for $\delta^{18}O$ and ± 0.37 ‰ for $\delta^{2}H$.

Data analyses

All statistical analyses and graphics were done with R 2.14.1 (R Development Core Team 2011). Mixed effects models were carried out by using the *lmer* function within the *lme4* package (Bates et al. 2011). Prior to analyses, all data were log transformed to meet the assumptions for mixed effects models that require normally distributed within-group errors. The maximum likelihood method was used to estimate the variance components. Block, plot identity (nested within block) and species identity were treated as random factors. Analyses were started from a null model containing the random factors. Fixed factors and interactions between the fixed factors were entered stepwise. Likelihood ratio tests (X^2) were applied to compare models and to test for a significant improvement of the model after adding the fixed effects.

To compare whether the $\delta^{18}O$ or $\delta^{2}H$ values in the xylem water of the samples taken after the labeling differ from the background samples, mixed-effect models were carried out, including sample type (i.e., back ground sample or labeled sample) as fixed factor separately for each labeling campaign.

Enrichment of the xylem water was then identified by calculating the difference of $\delta^{18}O$ or $\delta^{2}H$ values of the samples taken after the labeling and the respective average value of the plant background samples for each labeling time. To test if the enrichment in ^{18}O differs from the enrichment in ^{2}H, isotope (i.e., $\delta^{18}O$ vs. $\delta^{2}H$) was included as a fixed factor in the model in separate analyses for each campaign. Finally, effects of species richness, number of functional groups and functional group identity (i.e., grasses, legumes, small herbs and tall herbs) on uptake of ^{18}O- or ^{2}H-enriched water were tested for each labeling campaign by adding the fixed factors in the following order: species richness (SR, log-linear), functional group richness (FR, linear), functional group identity (FG), and the interaction between SR and FG.

Results

$\delta^{18}O$ and $\delta^{2}H$ values of soil water

Soil water in a depth of 6 to 10 cm, where the ^{18}O-enriched water was injected, displayed average $\delta^{18}O$ values of 65.5 ‰ (SD ± 39.45 ‰) in April, 106.7 ‰ (SD ± 44.14 ‰) in June, and 85 ‰ (SD ± 45.64 ‰) in September (Figure 1), highly enriched compared to the background values ($\delta^{18}O_{April}$ = -9.67 ‰ (SD ± 1.15 ‰), $\delta^{18}O_{June}$ = -5.08 ‰ (SD ± 0.83 ‰) and $\delta^{18}O_{September}$ = -2.79 ‰ (SD ± 1.98 ‰). Similarly, soil water in a depth of 26 to 30 cm, where the ^{2}H-enriched water was added, showed in average $\delta^{2}H$ values of 16.9 ‰ (SD ± 99.32 ‰) in April, 262.6 ‰ (SD ± 206.21 ‰) in June, and 144 ‰ (SD ± 171.22 ‰) in September, highly above the background values ($\delta^{2}H_{April}$ = -110.27 ‰ (SD ± 7.45 ‰), $\delta^{2}H_{June}$ = -93.14 ‰ (SD ± 10.24 ‰) and $\delta^{2}H_{September}$ = -50.12 ‰ (SD ± 7.61 ‰)). Soil layers above the target depth were enriched as well (Figure 1), most likely due to slow soil infiltration of the labeling solution injected into the holes. For $\delta^{18}O$, soil water at some plots in layers below the target depth was also enriched, probably caused by soil cracks or earthworm holes. However, two distinct soil layers imitating two different water sources were achieved at all three campaigns.

During the course of the growing season, background $\delta^{18}O$ and $\delta^{2}H$ values increased by about 7 ‰ and 60 ‰ in the target depth (6-10 cm for ^{18}O and 26-30 cm for ^{2}H), driven by enhanced water soil water evaporation at higher temperatures and changes in the isotopic composition of precipitation (Dansgaard 1964, Clark and Fritz 1997).

$\delta^{18}O$ and $\delta^{2}H$ values of xylem water

Xylem water after the labeling and pooled over all species richness levels displayed average $\delta^{18}O$ values of 14.24 ‰ (SD ± 14.21 ‰) in April, 28.88 ‰ (SD ± 21.01 ‰) in June, and 30.4 ‰ (SD ± 24.1 ‰) in September, well above the corresponding background values of -8.5 ‰ (SD ± 1.5 ‰) in April, -4.78 ‰ (SD ± 1.39 ‰) in June, and -3.35 ‰ (SD ± 1.27 ‰) in September. The $\delta^{18}O$ values of the xylem water after the labeling were significantly higher than the $\delta^{18}O$ values of the xylem water of the background samples at all three times (X^2_{April} = 209.82, $P_{April} < 0.001$; X^2_{June} = 220.37, $P_{June} < 0.001$, $X^2_{September}$ = 227.16, $P_{September} < 0.001$, Figure 2). In contrast, $\delta^{2}H$ values in the xylem water of the plants after the labeling did not differ significantly from background samples in April (X^2_{April} = 0.87, P_{April} = 0.350) and June (X^2_{June} = 1.19, P_{June} = 0.276), but in September ($X^2_{September}$ = 65.75, $P_{September} < 0.001$). While $\delta^{2}H$ values of the

xylem water of labeled plants were -65.37 ‰ (SD ± 14.75 ‰) in April, -43.13 ‰ (SD ± 16.78 ‰) in June and -19.16 ‰ (SD ± 9.02 ‰) in September, δ^2H values of background plants were -69.81 ‰ (SD ± 9.71) in April, -45.6 ‰ (SD ± 8.05 ‰) in June, and -29.71 ‰ (SD ± 7.53 ‰) in September (Figure 2).

The enrichment of xylem water in ^{18}O, i.e., the difference between the average seasonal background $\delta^{18}O$ value and the $\delta^{18}O$ values of the samples taken after the labeling, ranged in average between 22.74 ‰ and 33.75 ‰ during the growing season, in comparison to the much larger enrichment in the soil water that ranged between 75.12 ‰ and 111.78 ‰ at 6 to 10 cm soil depth. However, the enrichment of xylem water in ^2H only ranged between 2.47 ‰ and 10.55 ‰, despite a very large enrichment in the corresponding target depth of 26 to 30 cm soil depth (127.17 ‰ to 355.69 ‰), indicating preferential water uptake in the upper soil depth.

The enrichment of xylem water in ^{18}O differed significantly from the enrichment ^2H at each time (X^2_{April} = 126.35, $P_{April} < 0.001$; X^2_{June} = 208.86, $P_{June} < 0.001$; $X^2_{September}$ = 143.65, $P_{September} < 0.001$, Figure 3).

Enrichment of the xylem water in ^{18}O or ^2H was not affected by species richness or number of functional groups at any time (Table 1, Figure 3). Functional groups only differed in their ^{18}O enrichment in April, but not in June or September (P_{FG} = 0.005, Table 1), with legumes displaying lower and small herbs slightly higher ^{18}O enrichments compared to the other functional groups in April ($\delta^{18}O_{Legumes}$ = 11.61 ‰ (SD ± 8.14 ‰), $\delta^{18}O_{Small\ herbs}$ = 27.39 ‰ (SD ± 16.08 ‰), $\delta^{18}O_{Tall\ herbs}$ = 22.96 ‰ (SD ± 15.02 ‰), $\delta^{18}O_{Grasses}$ = 20.75 ‰ (SD ± 9.95 ‰). No difference among functional groups was found for ^2H enrichment at any time.

Discussion

With the present study, we tested if plant communities of increased species or functional group richness exhibit increased spatial or temporal complementarity in water use compared to low diverse communities. Our results suggest that the main water uptake was from the top soil layers in all mixtures and at all times, indicated by a higher enrichment of xylem water in ^{18}O (applied to the top soil layer) than in ^2H (applied to the deeper soil layer). We found no evidence for increased water exploitation from deeper

soil layers with increasing species richness or functional group richness nor effects of functional group identity on spatial or temporal exploitation of soil water. Thus, our results do not support the hypothesis of complementary water use as explanation for a positive biodiversity-ecosystem functioning relationship, neither spatially nor temporally.

These results, based on direct measurements of soil water use, contradict earlier studies that inferred water complementarity based on indirect approaches. For instance, Caldeira et al. (2001) studied soil moisture patterns and plant $\delta^{13}C$ in Mediterranean grasslands of varying species richness and interpreted lower foliar $\delta^{13}C$ values of plants growing in mixtures than in monocultures as a result of more complete water use due to higher stomatal conductance rates. Verheyen et al. (2008) considered complementary water use as the underlying mechanism for increased evapotranspiration with increasing species richness obtained from canopy surface temperature measurements. Van Peer et al. (2004) reported increased water consumption with increasing species richness in heat stressed, container-grown artificial grasslands based on soil moisture measurements. However, lower $\delta^{13}C$ values and thus higher stomatal conductance rates can also be the result of low light levels due to higher community biomass, which could in turn increase community evapotranspiration and lower canopy temperature. Thus, these indirect approaches cannot be used to unequivocally disentangle cause and effects.

On the other hand, studies using stable isotopes to directly test water uptake among coexisting species found strong evidence for water partitioning, typically in semi-arid ecosystems, where water availability is limited (Ehleringer et al. 1991, Casper and Jackson 1997, Dodd et al. 1998, Fargione and Tilman 2005, Nippert and Knapp 2007, Kulmatiski et al. 2010, Moreno-Gutiérrez et al. 2012). However, none of these studies tested different species richness levels. Thus, spatial niche differentiation seems more likely to allow for coexistence when water in upper soil layers is scarce than under conditions when water is not a limiting resource (see soil water content given in Figure 1). Under such conditions, water availability is closely linked to nutrient availability, both being be higher in upper than in deeper soil layers, thus favoring the development of a shallow rooting system (Schenk and Jackson 2002), even along a diversity gradient.

Furthermore, complementarity in belowground resources use (water, nutrients) is thought to result from an increasing variety of rooting depths among species with increasing species richness. Hence, vertical root biomass distribution is expected to change in favor

of increasing root biomass also in deeper soil layers with increasing species richness. However, Ravenek et al. (2014) did not find any shifts in relative root distribution along the vertical soil profile with increasing species richness or in plots with different functional group composition, despite a significant increase in total standing root biomass at higher species richness levels. Therefore, the increased root biomass production at higher species richness at 0 to 30 cm depth within the Jena Experiment (Bessler et al. 2012) is probably due to a more intense rooting over the whole soil profile or in the topsoil layer. These results give further support for a lack of vertical niche differentiation with increasing species richness, but rather show preferential resource uptake from the upper soil layers independent of species or functional group richness.

Clear experimental evidence for complementarity is also scarce for other soil resources, e.g., nitrogen. In two grassland studies, both conducting ^{15}N labeling experiments, neither spatial nor temporal complementarity of nitrogen uptake was found in more diverse grasslands compared to low diverse grasslands (Kahmen et al. 2006, von Felten et al. 2009). In both studies, the main nitrogen uptake was from the top soil layer (upper 3 cm).

Ecosystem processes have been found to be highly influenced by the functional group composition rather than by species richness alone (Hooper and Vitousek 1997, Tilman et al. 1997a). Kahmen et al. (2006) observed significant differences in nitrogen uptake among different functional groups (legumes, tall herbs, legumes, small herbs), irrespective of the species richness level. In our study, differences in water uptake among functional groups were not significant except for April 2011, the very start of the growing season when growth commences. Based on information derived from the literature, small herbs are assumed to have shallower roots than tall herbs, grasses and legumes in the Jena Experiment (Gubsch et al. 2011, Roscher et al. 2012), but roots of most species cover the depths studied with our labeling approach and root characteristics vary greatly among species within functional groups. This variation may explain the lack of a consistent functional group effect on water uptake patterns in our experiment.

In conclusion, our results suggest no increased complementarity in water use with increasing species richness. The main water uptake from the top soil layer is consistent with observed rooting patterns as well as with results on nitrogen uptake found in other temperate grasslands. If complementarity in water use differs between systems adapted to low vs. high water availability remains to be seen. Furthermore, since plant species are

often limited by multiple resources and differ in their resource requirements (Tilman et al. 1997b, Harpole and Tilman 2007), complementarity not only for a single resource, but for multiple resources might be the mechanism to explain the positive effects of high plant species richness on ecosystem processes.

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Table 1 Summary of the mixed-effects model testing the effects of species richness, functional group number and functional group identity on xylem water enrichment in ^{18}O and ^{2}H (i.e., difference between samples taken after the labeling and the background samples). Analyses were carried out for each campaign separately. Significant effects are formatted in bold.

	April				June				September			
	$\delta^{18}O$		$\delta^{2}H$		$\delta^{18}O$		$\delta^{2}H$		$\delta^{18}O$		$\delta^{2}H$	
	χ^2 ratio	*P*	*χ^2 ratio*	*P*	*χ^2 ratio*	*P*	*χ^2 ratio*	*P*	*χ^2 ratio*	*P*	*χ^2 ratio*	*P*
Species richness (SR, log-linear)	0.71	0.399	0.01	0.931	0.18	0.669	1.82	0.177	3.01	0.083	2.13	0.145
Functional group richness (FR, linear)	0.05	0.825	1.45	0.228	0.26	0.61	0.79	0.373	1.1	0.294	0.14	0.709
Functional group identity (FG)	12.94	**0.005**	3.79	0.285	6.11	0.106	2.13	0.546	2.62	0.453	5.82	0.121
SR x FG	0.19	0.801	1.6	0.66	2.37	0.499	1.73	0.63	0.85	0.837	2.58	0.461

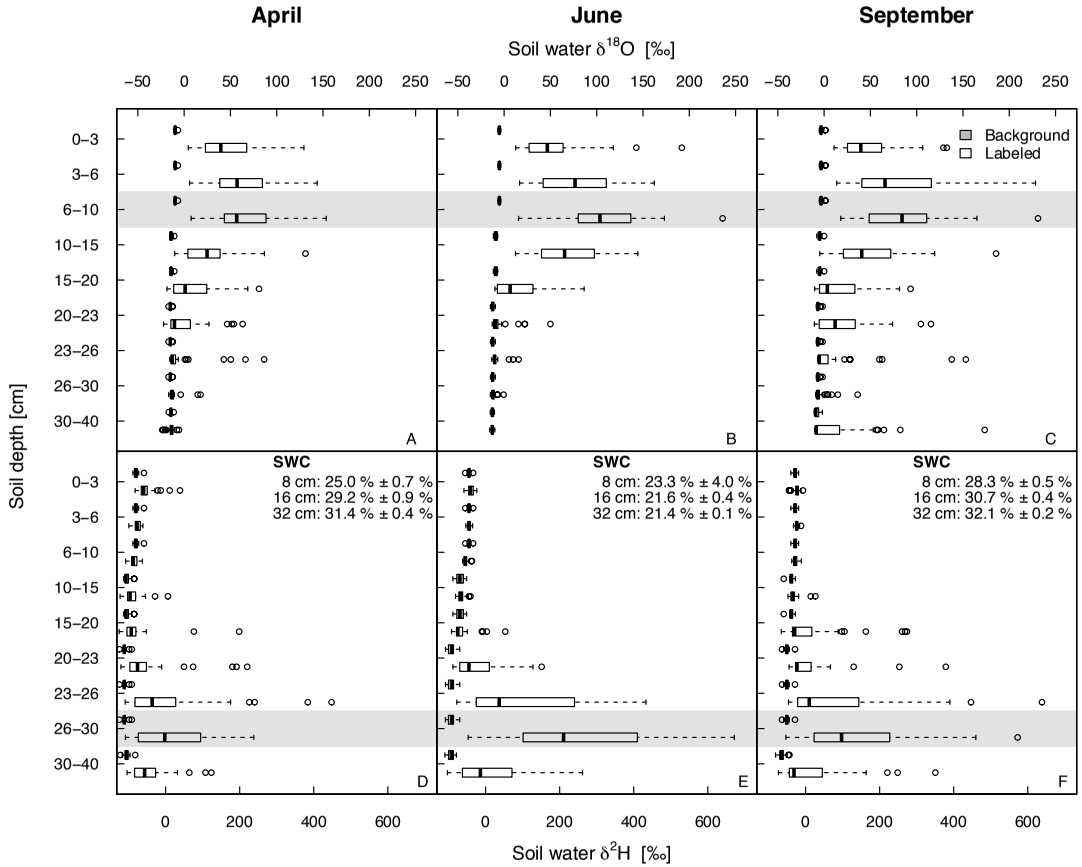

Figure 1 $\delta^{18}O$ (A-C) and $\delta^{2}H$ (D-F) values of soil water labeled with ^{18}O-enriched water in upper soil depths and with ^{2}H-enriched water in lower soil depths. Grey areas illustrate the depths of tracer application. Data are given for the natural background soil as well as after the labeling at three different times (April, June, September 2011), in each case pooled for all species richness levels. Values of soil water content (SWC) in 8, 16 and 32 cm are given as mean ± 1 SD for the 4-day labeling and harvest campaigns.

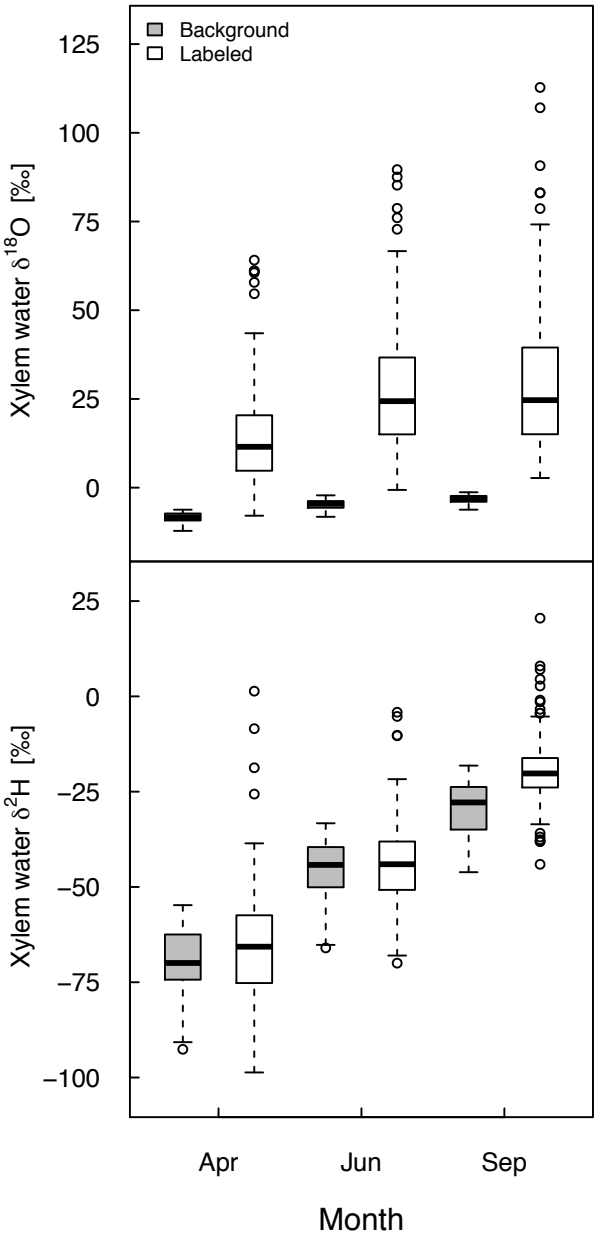

Figure 2 $\delta^{18}O$ (top) and $\delta^{2}H$ (bottom) values of xylem water. Data are given for the background samples and the samples taken after the labeling at three different times (April, June, September 2011), in each case pooled for all species richness levels. Outliers (at $\delta^{18}O$ = 141.7 ‰ and $\delta^{2}H$ =101.3 ‰ in June and at $\delta^{18}O$ = 187.3 ‰ in September) were removed for reasons of clarity. Results of the corresponding mixed-effects models are given in the running text.

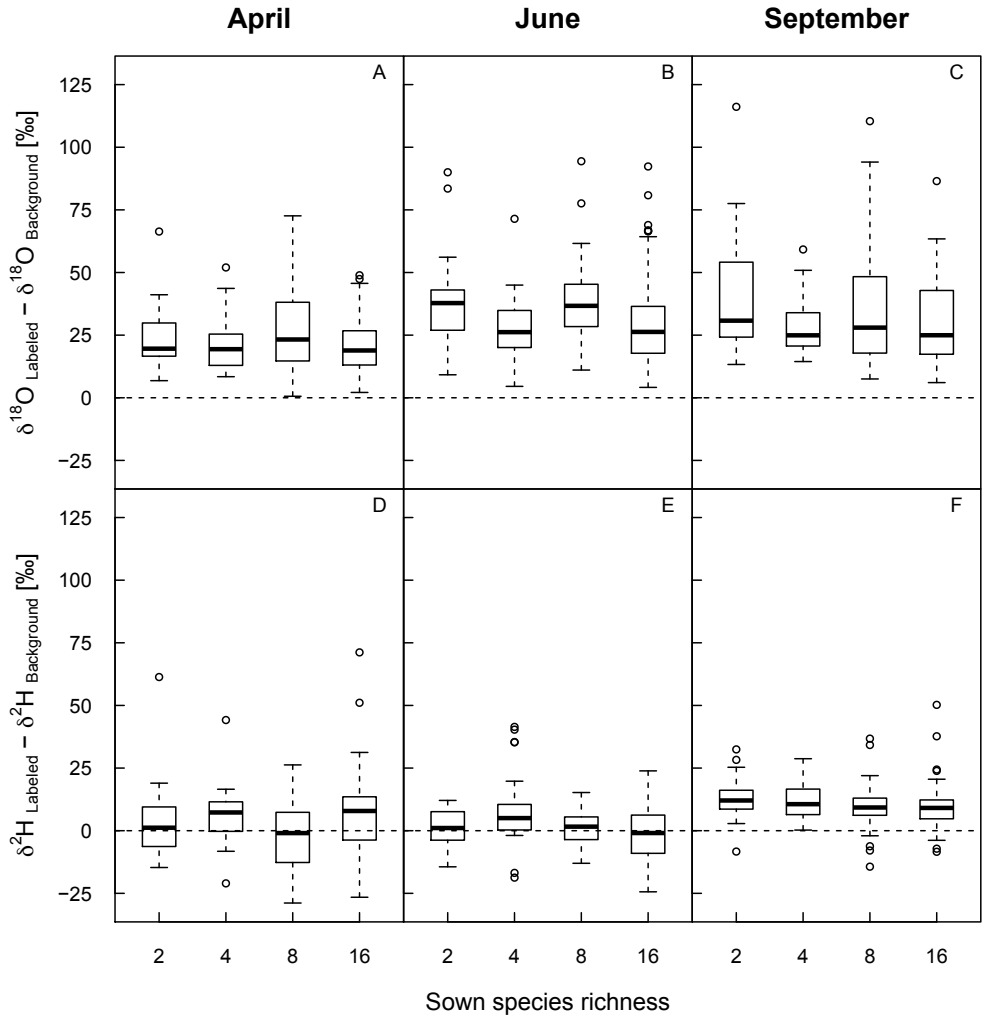

Figure 3 Differences in $\delta^{18}O$ (A-C) and $\delta^{2}H$ (D-F) values in the xylem water after the labeling compared to the corresponding background at three different times (April, June, September 2011) separately for each species richness level. Outliers (at $\delta^{18}O$ = 146.5 ‰ and $\delta^{2}H$ =146.9 ‰ in June and at $\delta^{18}O$ = 190.6 ‰ in September) were removed for reasons of clarity. Results of the corresponding mixed-effects models are given in Table 1.

Supporting information

Table S1 List of mixtures used for the tracer experiment

Plotcode	Sown species	Functional group
B1A02	*Alopecurus pratensis*	G
B1A02	*Bromus erectus*	G
B1A02	*Cardamine pratensis*	TH
B1A02	*Festuca rubra*	G
B1A02	*Heracleum sphondylium*	TH
B1A02	*Phleum pratense*	G
B1A02	*Ranunculus acris*	TH
B1A02	*Sanguisorba officinalis*	TH
B1A03	*Cynosurus cristatus*	G
B1A03	*Glechoma hederacea*	SH
B1A03	*Lotus corniculatus*	L
B1A03	*Medicago lupulina*	L
B1A03	*Phleum pratense*	G
B1A03	*Primula veris*	SH
B1A03	*Trisetum flavescens*	G
B1A03	*Veronica chamaedrys*	SH
B1A06	*Achillea millefolium*	TH
B1A06	*Alopecurus pratensis*	G
B1A06	*Anthoxanthum odoratum*	G
B1A06	*Anthriscus sylvestris*	TH
B1A06	*Avenula pubescens*	G
B1A06	*Bromus hordeaceus*	G
B1A06	*Campanula patula*	TH
B1A06	*Centaurea jacea*	TH
B1A06	*Geranium pratense*	TH
B1A06	*Heracleum sphondylium*	TH
B1A06	*Holcus lanatus*	G
B1A06	*Leucanthemum vulgare*	TH
B1A06	*Pimpinella major*	TH
B1A06	*Poa pratensis*	G
B1A06	*Poa trivalis*	G
B1A06	*Trisetum flavescens*	G
B1A07	*Ranunculus acris*	TH
B1A07	*Sanguisorba officinalis*	TH
B1A11	*Achillea millefolium*	TH
B1A11	*Anthriscus sylvestris*	TH
B1A11	*Campanula patula*	TH
B1A11	*Cardamine pratensis*	TH
B1A11	*Cirsium oleraceum*	TH
B1A11	*Crepis biennis*	TH
B1A11	*Daucus carota*	TH
B1A11	*Galium album*	TH
B1A11	*Geranium pratense*	TH
B1A11	*Heracleum sphondylium*	TH
B1A11	*Leucanthemum vulgare*	TH
B1A11	*Pastinaca sativa*	TH
B1A11	*Ranunculus acris*	TH
B1A11	*Rumex acetosa*	TH
B1A11	*Sanguisorba officinalis*	TH
B1A11	*Tragopogon pratensis*	TH
B1A14	*Anthriscus sylvestris*	TH
B1A14	*Daucus carota*	TH
B1A14	*Leontodon hispidus*	SH
B1A14	*Luzula campestris*	G
B1A14	*Plantago lanceolata*	SH
B1A14	*Trifolium campestre*	L
B1A14	*Trisetum flavescens*	G
B1A14	*Trifolium fragiferum*	L
B1A16	*Plantago lanceolata*	SH
B1A16	*Poa pratensis*	G
B1A17	*Alopecurus pratensis*	G
B1A17	*Daucus carota*	TH
B1A19	*Arrhenatherum elatius*	G
B1A19	*Campanula patula*	TH
B1A19	*Luzula campestris*	G
B1A19	*Prunella vulgaris*	SH
B2A01	*Anthoxanthum odoratum*	G
B2A01	*Knautia arvensis*	TH
B2A01	*Prunella vulgaris*	SH
B2A01	*Trifolium pratense*	L
B2A02	*Festuca rubra*	G
B2A02	*Trisetum flavescens*	G
B2A06	*Lathyrus pratensis*	L
B2A06	*Medicago lupulina*	L
B2A06	*Plantago lanceolata*	SH
B2A06	*Taraxacum officinale*	SH
B2A08	*Ranunculus acris*	TH
B2A08	*Trifolium campestre*	L
B2A09	*Ajuga reptans*	SH
B2A09	*Plantago lanceolata*	SH
B2A09	*Primula veris*	SH
B2A09	*Prunella vulgaris*	SH
B2A14	*Knautia arvensis*	TH
B2A14	*Leontodon hispidus*	SH
B2A14	*Luzula campestris*	G
B2A14	*Phleum pratense*	G
B2A14	*Sanguisorba officinalis*	TH
B2A14	*Trifolium dubium*	L
B2A14	*Trifolium hybridum*	L
B2A14	*Veronica chamaedrys*	SH
B2A16	*Knautia arvensis*	TH
B2A16	*Leontodon autumnalis*	SH

Table S1 continued

Plotcode	*Sown species*	Functional group
B2A16	*Plantago media*	SH
B2A16	*Vicia cracca*	L
B2A18	*Ajuga reptans*	SH
B2A18	*Alopecurus pratensis*	G
B2A18	*Anthriscus sylvestris*	TH
B2A18	*Bromus hordeaceus*	G
B2A18	*Campanula patula*	TH
B2A18	*Cardamine pratensis*	TH
B2A18	*Cynosurus cristatus*	G
B2A18	*Geranium pratense*	TH
B2A18	*Medicago lupulina*	L
B2A18	*Plantago media*	SH
B2A18	*Poa pratensis*	G
B2A18	*Primula veris*	SH
B2A18	*Ranunculus repens*	SH
B2A18	*Trifolium campestre*	L
B2A18	*Trifolium dubium*	L
B2A18	*Trifolium repens*	L
B2A19	*Plantago media*	SH
B2A19	*Taraxacum officinale*	SH
B2A20	*Plantago lanceolata*	SH
B2A20	*Trifolium dubium*	L
B2A22	*Achillea millefolium*	TH
B2A22	*Campanula patula*	TH
B2A22	*Centaurea jacea*	TH
B2A22	*Cynosurus cristatus*	G
B2A22	*Festuca pratensis*	G
B2A22	*Lathyrus pratensis*	L
B2A22	*Lotus corniculatus*	L
B2A22	*Onobrychis viciifolia*	L
B2A22	*Phleum pratense*	G
B2A22	*Poa trivalis*	G
B2A22	*Rumex acetosa*	TH
B2A22	*Sanguisorba officinalis*	TH
B2A22	*Trisetum flavescens*	G
B2A22	*Trifolium hybridum*	L
B2A22	*Trifolium repens*	L
B2A22	*Vicia cracca*	L
B3A03	*Phleum pratense*	G
B3A03	*Plantago media*	SH
B3A03	*Trifolium hybridum*	L
B3A03	*Vicia cracca*	L
B3A04	*Alopecurus pratensis*	G
B3A04	*Arrhenatherum elatius*	G
B3A04	*Cynosurus cristatus*	G
B3A04	*Dactylis glomerata*	G

Plotcode	*Sown species*	Functional group
B3A04	*Festuca rubra*	G
B3A04	*Holcus lanatus*	G
B3A04	*Poa trivalis*	G
B3A04	*Trisetum flavescens*	G
B3A05	*Anthoxanthum odoratum*	G
B3A05	*Anthriscus sylvestris*	TH
B3A05	*Bromus erectus*	G
B3A05	*Leucanthemum vulgare*	TH
B3A05	*Lotus corniculatus*	L
B3A05	*Onobrychis viciifolia*	L
B3A05	*Poa trivalis*	G
B3A05	*Trifolium hybridum*	L
B3A08	*Dactylis glomerata*	G
B3A08	*Festuca pratensis*	G
B3A09	*Alopecurus pratensis*	G
B3A09	*Anthoxanthum odoratum*	G
B3A09	*Arrhenatherum elatius*	G
B3A09	*Avenula pubescens*	G
B3A09	*Bromus erectus*	G
B3A09	*Bromus hordeaceus*	G
B3A09	*Cynosurus cristatus*	G
B3A09	*Dactylis glomerata*	G
B3A09	*Festuca pratensis*	G
B3A09	*Festuca rubra*	G
B3A09	*Holcus lanatus*	G
B3A09	*Luzula campestris*	G
B3A09	*Phleum pratense*	G
B3A09	*Poa pratensis*	G
B3A09	*Poa trivalis*	G
B3A09	*Trisetum flavescens*	L
B3A11	*Bromus erectus*	G
B3A11	*Plantago lanceolata*	SH
B3A11	*Poa trivalis*	G
B3A11	*Prunella vulgaris*	SH
B3A16	*Ajuga reptans*	SH
B3A16	*Glechoma hederacea*	SH
B3A16	*Lathyrus pratensis*	L
B3A16	*Leontodon hispidus*	SH
B3A16	*Medicago lupulina*	L
B3A16	*Onobrychis viciifolia*	L
B3A16	*Plantago media*	SH
B3A16	*Prunella vulgaris*	SH
B3A16	*Ranunculus repens*	SH
B3A16	*Taraxacum officinale*	SH
B3A16	*Trifolium campestre*	L
B3A16	*Trifolium fragiferum*	L

Table S1 continued

Plotcode	*Sown species*	Functional group
B3A16	*Trifolium hybridum*	L
B3A16	*Trifolium repens*	L
B3A16	*Veronica chamaedrys*	SH
B3A16	*Vicia cracca*	L
B3A19	*Taraxacum officinale*	SH
B3A19	*Trisetum flavescens*	G
B3A22	*Ajuga reptans*	SH
B3A22	*Anthoxanthum odoratum*	G
B3A22	*Bellis perennis*	SH
B3A22	*Bromus erectus*	G
B3A22	*Crepis biennis*	TH
B3A22	*Festuca rubra*	G
B3A22	*Galium album*	TH
B3A22	*Geranium pratense*	TH
B3A22	*Onobrychis viciifolia*	L
B3A22	*Phleum pratense*	G
B3A22	*Ranunculus repens*	SH
B3A22	*Rumex acetosa*	TH
B3A22	*Trifolium dubium*	L
B3A22	*Trifolium fragiferum*	L
B3A22	*Veronica chamaedrys*	SH
B3A22	*Vicia cracca*	L
B3A23	*Bromus hordeaceus*	G
B3A23	*Leucanthemum vulgare*	TH
B3A23	*Ranunculus repens*	SH
B3A23	*Trifolium fragiferum*	L
B3A24	*Ajuga reptans*	SH
B3A24	*Anthoxanthum odoratum*	G
B3A24	*Arrhenatherum elatius*	G
B3A24	*Avenula pubescens*	G
B3A24	*Bromus hordeaceus*	G
B3A24	*Festuca pratensis*	G
B3A24	*Glechoma hederacea*	SH
B3A24	*Lotus corniculatus*	L
B3A24	*Medicago x varia*	L
B3A24	*Poa trivalis*	G
B3A24	*Prunella vulgaris*	SH
B3A24	*Ranunculus repens*	SH
B3A24	*Taraxacum officinale*	SH
B3A24	*Trifolium pratense*	L
B3A24	*Trifolium repens*	L
B3A24	*Vicia cracca*	L
B4A02	*Anthriscus sylvestris*	TH
B4A02	*Arrhenatherum elatius*	G
B4A02	*Cynosurus cristatus*	G
B4A02	*Galium album*	TH

Plotcode	*Sown species*	Functional group
B4A02	*Glechoma hederacea*	SH
B4A02	*Heracleum sphondylium*	TH
B4A02	*Knautia arvensis*	TH
B4A02	*Leontodon hispidus*	SH
B4A02	*Luzula campestris*	G
B4A02	*Pastinaca sativa*	TH
B4A02	*Phleum pratense*	G
B4A02	*Plantago media*	SH
B4A02	*Poa pratensis*	G
B4A02	*Ranunculus acris*	TH
B4A02	*Ranunculus repens*	SH
B4A02	*Taraxacum officinale*	SH
B4A04	*Anthriscus sylvestris*	TH
B4A04	*Arrhenatherum elatius*	G
B4A04	*Plantago lanceolata*	SH
B4A04	*Trifolium campestre*	L
B4A06	*Ajuga reptans*	SH
B4A06	*Bellis perennis*	SH
B4A06	*Glechoma hederacea*	SH
B4A06	*Leontodon autumnalis*	SH
B4A06	*Primula veris*	SH
B4A06	*Prunella vulgaris*	SH
B4A06	*Taraxacum officinale*	SH
B4A06	*Veronica chamaedrys*	SH
B4A08	*Ajuga reptans*	SH
B4A08	*Anthoxanthum odoratum*	G
B4A08	*Avenula pubescens*	G
B4A08	*Bromus hordeaceus*	G
B4A08	*Festuca rubra*	G
B4A08	*Plantago lanceolata*	SH
B4A08	*Taraxacum officinale*	SH
B4A08	*Veronica chamaedrys*	SH
B4A10	*Achillea millefolium*	TH
B4A10	*Ajuga reptans*	SH
B4A10	*Bromus erectus*	G
B4A10	*Carum carvi*	TH
B4A10	*Festuca pratensis*	G
B4A10	*Pimpinella major*	TH
B4A10	*Plantago media*	SH
B4A10	*Primula veris*	SH
B4A14	*Bellis perennis*	SH
B4A14	*Plantago lanceolata*	SH
B4A16	*Anthriscus sylvestris*	TH
B4A16	*Phleum pratense*	G
B4A16	*Poa trivalis*	G
B4A16	*Primula veris*	SH

Table S1 continued

Plotcode	*Sown species*	Functional group
B4A16	*Sanguisorba officinalis*	TH
B4A16	*Taraxacum officinale*	SH
B4A16	*Trifolium dubium*	L
B4A16	*Trifolium fragiferum*	L
B4A18	*Alopecurus pratensis*	G
B4A18	*Bromus hordeaceus*	G
B4A18	*Carum carvi*	TH
B4A18	*Crepis biennis*	TH
B4A18	*Cynosurus cristatus*	G
B4A18	*Heracleum sphondylium*	TH
B4A18	*Lathyrus pratensis*	L
B4A18	*Leontodon autumnalis*	SH
B4A18	*Luzula campestris*	G
B4A18	*Onobrychis viciifolia*	L
B4A18	*Pimpinella major*	TH
B4A18	*Plantago media*	SH
B4A18	*Taraxacum officinale*	SH
B4A18	*Trifolium campestre*	L
B4A18	*Trifolium hybridum*	L
B4A18	*Veronica chamaedrys*	SH
B4A22	*Campanula patula*	TH
B4A22	*Cardamine pratensis*	TH
B4A22	*Geranium pratense*	TH
B4A22	*Knautia arvensis*	TH

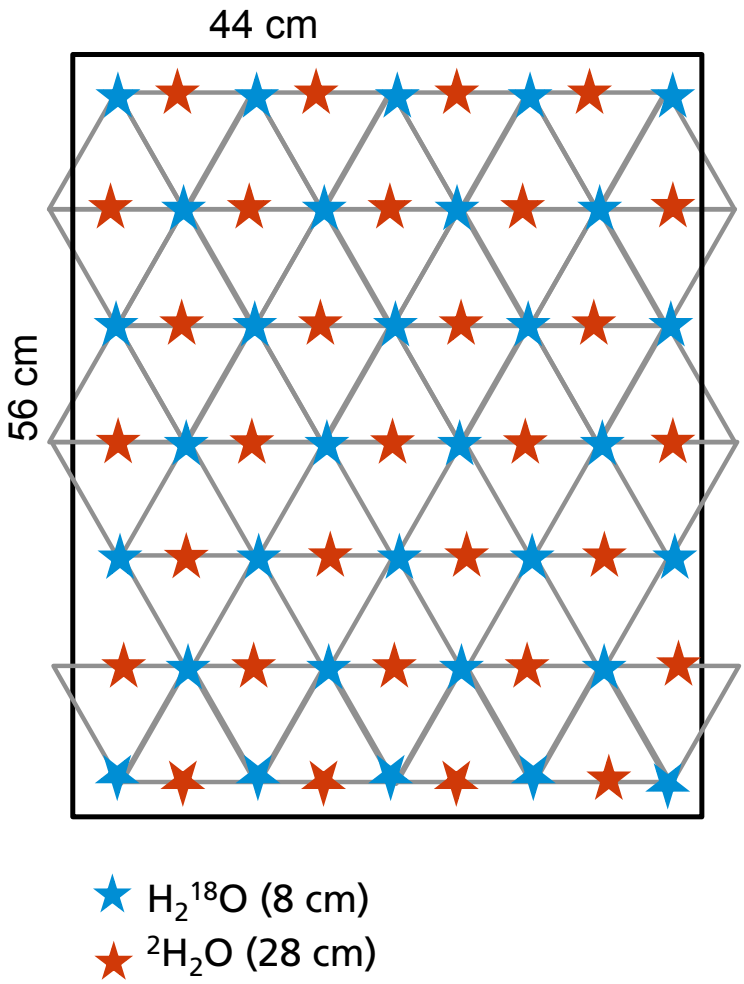

Figure S1 Scheme of application of the tracer solution

Chapter 2

Characterizing temporal changes in the light niche across a diversity gradient in grassland: light attenuation vs. leaf traits vs. functional dissimilarity

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Abstract

Complementarity in light use might increase light exploitation at increased plant diversity and could thus be an important mechanism for positive diversity-ecosystem functioning relationships. We addressed complementarity in light use and its temporal development by measuring vertical light profiles and leaf traits related to light use in 40 mixtures of varying species richness in a large grassland biodiversity experiment. Light attenuation within the canopy differed significantly among mixtures of varying species richness at peak biomass (late May, August), but neither at the beginning of the growing season (April) nor during regrowth after mowing (June, September). At peak biomass, light attenuation was 40% in 2-species mixtures and increased up to 80% in 16-species mixtures, suggesting more diverse light conditions throughout the canopy at high species richness. However, we found no effect of increased species or functional group richness on the expression of leaf traits related to light use, except for specific leaf area (SLA). Trait expression differed significantly within the growing season and among functional groups (except SLA) but did not coincide with the temporal patterns of light attenuation. Nevertheless, these different light use strategies of functional groups resulted in higher functional dissimilarity of leaf traits (except SLA) with increasing species richness at the community level. Thus, our results suggest that higher light attenuation in more diverse communities cannot be explained by the greater diversity in plastic leaf trait adjustment at functional group level, but that functional dissimilarity is the key to high complementary resource use in diverse plant communities.

Introduction

One central aim in current biodiversity research is to understand the mechanisms explaining positive effects of increasing species diversity on ecosystem processes (Hooper et al. 2005, Isbell et al. 2011). Niche complementarity is a frequently proposed mechanism, assuming that the chance to assemble species which differ in their spatial and/or temporal resource acquisition increases with increasing species and functional group richness. Niche separation in resource acquisition and resource use might result in reduced competition, more complete resource use and eventually increased community biomass production (Loreau and Hector 2001).

Light availability as a key resource for plant growth and the corresponding light niche can be studied with direct and indirect approaches, by (1) quantifying light attenuation, and (2) leaf traits related to light use, respectively. First, due to the unidirectional supply of light in plant canopies, the amount of available light is attenuated towards the ground (Monsi and Saeki 1953) and light quality also changes towards deeper canopy layers (Jones 1992). Several studies in experimental grasslands using direct approaches have shown that light availability at the ground and at peak biomass decreases with increasing species richness (Naeem et al. 1999, Wacker et al. 2009), as a result of increased biomass production and canopy density (Spehn et al. 2005, Lorentzen et al. 2008, Vojtech et al. 2008). Differences in canopy architecture and leaf positioning within canopies in plant communities of varying diversity are thought to improve leaf exposure to light and reduce self-shading, therefore not only maximizing the use of aboveground space, but also use light niches as much as possible. However, the temporal development of light attenuation as a function of diversity is often not known. Second, as individuals or species differing in growth height are exposed to light conditions of varying quality and quantity within the canopy, morphological and physiological adjustments of leaves to these conditions might also contribute to the complementarity in light use at the community level. It is well known that species exposed to low light conditions within the canopy produce leaves that are characterized by a high leaf area per leaf biomass or a high chlorophyll content (Evans and Poorter 2001, Valladares and Niinemets 2008), while leaves of species in upper layers exposed to high light conditions tend to have thicker leaves (Körner 1993, Anten 2005). Small-statured species increased specific leaf area (SLA) and chlorophyll concentrations, while decreasing leaf nitrogen per unit area when growing in mixtures compared to monocultures (Daßler et al. 2008, Roscher et al. 2011a). Furthermore, leaf morphological traits (such as SLA) at peak canopy development have been shown to differ among species within the functional groups of grasses and legumes, suggesting increased complementarity in light acquisition (Gubsch et al. 2011, Roscher et al. 2011b). Thus, leaf traits can respond rather plastically to changing light availability. However, although light use has often been investigated in terms of spatial niche differentiation, its role for temporal niche differentiation has rarely been assessed.

In the present study, we addressed complementarity in light use and its temporal development using both direct and indirect approaches: we measured light attenuation as well as different morphological and physiological leaf traits related to light acquisition in

plant communities of increasing species richness in a large grassland biodiversity experiment (Jena Experiment, Roscher et al. 2004), which is based on a pool of 60 grassland species assigned to four plant functional groups. Specifically, the traits were specific leaf area (SLA), leaf dry matter content (LDMC), leaf greenness (as surrogate for chlorophyll content) and stomatal conductance. SLA is often measured to assess light acquisition strategies; larger values of SLA are expressed under low light conditions as a larger leaf area per leaf mass achieved through the formation of thinner leaves enables increased light capture (Weiher et al. 1999, Hodgson et al. 2011). LDMC is also known as a trait indicating adjustment to light conditions; LDMC correlates positively with irradiance (Poorter et al. 2010). Shaded leaves usually have higher chlorophyll concentrations than sun leaves (Valladares and Niinemets 2008). We estimated chlorophyll content using a chlorophyll meter, which enables fast and non-destructive assessment of leaf greenness. In addition to gradients in light availability between upper and lower canopy layers, temperature and vapor pressure deficit decrease within the canopy of closed vegetation stands (Niinemets and Valladares 2004), which eventually affects gas exchange. Therefore, stomatal conductance, which is expected to decrease at low light availability (Valladares and Niinemets 2008), was assessed.

Thus, we addressed the following questions: (i) How does light attenuation within the canopy change depending on species and functional group richness as well as time of the year? We expected that light attenuation along the vertical canopy profile and thus the the potential presence of light niches increase with increasing species richness and that plant diversity effects on the light niche are stronger shortly before mowing when the canopy is fully developed rather than during regrowth or at the beginning of the growing season. (ii) How do leaf traits vary with increasing species and functional group richness as well as throughout the growing season? Since light attenuation is expected to increase with increasing species and functional group richness, we expected to find effects of increased plant diversity on the expression of leaf traits. In more detail, we expected SLA, leaf greenness and g_s to increase but LDMC to decrease with increasing species richness. Furthermore, we hypothesized that species adjust leaf traits plastically to temporal changes in light availability. (iii) Do functional groups differ in their strategies in light use and, thus, occupy different light niches? We expected to find small-statured species to adjust more plastically to changes in light conditions than tall-statured species. (iv) Does functional dissimilarity of leaf traits within a community vary with increasing species and

functional group richness and throughout the growing season? Increasing light attenuation along the canopy profile with increasing species richness and at peak biomass times might led to increased spatial variation in light availability. Thus, we expected increased differences (or functional dissimilarity) in leaf trait expression among species as indicator for complementary light use at the community level.

Material and Methods

Study site

The Jena Experiment is the largest European grassland biodiversity experiment (Roscher et al. 2004). It has been established in 2002 and is located in the floodplain of the River Saale close to the city of Jena (Germany; 50°55'N, 11°35'E, 130 m a.s.l.). Mean annual air temperature is 9.3°C, and annual precipitation sums up to 587 mm (Kluge and Müller-Westermeier 2000). The experiment consists of 82 plots, covering a plant species richness gradient of 1, 2, 4, 8, 16 and 60 species, combined with a gradient of 1, 2, 3 and 4 functional groups (grasses, legumes, small herbs and tall herbs). Plots are arranged in four blocks to account for variation in soil texture caused by different distances to the river. Species richness levels are equally replicated within each block. The species mixtures were randomly assembled out of a pool of 60 grassland species common in Central Europe. Further details are given in Roscher et al. (2004). For the present study, a subset of 40 plots were chosen, including mixtures of 2, 4, 8 and 16 species, each with 10 replicates, distributed equally among the experimental blocks. The plots were weeded regularly, i.e., three times in 2011 (4 to 11 April 2011, 13 to 15 June 2011, 12 to 14 September 2011). Management mimics extensively used hay meadows with no fertilization and mowing twice per year. Mowing took place 30 to 31 May 2011 and 29 to 30 August 2011.

Leaf trait measurements

The leaf traits measured were specific leaf area (SLA), leaf dry matter content (LDMC), leaf greenness and stomatal conductance (g_s). Leaf traits were assessed for all species available in each plot. Measurements were repeated five times during the growing season: during 14- to 17-Apr-, 24- to 27-May-, 23- to 26-Jun-, 23- to 26-Aug- and 22- to 25-Sep-2011, resulting in two measurement campaigns at peak biomass shortly before mowing

(May and August), one at the beginning of the growing season (April), and two during the regrowth phase after mowing (June and September).

Leaf greenness (unitless), an estimate of chlorophyll concentration, was assessed by measuring the absorption of two different wavelengths (650 nm and 940 nm) with a portable chlorophyll meter (SPAD-502, Konica-Minolta, Osaka, Japan) on a young, but fully expanded leaf of three shoots per species per plot. We found a good correlation (r^2 = 0.69, $P < 0.001$) between measured leaf greenness values of the chlorophyll meter and chlorophyll concentrations from leaf extracts sampled from all species included in our study (data not shown). For the same leaves, stomatal conductance (mmol m^{-2} s^{-1}) was measured with a portable porometer (SC-1 Leaf porometer, Decagon Devices, Pullman, USA) in the Auto mode for 30 seconds. After finishing these measurements, which critically depend on stable weather conditions, three to five fully expanded leaves of different shoots per species per plot were collected. Leaf samples were put in moist tissue paper and stored at 4 °C for 6-10 hours in sealed plastic bags to promote rehydration. Then, leaves were blotted dry with tissue paper to remove any water droplets and immediately weighed to determine their fresh weight. Afterwards, the leaf area was quantified with a portable LI-3000A leaf area meter (LICOR, Lincoln, USA). All samples were then dried for 48 hours at 70 °C and weighed (dry weight). SLA was calculated as the ratio of leaf area to dry weight in mm^2 mg^{-1}, LDMC as the ratio of dry weight to fresh weight in mg g^{-1}.

Measurements of canopy characteristics

In parallel to the leaf trait measurements, canopy height (cm) was determined at five individual points within each plot. Light intensity (PPFD in µmol m^{-2} s^{-1}) along a canopy profile was measured at five heights (3, 10, 20, 30 and 150 cm above soil surface) once in each plot, using five PAR sensors (PQS 1, Kipp&Zonen, Delft, The Netherlands) fixed on a portable rod and placed into the canopy for single point measurements. We calculated relative light transmission as the ratio of light intensity within the canopy divided by the light intensity at reference height (150 cm) for each height. Light attenuation was then calculated as (1-relative light transmission at 3 cm above soil surface) and expressed in percent.

Data analyses

All statistical analyses and graphics were done using the statistical software R 2.14.1 (R Development Core Team 2011), including the packages *lme4* (Bates et al. 2011) and *multcomp* (Hothorn et al. 2008). Values of leaf greenness and stomatal conductance of the three different shoots were averaged per species per plot for each sampling campaign. Plant trait data were analyzed with mixed-effects models using the *lmer* function within the *lme4* package. Prior to analyses, data were log-transformed to achieve normally distributed within-group errors, a requirement for linear mixed models. The analysis was started with a constant null model containing the following random effects: block, plot identity (nested within block) and species identity. Fixed effects and interactions were added stepwise in the following order: time of year (Time), species richness (SR, log-linear), functional group richness (FR, linear), functional group identity (FG.ID), Time x SR, Time x FR, Time x FG.ID, SR x FG.ID and SR x FG.ID x Time. The maximum likelihood method and likelihood ratio tests (χ^2 ratio) were used to test for a significant improvement of the model after step-wise adding the fixed effects. Tukey's HSD tests were used to identify differences among times and functional groups by applying the *glht* function of the *multcomp* package. Additionally, the effects of time of year, species richness and their interaction on the leaf traits were analyzed separately for each functional group.

The procedure of statistical analyses of relative light transmission, light attenuation and canopy height were similar to the analyses described above, with block and plot identity (nested within block) as random effects and time of year (Time), species richness (SR, log-linear), functional group richness (FR), and height of measurement (not for canopy height) as fixed effects. Since relative light transmission is a percentage variable, data was transformed using arcsine square root transformation to fulfill the requirement of normally distributed within-group errors for the mixed model. Canopy height was log-transformed prior to analysis as well.

Furthermore, to quantify the dissimilarity in leaf trait expression within the community, functional trait diversity was calculated separately for each leaf trait as quadratic entropy of Rao (Rao 1982)

$$FD_Q = \sum_{i=1}^{S} \sum_{j=1}^{S} d_{ij} p_i p_j,$$

where S is the number of species in the community, d_{ij} is the pairwise Euclidean distance in trait values of species *i* and *j*, p_i and p_j are the abundance values of species *i* and *j* in the community (Botta-Dukát 2005). In the present study, the abundance of species was given as presence = 1 and absence = 0. Data of all traits were log-transformed prior calculation to fulfill requirement of normality. Calculations were done using the *FD* package in R (Laliberté and Legendre 2010, Laliberté and Shipley 2011). The effect of time of year (Time), species richness (SR, log-linear), functional group number (FR, linear) and their interactions on functional diversity of each trait was tested similar to the procedure described above with block and plot identity (nested within block) as random factors. Functional diversity of each trait was log-transformed prior to analysis to meet the assumption of normally distributed within-group errors. Tukey's HSD tests were used to identify differences among times.

Results

Canopy characteristics

Light availability within the canopy differed significantly among species richness levels, height of measurement and time of year (Table 1). Differences were most pronounced at peak biomass in May and August (Fig. 1), with most pronounced profiles in the 16-species mixtures. Light transmission at the top and mid canopy (i.e., 10 and 20 cm above soil surface) was lower in mixtures of increased species richness compared to low diversity mixtures (Table 1, Fig. 1). Average values of relative light transmission at 3 cm above soil surface were 0.32 in May and 0.20 in August 2011 (i.e. at estimated peak development of the canopy) in the 16 species mixtures. Thus, light attenuation at 3 cm above soil surface reached values of 68% in May and of 80% in August 2011. In May and August, light attenuation tended to be higher in the 4-species (52% and 64% light attenuation at 3 cm in May and August, respectively) than in the 8-species mixtures (43% and 46% light attenuation 3 cm in May and August, respectively; Fig. 1). In contrast, in April as well as in June and September, relative light transmission were almost unchanged throughout the canopy profile and light attenuation at 3 cm above soil surface was typically smaller than 25% (Fig. 1, Fig. 2B).

Canopy heights did not significantly differ along the species richness gradient, but strongly among times of the year (Table 1). Tallest canopies were found in May and

August (Fig. 2A), when the canopy was fully developed and thus light attenuation was the highest (Fig. 2B). In accordance with the patterns of light attenuation, canopy height was lower in the 8-species mixtures than in 4-species mixtures and similar in the 2-species mixtures in May and August (Fig. 2A).

Variation of leaf trait expression with time and diversity

All leaf traits measured differed significantly with time (Table 2), but temporal differences varied with species richness (LDMC and leaf greenness; Table 2) and functional group identity (LDMC, leaf greenness, g_s, Table 2). None of the measured leaf traits varied with increasing species richness throughout the growing season (except SLA) or with functional group richness (Table 2). SLA increased with increasing species richness (Fig. S1 A-E), irrespective of time (non-significant interaction Time x SR; Table 2). Separate analyses of traits for May and August, when canopy was fully developed and light conditions differed among the species richness levels, also did not reveal effects of species richness on the trait expression. (ESM, Table S1).

Differences among plant functional groups in trait expression and their variation with time and diversity

The four functional groups differed in LDMC, leaf greenness and g_s, but not in SLA (Table 2). Expression of all traits varied temporally for each functional group (significant interactions of FG.ID x Time in the full model as well as in the separate analyses for each functional group, Table 2). Furthermore, SLA and leaf greenness differed along the species richness gradient for specific functional groups (significant SR x FG.ID interaction in full model and significant effect of SR in separate analyses for small herbs, grasses and legumes, Table 2).

Specifically, while functional groups did not differ in SLA in general, the temporal patterns in trait values varied among functional groups. Small herbs and tall herbs showed highest SLA values in August and September (Fig. 3 A-D), while SLA of grasses and legumes hardly differed over the growing season. Furthermore, small herbs and grasses showed an increase in SLA with increasing species richness (Fig. 4 A and C). Grasses displayed highest values of LDMC (as indicated by the multiple comparisons; Fig. 4 E-H). The temporal patterns of LDMC were similar for all functional groups, with increasing values from April to May and decreasing values towards September (Fig. 3 E-H). Legumes had highest values of leaf greenness compared to other functional groups,

which showed similar values (Fig. 4 I-L). Furthermore, leaf greenness of legumes increased from April to May and stayed high during the growing season, while decreasing for small herbs, tall herbs and legumes (Fig. 4I-L). Legumes showed increasing values of leaf greenness along the species gradient, while increasing species richness did not affect leaf greenness in non-legume functional groups (Table 2, Fig. 3 I-L). Lowest values of g_s were observed for grasses, while the highest values were found for tall herbs (Fig. 4 M-P). Stomatal conductance of tall herbs and legumes decreased from April to May, while it increased after mowing in June and was lower again in August and September. Furthermore, g_s were highest in June for small herbs and lowest in May for grasses, while the other seasons did not differ significantly in these functional groups (Fig. 3 M-P). Differential effects of species richness on leaf trait expression of different functional groups did not depend on season (non-significant interaction SR x FG.ID x Time; Table 2).

Functional dissimilarity of leaf traits

Functional dissimilarity of all traits significantly differed throughout the growing season (Table 3; except for leaf greenness) and increased with increasing species richness (Table 3, Fig. 5 F-T; except for SLA). Moreover, functional dissimilarity of LDMC and leaf greenness increased with functional group richness. However, the effects of species richness and functional group richness did not vary with time (non-significant interaction Time x SR and Time x FR interactions Table 3).

Discussion

As light attenuation within the canopy increases (and relative light transmission decreases) with increasing plant species richness, while concurrently biomass production also increases, the aim of this study was to assess if species growing in more diverse mixtures use light more effectively than species in low diverse mixtures. We used direct measurements of light intensity to describe the light availability and therefore the potential presence of light niches within the canopy as well as their temporal development. Furthermore, we measured morphological and physiological leaf traits and analyzed if species growing in communities of increased plant diversity adjust to spatial and temporal variations in light availability and therefore increase complementary light use.

How does light attenuation within the canopy change depending on species and functional group richness as well as time of the year?

We found large temporal variations in the vertical light profiles due to the management of the grassland, with strongest light attenuation in May and August at peak biomass before mowing. In contrast, when canopies were short as in April at the beginning of the growing season and during regrowth after mowing in June and September, the low canopy heights in all mixtures exerted no effect on the vertical light profiles, which in turn did not differ among communities of varying species richness. According to our expectations, we found increased light attenuation from 2 to 16-species mixtures at peak biomass times due to higher and denser canopies. However, averaged light attenuation in the 16-species mixtures (May 68%, August 80% in 2011) was lower compared to other studies (97% in 32-species mixtures, 87% in 8-species mixtures; Spehn et al. (2000)), probably due to lower canopy biomass and/or density due to the rather dry spring conditions compared to other years (Marquard et al. 2013).

Contrary to our expectations, light attenuation in May and August was higher in the 4- than in the 8-species mixtures, although this was in line with their canopy height. Differences in species composition such as a higher proportion of grasses in the 8-species mixtures than in the 4-species mixtures might explain this unexpected pattern of light attenuation: Nine of the ten mixtures with eight sown species contained grass species, while only five of the ten 4-species mixtures did. Grasses are known to express vertically oriented leaves in contrast to herb species with more horizontally arranged leaves. Thus, mixtures containing more grasses have a lower light attenuation towards the ground (Jones 1992) than mixtures containing less grasses and therefore more plants with rather horizontally orientated leaves (e.g. herbs, legumes).

In brief, direct measurements of light availability along a vertical canopy profile clearly showed that light attenuation strongly changed over time and was stronger in high diverse compared to low diverse mixtures at peak biomass times. If leaf traits respond to these changing light conditions, we would expect similar patterns in leaf trait expression during the growing season as well as along the species richness gradient.

How do leaf traits vary with increasing species and functional group richness as well as throughout the growing season?

All leaf traits varied during the growing season, but their temporal patterns differed among the studied traits and did not reflect the temporal variations in light availability (except for g_s). Stomatal conductance showed lowest values at both times of peak biomass (ESM, Fig. S1), which might be caused by the lower light availability within the canopy at these times or lower soil water potential. Contrary to our expectations leaf traits did not change significantly with increasing species or functional group richness, except for SLA that slightly increased with increasing species richness. Hence, although light attenuation showed pronounced temporal variations, particularly with increasing species richness at peak canopy development, we did not find an overall adjustment of the measured leaf traits to these changing light conditions, neither temporally nor along the diversity gradient. Since SLA and LDMC were found to reflect also soil fertility (Al Haj Khaled et al. 2005, Hodgson et al. 2011, Pérez-Harguindeguy et al. 2013), the effect of light availability on leaf trait expression might be superimposed by nutrient availability. As legumes are well known to positively affect plant available nitrogen through the fixation of atmospheric N_2 (Hartwig 1998), we tested the effects of legume presence/absence in our experimental plant communities using additional models (legume presence fitted before species richness, see Table S2 in ESM). These models provided further evidence that legume presence had positive effects on leaf greenness, and species richness effects on leaf greenness became statistically significant after accounting for legume presence. In contrast, legume presence did not influence trait values of SLA, LDMC and g_s, while positive effects of increased species richness on SLA disappeared, when fitted after legume presence. Thus, improved soil fertility through legume presence might have affected SLA in our study, which is in line with a previous study on grasses (Gubsch et al. 2011). Furthermore, the anatomical constitution of the leaves might limit adaption to changing light conditions as suggested by Niinemets (2007) and Hallik et al. (2009), who did not find a relationship between SLA and light conditions either. Thus, leaf trait expression – often used as indirect measurement of resource niches – did not reflect variable light conditions and the potential presence of light niches observed via direct measurements, possibly due to a functional trade-off to optimize the use of other resources than light, such as nutrients.

Do functional groups differ in their strategies in light use and thus occupy different light niches?

The functional groups differed significantly regarding all plant traits, except SLA. SLA of small herbs and grasses increased with increasing species richness, probably indicating an adjustment to increased light attenuation or improved nitrogen nutrition, in line with previous results for small herbs (Daßler et al. 2008, Roscher et al. 2011a) and for grasses (Gubsch et al. 2011). Furthermore, the functional groups displayed temporal changes in leaf trait expression, although the patterns were not in line with those in light availability. SLA values of small herbs and tall herbs were highest in August and September, while grasses and legumes expressed only slight temporal changes. Although light attenuation suggested the strongest presence of light niches in May and August, increasing SLA values might also reflect reduced investment in structural tissues towards the end of the growing season. The temporal patterns of LDMC were rather similar for all functional groups, with highest values in May and decreasing values towards the end of the growing season, although we found a significant interaction of functional group identity with time. In general, grasses expressed higher values of LDMC compared to the other functional groups, in line with other studies (Al Haj Khaled et al. 2005, Ansquer et al. 2009).

In terms of leaf greenness, legumes clearly differed from all other functional groups, which displayed rather similar values. Higher chlorophyll concentrations in legumes, as indicated by the higher leaf greenness values compared to the other functional groups, might be due to their ability to fix atmospheric nitrogen in symbiosis with root bacteria. Due to this additional nitrogen source, they are less dependent on the soil nitrogen pool (Temperton et al. 2007) and might be able to invest more nitrogen into light harvesting compounds such as chlorophyll. We had furthermore expected to find increased leaf greenness in small herbs, as higher chlorophyll content is often suggested as a mechanism to adapt to low light environments (Valladares and Niinemets 2008, Roscher et al. 2011a), but leaf greenness was found to be similar for small herbs, tall herbs and grasses, maybe due to insufficient sensitivity of the chlorophyll meter used. The temporal patterns of g_s differed among the functional groups, with grasses showing lowest values in g_s compared to the other functional groups. This is in line with the 'low nutrient strategy' grasses are often associated with. Characterized by dense tissues, low nitrogen concentrations and low rates of physiological activity, this strategy seems to enable

grasses to be less dependent on water and less susceptible to herbivory than other functional groups (Craine et al. 2002). Thus, our analyses revealed that the expression of leaf traits differed strongly among functional groups, clearly suggesting differences in light use niches, but potentially also confounding effects of covarying environmental factors (e.g. nutrient availability) at increased species and functional group richness.

Does functional dissimilarity of leaf traits within a community vary with increasing species and functional group richness and throughout the growing season?

Strongest vertical light profiles in light attenuation as found in highly diverse communities suggested increased variation in light availability and therefore the potential presence of light niches available to plant species and functional groups. Consequently, we expected variations of leaf traits within the community calculated as average functional dissimilarity to be larger in highly diverse communities, thus, increasing the opportunities for complementary light use. In our study functional dissimilarity of all traits at community level (except SLA) increased with increasing species richness at each time of year and increasing functional richness (except SLA, g_s only as a trend) throughout the growing season suggesting that the absence of species richness effects at the single trait level was compensated when species composition was included at the community level (Petchey et al. 2009). Furthermore, highly diverse communities might increase the variation in trait expression in response to multipe resources compared to only one resource such as light, thus increasing the diversity in overall resource use strategies (Roscher et al. 2012) throughout the year. For example, Milcu et al. (2014) observed higher dissimilarity in leaf nitrogen concentrations in highly diverse compared to less diverse communities at the Jena Experiment, which might indicate optimization of canopy photosynthesis according to leaf nitrogen as well as light availabilities. Thus, although existing light niches at the functional group level did not change along the species richness gradient, functional dissimilarity at the community level clearly increased with increasing plant diversity, enabling diverse communities to use light more effectively, as seen in the higher light attenuation observed in this study.

Conclusions

Comparing two different approaches often used to infer light niches in plant communities yielded different results. While direct measurements of vertical light profiles revealed a large potential for light niches being present along the species richness gradient at peak

biomass, indirect measurements of leaf morphological and physiological traits related to light did not support the use of this potential. Leaf trait expression did not change with plant diversity and did not follow the same temporal pattern as light profiles within the canopy. Although other resources than light also impact on leaf traits, e.g., nitrogen, the observed differences suggest that the community level bears further mechanisms how complementarity in resource use can be expressed.

One of these mechanisms is functional dissimilarity that takes not only leaf traits but also community composition into account. Although leaf traits were not affected by plant diversity, they differed among functional groups. Consequently, functional dissimilarity increased with species richness throughout the growing season, independent of management. This enabled species-rich communities to use light more effectively (at peak biomass) than species-poor communities as clearly demonstrated by the light attenuation profiles. Our results seem to suggest that although functional dissimilarity does not allow identifying the underlying mechanisms of the diversity effect, it might be the better "currency" to evaluate complementarity between plant communities of varying diversities.

Acknowledgements

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Table 1 Summary of the mixed-effects models testing the effects of time of year, species richness, and height of the measurement on relative light transmission, light attenuation, and the effects of time of year and species richness on canopy height.

	Relative light transmission		Light attenuation			Canopy height	
	X^2 ratio	*P*	X^2 ratio	*P*		X^2 ratio	*P*
Time	181.01	**< 0.001**	148.98	**< 0.001**	Time	211.68	**< 0.001**
Species richness (SR, log-linear)	11.36	**< 0.001**	9.83	**0.002**	Species richness (SR, log-linear)	1.65	0.199
Measurement height	132.35	**< 0.001**	341.38	**< 0.001**	Time x SR	3.43	0.489
Time x SR	13.74	**0.008**	15.33	**0.004**			
Time x Measurement height	214.14	**< 0.001**	314.46	**< 0.001**			
SR x Measurement height	12.71	**0.005**	26.05	**< 0.001**			
Time x SR x Measurement height	11.06	0.524	19.13	0.262			

Fixed effects were added stepwise to an initial null model. Variance components were estimated using maximum likelihood method. χ^2 ratio tests were used to test for significant improvement of the model after adding fixed effects. Significant factors and interactions are formatted in bold.

Table 2 Summary of the mixed-effects models testing the effects of time of year, species richness, functional group richness and functional group identity on specific leaf area (SLA), leaf dry matter content (LDMC), leaf greenness, and stomatal conductance.

		SLA		LDMC		Leaf greenness		Stomatal conductance	
		X^2 ratio	*P*	X^2 ratio	*P*	X^2 ratio	*P*	X^2 ratio	*P*
Time		138.80	**< 0.001**	296.67	**< 0.001**	143.55	**< 0.001**	108.33	**< 0.001**
Species richness (SR, log-linear)		3.90	**0.050**	0.34	0.560	1.33	0.249	0.61	0.435
Functional group richness (FR)		0.63	0.430	0.21	0.644	1.45	0.229	1.22	0.270
Functional group identity (FG.ID)		5.06	0.170	56.95	**< 0.001**	27.70	**< 0.001**	58.34	**< 0.001**
Time x SR		3.60	0.463	13.21	**0.010**	10.44	**0.034**	3.68	0.451
Time x FR		3.77	0.439	4.77	0.312	3.26	0.516	2.54	0.647
Time x FG.ID		76.24	**< 0.001**	50.06	**< 0.001**	109.00	**< 0.001**	36.32	**< 0.001**
SR x FG.ID		11.30	**0.010**	4.17	0.244	10.36	**0.016**	3.43	0.330
SR x FG.ID x Time		19.43	0.079	9.56	0.654	12.68	0.393	5.33	0.946
Small herbs									
	Time	90.93	**< 0.001**	81.49	**< 0.001**	101.50	**< 0.001**	24.97	**< 0.001**
	SR	5.46	**0.020**	0.57	0.460	0.73	0.393	2.54	0.111
	Time x SR	9.04	0.060	13.38	**0.010**	8.35	0.080	2.44	0.655
Tall herbs									
	Time	224.73	**< 0.001**	103.64	**< 0.001**	74.75	**< 0.001**	38.10	**< 0.001**
	SR	0.04	0.842	4.20	**0.040**	1.86	0.173	0.08	0.774
	Time x SR	6.22	0.184	2.47	0.649	8.96	0.062	0.89	0.926
Grasses									
	Time	22.05	**< 0.001**	125.62	**< 0.001**	58.71	**< 0.001**	45.98	**< 0.001**
	SR	4.24	**0.040**	0.14	0.707	0.40	0.526	0.28	0.597
	Time x SR	2.84	0.585	2.08	0.721	5.47	0.243	2.50	0.645
Legumes									
	Time	10.59	**0.032**	35.83	**< 0.001**	23.70	**< 0.001**	31.19	**< 0.001**
	SR	1.52	0.217	0.25	0.620	4.10	**0.043**	0.41	0.520
	Time x SR	5.77	0.217	4.49	0.344	5.43	0.246	2.64	0.619

Fixed effects were added stepwise to an initial null model. Variance components were estimated using maximum likelihood method. χ^2 ratio tests were used to test for significant improvement of the model after adding fixed effects. Effects of time of year and species richness were tested for each functional group in separate models. Significant factors and interactions are formatted in bold.

Table 3 Summary of the mixed-effects models testing the effects of time of year, species richness and functional group richness on functional diversity of specific leaf area (FD_Q SLA), leaf dry matter content (FD_Q LDMC), leaf greenness (FD_Q Leaf greenness), and stomatal conductance (FD_Q g_s).

	FD_Q SLA		FD_Q LDMC		FD_Q Leaf greenness		FD_Q g_s	
	X^2 ratio	*P*	X^2 ratio	*P*	X^2 ratio	*P*	X^2 ratio	*P*
Time	20.88	**< 0.001**	12.02	**0.017**	0.81	0.937	15.75	**0.003**
Species richness (SR, log-linear)	1.96	0.162	8.27	**0.004**	13.25	**< 0.001**	5.03	**0.025**
Functional group richness (FR)	1.68	0.195	8.80	**0.003**	9.10	**0.003**	3.29	0.070
Time x SR	5.74	0.219	2.98	0.561	6.18	0.186	1.66	0.799
Time x FR	1.19	0.880	4.37	0.358	1.38	0.848	5.53	0.237
SR x FR	0.13	0.721	0.00	0.983	0.40	0.526	2.02	0.155
Time x SR x FR	5.89	0.207	5.17	0.271	5.76	0.218	7.64	0.106

Fixed effects were added stepwise to an initial null model. Variance components were estimated using maximum likelihood method. χ^2 ratio tests were used to test for significant improvement of the model after adding fixed effects. Significant factors and interactions are formatted in bold.

List of figures

Fig. 1 Relative light transmission as a function of height and species richness between April and September 2011. Different symbols indicate species richness levels (SR). Means and ± 1 SE (N = 5-10) are presented. Symbols are connected by lines for reasons of clarity.

Fig. 2 Canopy height (A) and light attenuation (B) averaged per species richness level between April and September 2011. Different symbols indicate species richness levels (SR). Means and ± 1 SE ($N_{Canopy\ height}$ = 10, $N_{Light\ attenuation}$ = 5-10) are presented. Arrows in lower panel indicate times of mowing.

Fig. 3 Boxplots of specific leaf area (SLA; A-D), leaf dry matter content (LDMC; E-H), leaf greenness (I-L) and stomatal conductance (g_s; M-P) for each functional group between April and September 2011. Small letters indicate significant differences among times for each functional group per trait. Range of number of replicates over all times for each functional group: $N_{Small\ herbs}$ = 70-73, $N_{Tall\ herbs}$ = 50-74, $N_{Grasses}$ = 50-74, $N_{Legumes}$ = 24-41. Some outliers were removed for reasons of clarity (N = 1 – 6 per boxplot).

Fig. 4 Boxplots of specific leaf area (SLA; A-D), leaf dry matter content (LDMC; E-H), leaf greenness (I-L), and stomatal conductance (g_s; M-P) for each functional group along the species richness gradient. Small letters indicate significant differences among functional groups per trait. Range of number of replicates over all species richness levels for each functional group: $N_{Small\ herbs}$ = 35-135, $N_{Tall\ herbs}$ = 15-177, $N_{Grasses}$ = 25-166, $N_{Legumes}$ = 6-84.

Fig. 5 Boxplots of functional diversity of specific leaf area (FD_Q SLA; A-D), leaf dry matter content (FD_Q LDMC; E-H), leaf greenness (FD_Q Leaf greenness; I-L), and stomatal conductance (FD_Q g_s; M-P) along the species richness gradient separately for April to September 2011. Small letters indicate significant differences among times of the year over all species richness levels for each trait. Range of number of replicates over all

species richness levels for each functional group: $N_{Small\ herbs}$ = 35-135, $N_{Tall\ herbs}$ = 15-177, $N_{Grasses}$ = 25-166, $N_{Legumes}$ = 6-84. N = 10 plots per species richness level per time

Figure 1

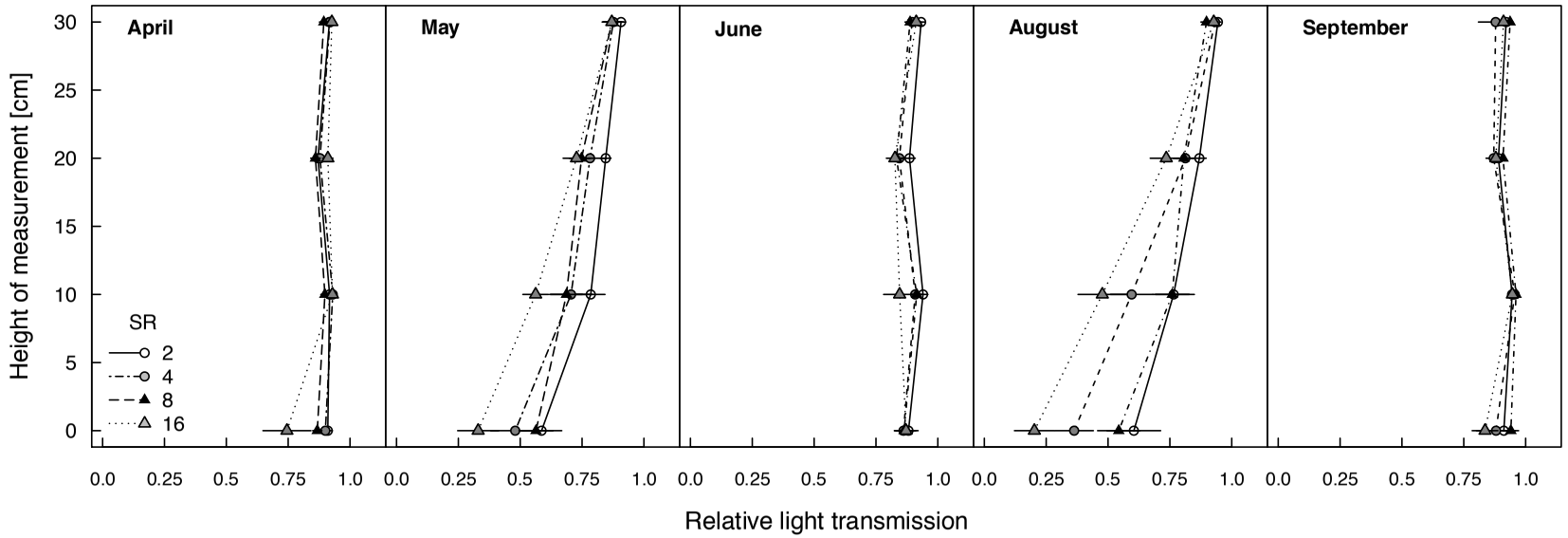

April
May
June
August
September
Height of measurement [cm]
0
5
10
15
20
25
30
SR
2
4
8
16
0.0
0.25
0.5
0.75
1.0
Relative light transmission

Figure 2

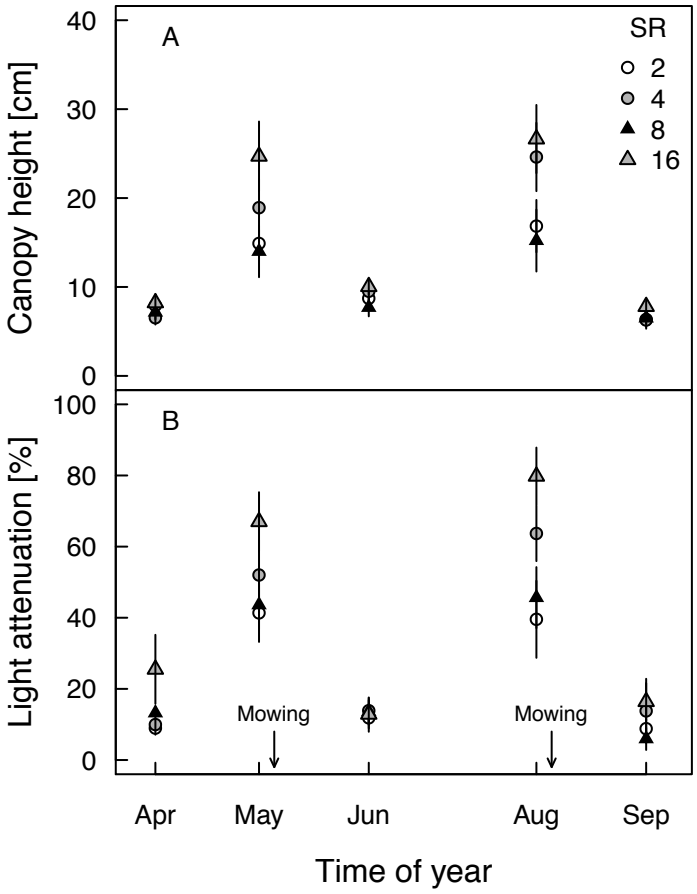

A
B
SR
2
4
8
16
Canopy height [cm]
Light attenuation [%]
Mowing
Mowing
Apr
May
Jun
Aug
Sep
Time of year

Figure 3

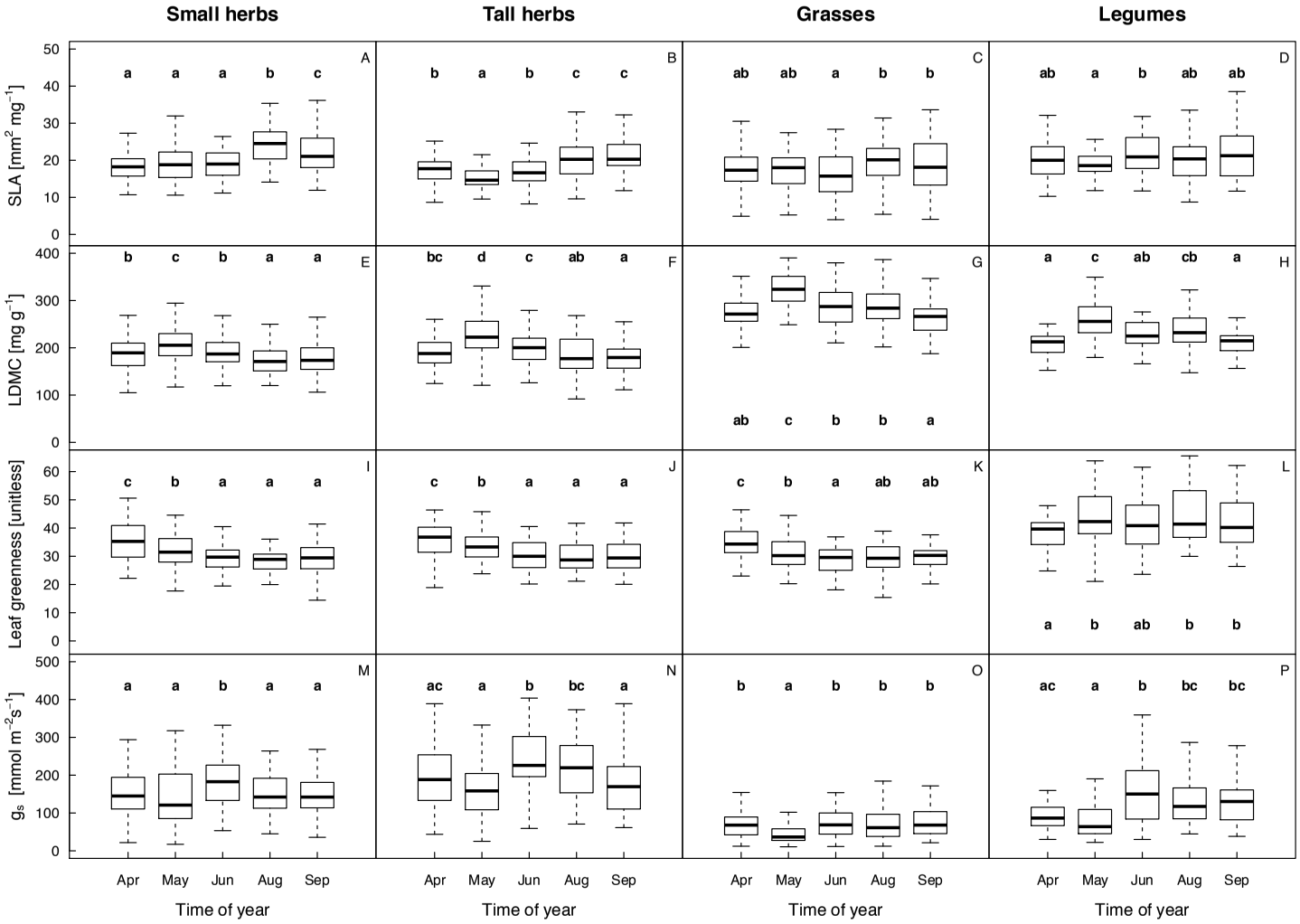
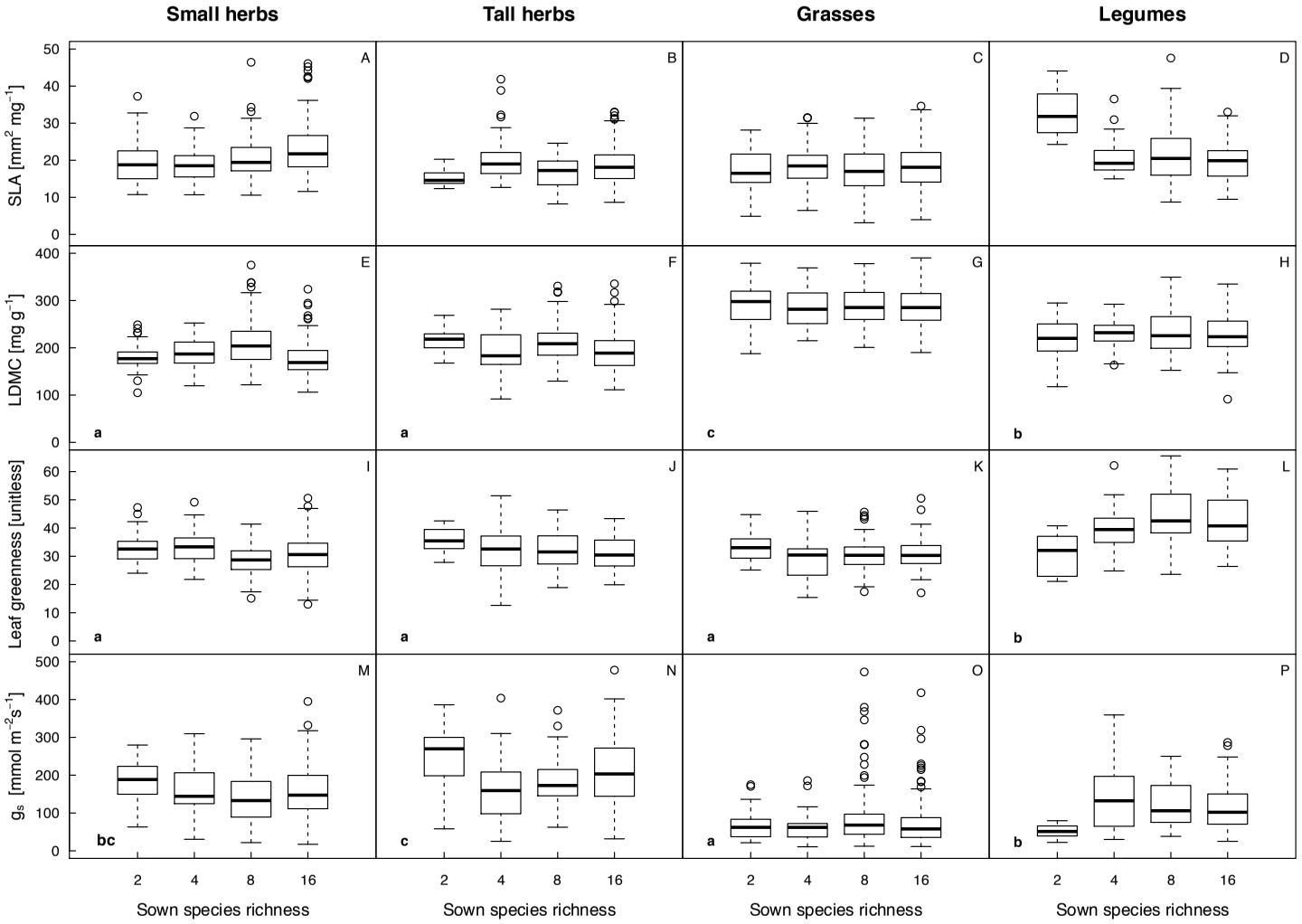

Small herbs
Tall herbs
Grasses
Legumes
SLA [mm² mg⁻¹]
LDMC [mg g⁻¹]
Leaf greenness [unitless]
gs [mmol m⁻²s⁻¹]
Apr
May
Jun
Aug
Sep
Time of year

Figure 4


Small herbs
Tall herbs
Grasses
Legumes
SLA [mm² mg⁻¹]
LDMC [mg g⁻¹]
Leaf greenness [unitless]
gs [mmol m⁻²s⁻¹]
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
a
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a
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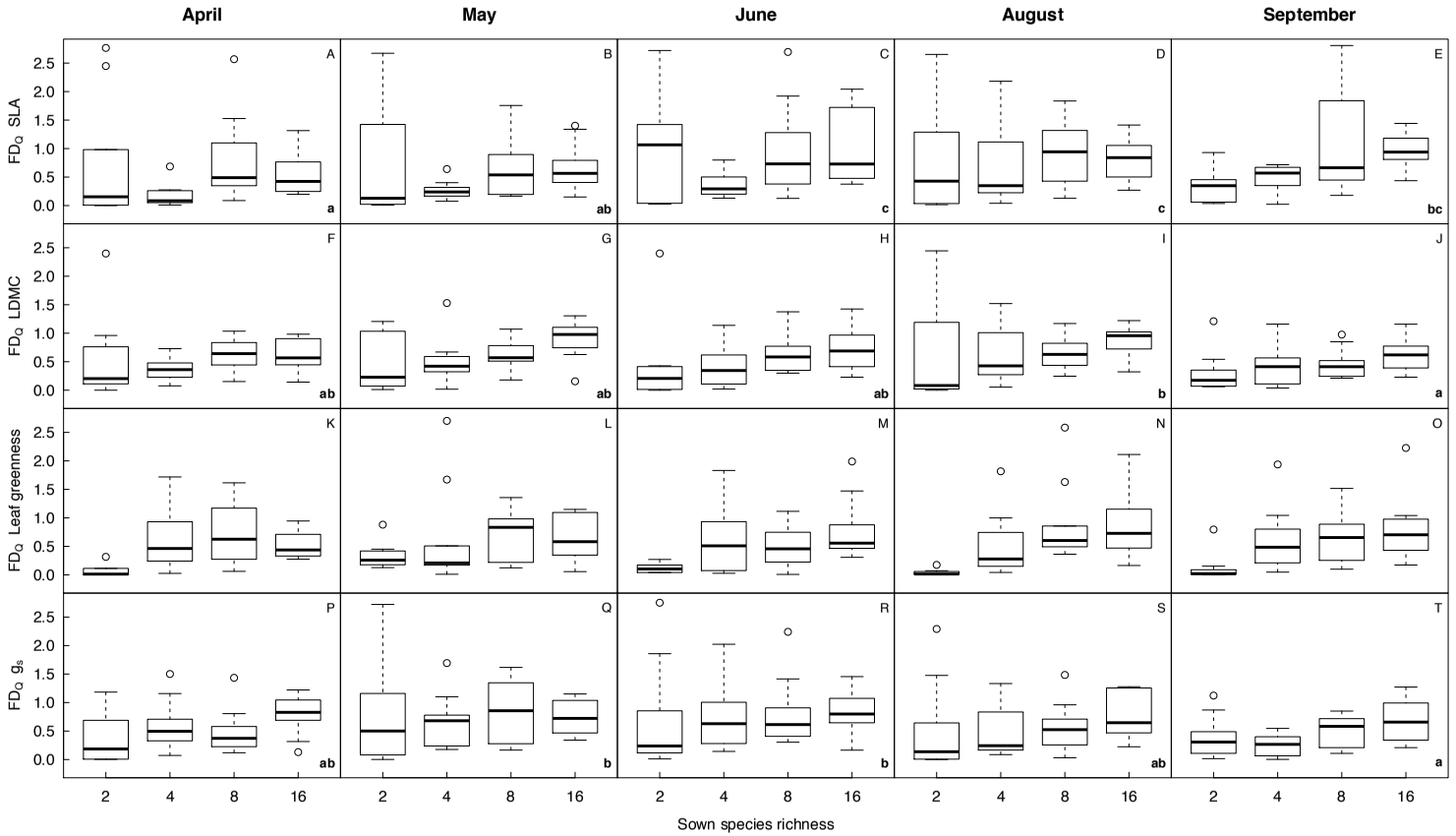
c
a
b
2
4
8
16
Sown species richness

Figure 5


April
May
June
August
September
FDQ SLA
FDQ LDMC
FDQ Leaf greenness
FDQ gs
A
B
C
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Sown species richness

Supporting information

Fig. S1 Boxplots of specific leaf area (SLA; A-E), leaf dry matter content (LDMC; F-J), leaf greenness (K-O) and stomatal conductance (g_s; P-T) along the species richness gradient separately for April to September 2011. Small letters indicate significant differences among times of the year over all species richness levels for each trait. Range of number of replicates over all times and the four traits for each species richness level: $N_{2\text{-species}}$ = 15-20, $N_{4\text{-species}}$ = 33-38, $N_{8\text{-species}}$ = 53-70, $N_{16\text{-species}}$ = 90-117.

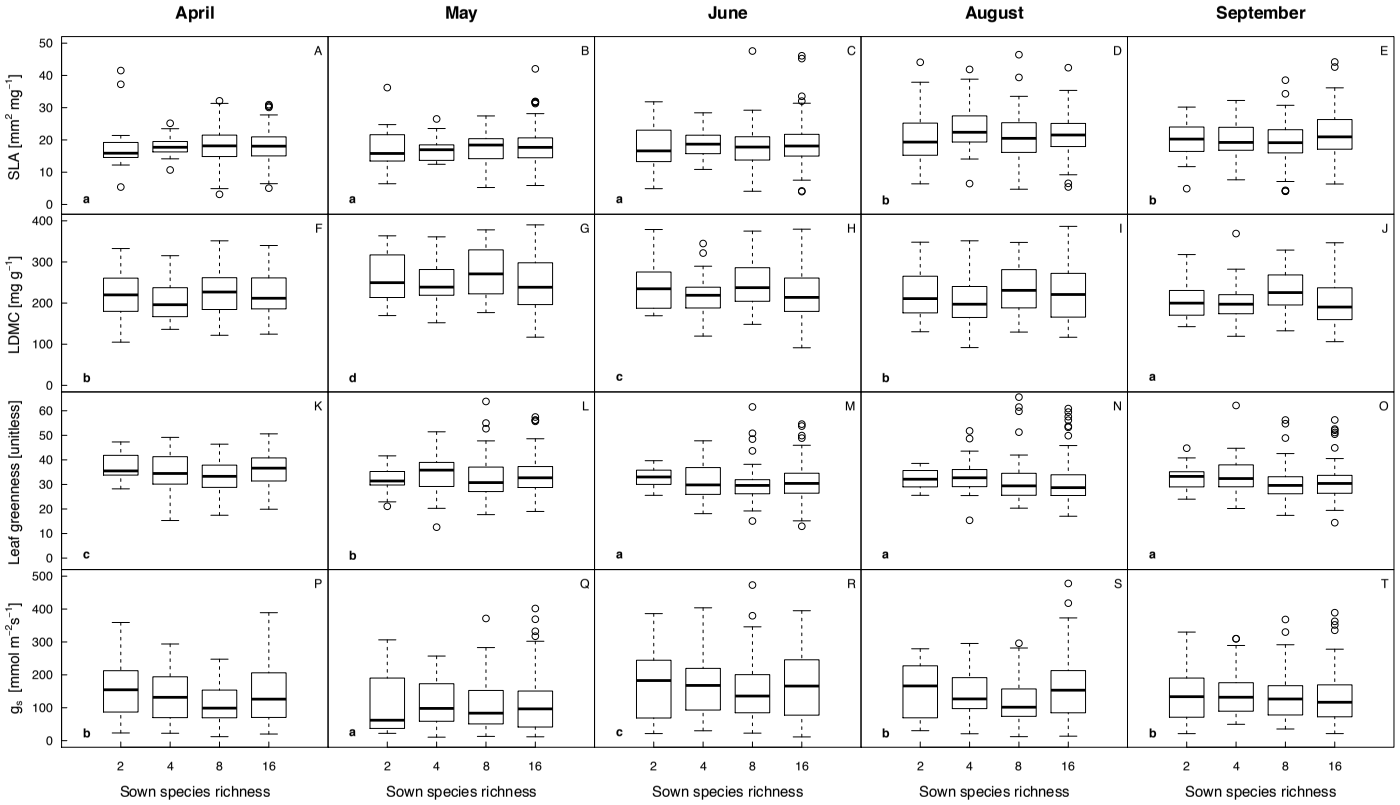

Table S1 Summary of the mixed-effects models testing the effects of species richness, functional group richness and functional group identity on specific leaf area (SLA), leaf dry matter content (LDMC), leaf greenness, stomatal conductance measured in May and August 2011. Fixed effects were added stepwise to an initial null model. Variance components were estimated using maximum likelihood method. χ^2 ratio tests were used to test for significant improvement of the model after adding fixed effects. Significant factors and interactions are formatted in bold.

	SLA				**LDMC**				**Leaf greenness**				**Stomatal conductance**			
	May		**August**		**May**		**August**		**May**		**August**		**May**		**August**	
	***X*² ratio**	***P***	***X*² ratio**	***P***	***X*² ratio**	***P***	***X*² ratio**	***P***	***X*² ratio**	***P***	***X*² ratio**	***P***	***X*² ratio**	***P***	***X*² ratio**	***P***
Species richness (SR, log-linear)	2.19	0.139	0.84	0.361	1.22	0.270	0.89	0.345	0.19	0.664	3.65	0.056	0.31	0.580	0.02	0.902
Functional group richness (FR)	0.14	0.711	0.46	0.500	0.43	0.510	0.86	0.354	3.57	0.059	1.05	0.305	0.05	0.819	0.53	0.466
Functional group identity (FG.ID)	8.75	**0.033**	6.50	0.090	46.66	**< 0.001**	51.46	**< 0.001**	20.98	**< 0.001**	40.03	**< 0.001**	47.62	**< 0.001**	51.19	**< 0.001**
SR x FG.ID	10.04	**0.018**	10.14	**0.017**	10.12	**0.018**	0.35	0.950	6.13	0.105	7.59	0.055	3.44	0.329	3.64	0.304

Table S2 Summary of the mixed-effects models testing the effects of time of year, legume presence, species richness, functional group richness and functional group identity on specific leaf area (SLA), leaf dry matter content (LDMC), leaf greenness, and stomatal conductance.

	SLA		LDMC		Leaf greenness		Stomatal conductance	
	X^2 ratio	*P*	X^2 ratio	*P*	X^2 ratio	*P*	X^2 ratio	*P*
Time	138.80	**< 0.001**	296.67	**< 0.001**	143.54	**< 0.001**	108.33	**< 0.001**
Legumes presence	3.34	0.068	1.75	0.187	9.52	**0.002**	0.60	0.441
Species richness (SR, log-linear)	2.73	0.098	0.04	0.839	5.14	**0.023**	0.91	0.341
Functional group richness (FR)	0.13	0.719	0.46	0.496	5.86	**0.016**	0.40	0.525
Functional group identity (FG.ID)	4.91	0.179	56.73	**< 0.001**	26.39	**< 0.001**	58.45	**< 0.001**
Time x SR	3.61	0.462	13.24	**0.010**	9.97	**0.041**	3.66	0.454
Time x FR	3.76	0.439	4.77	0.312	3.20	0.525	2.53	0.640
Time x FG.ID	76.14	**< 0.001**	50.03	**< 0.001**	107.83	**< 0.001**	36.26	**< 0.001**
SR x FG.ID	10.24	**0.017**	3.76	0.288	15.22	**0.002**	3.59	0.309
SR x FG.ID x Time	19.37	0.080	9.53	0.657	12.86	0.379	5.27	0.948

Chapter 3

Functional diversity of leaf nitrogen concentrations drives grassland carbon fluxes

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Abstract

Little is known about the role of plant functional diversity for ecosystem-level carbon (C) fluxes. To fill this knowledge gap, we translocated monoliths hosting communities with four and 16 sown species from a long-term grassland biodiversity experiment ("The Jena Experiment") into a controlled environment facility for ecosystem research (Ecotron). This allowed quantifying the effects of plant diversity on ecosystem C fluxes as well as three parameters of C uptake efficiency (water and nitrogen use efficiencies and apparent quantum yield). By combining data on ecosystem C fluxes with vegetation structure and functional trait-based predictors, we found that increasing plant species and functional diversity led to higher gross and net ecosystem C uptake rates. Path analyses and light response curves unravelled the diversity of leaf nitrogen concentration in the canopy as a key functional predictor of C fluxes, either directly or indirectly via LAI and above-ground biomass.

Introduction

A large body of empirical studies support the emerging consensus that biodiversity loss reduces the efficiency by which ecological communities capture biologically essential resources (Cardinale et al. 2012). Spread over more than 20 years, the bulk of these studies focused on biomass production (Tilman et al. 2001; Duffy 2003; Cardinale et al. 2012). However, little information is available about the effects of plant species richness on the carbon (C) fluxes underpinning the capacity of ecosystems to increase biomass and act as C sinks (Stocker et al. 1999; Johnson et al. 2008; Klumpp & Soussana 2009; Hirota et al. 2010). In this context, to achieve a thorough mechanistic understanding, it is not only necessary to understand the effects of plant species richness on the net ecosystem CO_2 exchange (NEE) but also to understand the contribution of component fluxes such as gross ecosystem productivity (GPP) and ecosystem respiration (Reco) to the total ecosystem C flux. The handful of studies that explicitly attempted to partition the C flux generally found that decreasing plant diversity significantly decreased NEE and GPP, with inconsistent effects on Reco (Stocker et al. 1999; Hirota et al. 2010). However, the underlying mechanism why canopies containing leaves of more species are able to fix more C is little understood because very few studies looked beyond biomass-related effects (Vojtech et al. 2008; Wacker et al. 2009). Consequently, even less is known if

(and how) plant diversity affects key ecosystem parameters related to C uptake efficiency such as water use efficiency (WUE; carbon gain at the expense of water loss), nitrogen use efficiency (NUE; C gain per N content per leaf area) and canopy apparent quantum yield (AQY; C gain per available light quantum flux density) – all of which being important functional parameters that intimately couple the uptake of C with the major growth limiting factors (water, nitrogen and light).

Recently, several studies showed that the diversity of functional traits, not the taxonomic richness, ultimately drives biodiversity–ecosystem functioning relationships because traits are better at capturing the functional complementarity of a community (Cadotte et al. 2009; Flynn et al. 2011; Milcu et al. 2013). There is evidence that the use of species traits in ecology significantly contributes to achieving a predictive framework for ecosystem functioning (Wright et al. 2004; Violle et al. 2007; Reiss et al. 2009; Osnas et al. 2013). However, to date, we know little about which are the functional traits that drive the different components of the ecosystem-level C fluxes (GPP, Reco and NEE) and the relevant C uptake efficiency parameters (WUE, NUE and AQY), and how functional trait–ecosystem functioning relationships vary with plant species richness.

To address this knowledge gap, we took advantage of a long-term biodiversity experiment ('The Jena Experiment'; Roscher et al. 2004) and an advanced controlled environment facility for ecosystem research (Ecotron), which allows for continuous measurements of ecosystem-level C and water fluxes. Large monoliths originating from ecosystems sown with four and 16 species established 9 years ago were used to test the overarching hypothesis that increasing plant diversity leads to increased C uptake, and that the increased functional complementarity at higher diversity is better captured by functional trait-based diversity indices than by species richness. Specifically, we aimed to (1) quantify the effect size of plant diversity on ecosystem C fluxes (GPP, Reco and NEE) and three parameters of C uptake efficiency (WUE, NUE and AQY) during the growing season, (2) identify the most relevant/predictive plant functional trait- based metrics [community weighted means and Rao's quadratic entropy diversity index (FD_Q) derived from functional traits] and (3) explore the nature of the interactions between species richness, functional trait-based predictors and the conventional metrics of vegetation structure that underpin the C fluxes using path analysis.

Materials and Methods

Plant communities

Plant communities originated from the long-term Jena Experiment (50° 55′ N, 11° 35′ E, 130 m above sea level; mean annual temperature 9.3 °C, mean annual precipitation 587 mm). Located on the floodplain of the Saale River (Jena, Germany), the site was a former arable field until May 2002, when 82 large plots (20 x 20 m) varying in plant species richness (1–60 species), plant functional groups (grasses, herbs and legumes) and plant identity were established, simulating a random loss of species from the local Arrhenatherion grasslands (Roscher et al. 2004). Twelve plots were selected according to the following criteria: (1) all three plant functional groups were present, (2) realised species numbers were close to sown species richness and (3) plots were equally distributed across the experimental blocks of the field site to account for different soil textures. The selected plots (Table S1) included two sown diversity levels (four and 16 species) with six independent replicates per diversity level and met the aforementioned criteria with the exception of one plot were no grasses had been sown. Soil monoliths selected to be representative (as percentage vegetation cover and standing biomass) of the plots they originate from, were extracted in lysimeters (2 m^2, diameter of 1.6 m and 2 m depth, weighing 7–8 tonnes) in December 2011 following an established non-compacting extraction method (see Supporting Information). After extraction, the lysimeters were buried to the surface level near the experimental field. This facilitated the recovery after the extraction disturbance, while being exposed to the same environmental conditions as the plots, before being transported to the Ecotron facility at the end of March 2012.

The CNRS Ecotron facility

The Montpellier European Ecotron is a new experimental infrastructure developed by the Centre National de la Recherche Scientifique (CNRS, France) to study the response of ecosystems to global changes. The lysimeters were allocated randomly to the 12 controlled environment units of the macrocosms platform. Each unit consists of a 30 m^3 transparent dome situated on top of a dedicated lysimeter room. A material highly transparent to light and UV radiation (250 µm thick Teflon-FEP film, DuPont, USA) is used as cover for the domes. Within each dome, the main abiotic (air temperature, humidity and CO_2 concentration) characteristics of the atmospheric compartment of ecosystems are controlled, and the soil surface and canopy of the lysimeter are exposed to

natural sunlight and the controlled atmosphere (Fig. S1). Air speed at 50 cm above the soil is 1 (± 0.3 SEM.) m s^{-1} as measured by a thermoelectric flow sensor (FV-A605-TA; Ahlborn Mess- und Regelungstechnik GmbH, Holzkirchen, Germany). The lysimeter room hosts the soil monolith, the weighting system (4 CMI-C3 shear beam load cells per lysimeter, Precia-Molen, Privas, France), soil sensors, a soil temperature control system and Marriott's bottles emulating a constant belowground water table. The lysimeters were kept under controlled conditions in the Ecotron for 4 months, throughout the phase of high vegetative growth (end of March to end of July), until the experiment ended with a destructive harvest. The imposed climatic regime aimed to simulate the average climatic conditions in the Jena Experiment since 2002. As the spring–summer conditions of year 2007 were very close to the average temperature and precipitation regimes, the 10-min-interval field recorded weather data containing the daily profiles of air temperature and humidity were imposed as climatic set points in the Ecotron. Throughout the experiment the averaged air temperature achieved was close to the set point (14.0 °C vs. 14.9 °C in Jena). However, the achieved averaged air humidity was somewhat lower (58.9% RH vs.73.4% RH in Jena) as the humidifying system had to be occasionally stopped to prevent wetting the vegetation when the set points were higher than 80% RH. The monoliths were exposed to slightly higher temperatures during transport and prior to installation in the Ecotron. Consequently, we opted for increasing the precipitation by + 28% relative to 2007 (Fig. S2) in order to achieve similar soil moisture conditions (Fig. S3). As the incoming radiation [as estimated from the HelioClim-1 database for the two locations (Blanc et al. 2011)] in Jena is in average 37% lower than in Montpellier during the April to July period, a black shading mesh was added on the inside of each dome, which reduced the incoming radiation by 44%. Target plant communities were maintained by regular weeding, and the aboveground biomass was mown at the end of April and at the end of July to recreate the mowing management of the Jena Experiment. The final harvest took place at the time of the July mowing and included destructive soil and root sampling.

Carbon flux measurements

The CNRS Ecotron was designed to continuously measure CO_2-NEE (NEE = GPP-Reco) by sequentially measuring the CO_2 concentration at the inlet and outlet of each dome (every 12 min) using a multiplexer system coupled with two LI-7000 CO_2/H_2O analysers (LI-COR Biosciences, Lincoln, NE, USA). We used the Reichstein et al. (2005) C flux

partitioning algorithm to estimate the daytime ecosystem respiration (Reco-day) based on an exponential regression model (Lloyd & Taylor 1994) (see Supporting Information). This allowed for the estimation of ecosystem respiration over 24h (Reco = Reco-night + Reco-day) and gross primary production (GPP = NEE-day - Reco-day). Ecosystem WUE was estimated as the ratio of GPP to ecosystem evapotranspiration derived from measurements by lysimeter weight changes over 24h. Although NEE is continuously measured in the Ecotron, the CO_2 fluxes were frequently and unavoidably disturbed during experimental work (watering, weeding, mowing, sampling, checks, etc.) by the respiration of the persons entering the domes. Hence, testing diversity effects on undisturbed CO_2 fluxes and water use efficiency could only be done with values of four undisturbed days for each month.

Light response curves

The response of ecosystem NEE to the available photon flux density measured as photosynthetic active radiation (PAR) was explored during 3 days with available clear sky from sunrise to midday (in July). Although several other equations have been used to describe photosynthetic light response curves, we chose the non-rectangular hyperbola as it is commonly used in ecophysiological studies. It gives an excellent phenomenological description of leaf and canopy photosynthesis and is partly derived from a Michaelis and Menten's mechanistic understanding of biochemical reactions within the chloroplast (Thornley 1976, 1998). In addition, it estimates four biologically meaningful parameters: (1) AQY, the apparent canopy quantum yield (μmol CO_2 μmol photons^{-1}), a measure of maximum photochemical efficiency estimated as the slope of the photosynthetic CO_2 uptake in the morning when PAR was below 200 μmol m^{-2} s^{-1}, and with the assumption of no other limiting factors; (2) maxNEE, maximum rate of NEE at saturating light intensity; (3) θ, the curvature of the hyperbola, a unitless parameter defining the degree of curvature between light limited and CO_2 limited parts of the response and (4) Reco-night, the rate of night-time respiration. The equation is:

$$\mathrm{NEE} = \frac{\mathrm{AQY}*\mathrm{PAR}+\mathrm{maxNEE}-\sqrt{(\mathrm{AQY}*\mathrm{PAR}+\mathrm{maxNEE})^2-4*\Theta*\mathrm{AQY}*\mathrm{PAR}*\mathrm{maxNEE}}}{2\Theta} - \mathrm{Reco\text{-}night}$$

Vegetation structure and functional trait predictors

As predictors of C fluxes we tested: (1) a set of 12 vegetation structure-related predictors directly measured at the final harvest in July, including aboveground shoot biomass (ShootBM), total root biomass (RootBM), root biomass by depth (0–5, 5– 10, 10–20, 20–30, 30–60 cm), total biomass (TotalBM), shoot biomass of legumes (LegBM), shoot biomass of grasses (GrassBM), shoot biomass of herbs (HerbBM), leaf area index (LAI), leaf to shoot biomass ratio (Leaf-to-ShootBM-ratio) and percentage bare ground (% Bare-ground), and (2) a set of functional trait-based predictors including functional diversity indexes and community weighted means (CWM) calculated from ten plant functional traits that have been previously shown to be linked to plant photosynthetic rates, transpiration and light interception (see Table S2 for an overview of response variables and predictors). We chose a functional diversity measure based on Rao's quadratic entropy (FD_Q) (Botta Dukát 2005) as index of functional diversity as it incorporates information about functional distance as well as functional evenness (abundance-weighted) of a community. For each plant species, the following functional traits originate from in situ plot measurements taken before the final destructive harvest: stomatal conductance (μmol m^{-2} s^{-1}), specific leaf area (SLA; mm^2 mg^{-1}), leaf greenness (unitless measure of foliar chlorophyll content), leaf dry matter content (LDMC; mg g^{-1}), leaf N concentration (leafN%; mg N g^{-1} leaf), species-specific plant height (cm) and specific leaf nitrogen (SLN; g N m^{-2} leaf). Literature surveys were used for seasonality of foliage (ordinal, 1 = summer green, 2 = partly evergreen, 3 = evergreen), rooting type (ordinal, 1 = long-living primary root system, 2 = secondary fibrous roots in addition to the primary root system, 3 = short living primary root system, extensive secondary root system) and rooting depth (cm) as used by Roscher et al. (2004). FD_Q was calculated for each of the ten functional traits separately, all available traits simultaneously (FD_Q-all) and only leaf-related traits (FD_Q-leaf).

FD_Q and CWM for each trait were calculated using the 'FD' package (Laliberté & Legendre 2010) available through the R statistical package version 2.15.0 (R Core Team 2012).

Statistical analyses

Temporal dynamics of NEE, GPP, Reco and WUE as affected by sown species richness (Sdiv) were analysed by repeated measures ANOVA, followed by contrast analysis

(planned comparisons) testing for species richness effects at each month. As the plant diversity effect generally increased with time after the April weeding and mowing and the plant trait data were collected in July, the mechanisms through which plant species richness affect the CO_2 fluxes were explored in further detail using the data from the last experimental month (July 2012). Two approaches were used. First, we aimed at identifying the most important predictors by fitting simple univariate regression models for each predictor as well as any potential covariates such as soil texture (% clay content in soil) and climatic conditions (air temperature, air humidity and soil moisture). These were then simultaneously run through a model averaging procedure (*dredge* function in *MuMIn* package, R software) to select the most relevant predictors based on Akaike weights (AICw), which represent the probability that a particular model is the best fit to the observed data (Burnham & Anderson 2002). The predictors found within the 95% confidence interval (cumulative AICw ≤ 0.95) were selected and simultaneously included in a multivariate linear fitting regression model using the *lm* (package *stats*, R software) function. The multivariate regression models were then simplified to reach the most parsimonious models by using the automatic model simplification '*step*' procedure based on AICc (Venables & Ripley 2002).

The second approach aimed to understand the importance and relationship between the different predictors of NEE, GPP, Reco, WUE, NUE and AQY with the help of path analysis, which allows the evaluation of multiple and complex causal hypotheses. For each response variable, full models were designed to test whether the functional trait-based predictors were directly or indirectly (via vegetation structure) affecting the response variables. As FD_Q was inherently weighted by realised species percentage cover, we used realised species richness (Rdiv) in the path analyses [note the high correlation between Rdiv and Sdiv (Pearson's $r_{\text{realised, sown}} = 0.93$)]. The model also tested the importance of the pathways between Rdiv and the functional and vegetation structure predictors as well as a direct pathway between Rdiv and the response variable (see Fig. S4 for a simplified schematic of the full model). A LAI-to-ShootBM pathway was also included; while the measured LAI obviously does not have an immediate mechanistic effect on the standing biomass, the cumulative invest- ment in light acquisition by the community represented by LAI, will have had an effect on biomass production.

Because the functional diversity of leaf nitrogen concentration (FD_Q-leafN%) and of specific leaf nitrogen (FD_Q-SLN) turned out to be particularly important, we also

performed path analyses to understand their relationship with the other predictors. Minimal adequate models were achieved using the 'specification search' procedure by AIC as available in *SPPS Amos 20* statistical package (Arbuckle 2011). Good model fits were indicated by non-significant differences between the predicted and observed covariance matrices (χ^2 tests with $P > 0.05$), lower AIC, lower Root Mean Squared Error Approximation (RMSEA < 0.05) and higher Comparative Fit Index (CFI > 0.90) (Grace 2006; Arbuckle 2011).

Results

Species richness effects

Realised species richness (Rdiv) in the Ecotron was very similar to the Jena field-recorded values in the summer of 2012 (Pearson's $r_{Ecotron,\ field} = 0.96$). The effect of sown plant species richness (Sdiv) on C fluxes (GPP, Reco and NEE) became significant after the first mowing and weeding at the end of April (Fig. 1). Hence, we further concentrate on the results from June and July, at least 1 month after the first mowing. GPP was 49.2% ($F_{1/10} = 8.11$, $P = 0.046$) higher in June and 47.1% ($F_{1/10} = 8.11$, $P = 0.017$) in July in communities with 16 relative to four sown species (Fig. 1a). Reco was not significantly affected by Sdiv in June and only a marginally statisti- cally significant increase was detected in July ($F_{1/10} = 4.71$, $P = 0.055$) (Fig. 1b). NEE was 48.5% ($F_{1/10} = 6.16$, $P = 0.032$) and 35.1% ($F_{1/10} = 4.28$, $P = 0.065$) higher in June and July, respectively, in communities with 16 sown plant species (Fig. 1c).

WUE was 51.6% higher ($F_{1/10} = 6.13$, $P = 0.032$) in June and 37.6% higher in July ($F_{1/10} = 6.55$, $P = 0.028$) in communities with 16 sown plant species relative to four species (Fig. 2a). July measurements of NUE were 65.3% higher in the plots with 16 species ($F_{1/10} = 11.13$, $P = 0.007$; Fig. 2b) whereas the AQY was marginally significantly higher (+ 34.6%; $F_{1/10} = 3.7$, $P = 0.082$; Fig. 2c).

Most parsimonious predictors

When only vegetation structure-related predictors were included (Table S3), GPP increased with both ShootBM and LAI, and was best predicted by the ShootBM + LAI ($r^2 = 0.87$, $P < 0.001$) model. Reco also increased with Shoot- BM ($r^2 = 0.66$, $P = 0.001$), whereas NEE increased with LAI ($r^2 = 0.44$, $P = 0.019$). In the models based on

functional trait predictors, GPP increased with the functional diversity of leaf N concentrations (FD_Q-LeafN%) in the canopy ($r^2 = 0.46$, $P < 0.017$). Reco also increased with FD_Q -LeafN% ($r^2 = 0.49$, $P = 0.011$), whereas NEE increased with the community weighted means of the height of plant individuals (CWM-height) ($r^2 = 0.39$, $P = 0.029$).

WUE increased with ShootBM ($r^2 = 0.69$, $P < 0.001$), NUE decreased with the percentage bare ground in the community ($r^2 = 0.59$, $P < 0.003$) and AQY increased with the biomass of legumes ($r^2 = 0.35$, $P = 0.04$). When only functional trait-derived indices were considered, WUE ($r^2 = 0.40$, $P = 0.028$) and NUE ($r^2 = 0.52$, $P = 0.008$) increased with FD_Q-leafN% (Fig. 2d, e) whereas AQY with FD_Q-SLN ($r^2 = 0.29$, $P = 0.011$) (Fig. 2f).

Path analyses

Minimal adequate models were achieved for all response variables (Table 1, Fig. 3). No direct pathway between realised species richness (Rdiv) and the C-uptake-related response variables was retained in the minimal models, indicating that indirect pathways explained the effect of Rdiv through several response variables, notably the FD -leafN% and FD –SLN (Fig. 3).

For GPP ($r^2 = 0.94$), the effect of Rdiv occurred via increasing FD_Q-leafN%, which in turn increased LAI with direct and indirect (via ShootBM) effects on GPP (Fig. 3, Table 1). The Rdiv effect on GPP was also explained by increasing functional diversity calculated from all leaf functional traits (FD_Q-leaf). Reco was affected by Rdiv through increasing FD_Q-leafN%, which directly and indirectly (via a LAI–to- ShootBM pathway) further affected Reco ($r^2 = 0.83$). For NEE, the Rdiv effect occurred via increasing FD_Q-leafN%, which in turn directly and indirectly (via increasing the LAI) increased NEE ($r^2 = 0.74$). In addition, the community weighted mean of the height of individual species (CWM-height) was also retained as a significant predictor for NEE, but this predictor was not correlated with Rdiv.

The Rdiv effect on WUE ($r^2 = 0.89$) was explained through two pathways. The first pathway showed an increase in WUE with increasing FD_Q-leafN% via an LAI-to-ShootBM pathway. The second pathway identified an increase in WUE with increasing diversity of specific leaf nitrogen in the canopy (FD_Q-SLN) (Fig. 3, Table 1). NUE was

also affected by Rdiv through two pathways, increasing with FD_Q-leafN% and decreasing with the percentage of bare ground in the community (Fig. 3, $r^2 = 0.77$). AQY increased with FD_Q-SLN, the amount of legume biomass (LegBM) and the community weighted means of the rooting depth (CWM-rooting depth) (Fig. 3, $r^2 = 0.76$). However, increasing Rdiv only increased the FD_Q-SLN and did not significantly influence the other two predictors (Fig. 3).

As FD_Q-leafN% and FD_Q-SLN proved to be important predictors, we further conducted two additional path analyses aiming to explore the relationships between these predictors and the remaining explanatory variables. We found, alongside the presence of legumes (LegBM), that parameters related to light acquisition strategy such as diversity of the height of individuals (FD_Q-height) and diversity of specific leaf area of the species in the community (FD_Q-SLA) were important predictors of FD_Q-leafN% ($r^2 = 0.85$). However, a direct/non-explained pathway from Rdiv to FD_Q-leafN% was also retained in the model. FD_Q-SLN increased with the functional diversity calculated from all leaf traits (FD_Q-leaf, $r^2 = 0.50$). Scatterplots with regression lines depicting the strength of the pathways presented in Fig. 3 are available in Fig. S5.

Light response curves

As we found little hysteresis between morning and afternoon values of NEE (Fig. 4a), we further fitted non-rectangular hyperbola regression curves for the available 3 days with clear sky from sunrise to midday. We further tested the most parsimonious predictors for the four fitted parameters (see methods). As expected, the predictors for Reco-night and maxNEE were generally in line with the results for Reco and GPP (Table 2). The curvature (θ) was best predicted by the leaf-to-shoot biomass ratio when only vegetation structure predictors were included. When functional trait-based indices were included (Table 2) a model containing the functional diversity of stomatal conductance and community weighted means of the height of individuals (FD_Q-gs + CWM-height) was retained. As FD_Q-SLN and FD_Q-leafN% appear as important predictors for the light response curves (Table 2) and path analyses (Fig. 3), we further contrasted the non-rectangular hyperbola fitted for communities with the three lowest and highest FD_Q-SLN (Fig. 4b) and FD_Q-leafN% (Fig. 4c) to emphasise the importance of functional diversity of leaf N concentrations. We found an increase in AQY of 35.3% ($F_{1/4} = 11.16$, P =

0.029) in communities with higher FD_Q-SLN (Fig. 4b) and an increase of 39.1% in maxNEE ($F_{1/4} = 14.09$, $P = 0.020$) in communities with higher FD_Q-leafN% (Fig. 4c).

Discussion

By combining data on ecosystem-level C fluxes (GPP, Reco and NEE), C uptake efficiencies (WUE, NUE and AQY), vegetation structure and functional trait predictors, this study provides novel insights into the importance of functional diversity and species richness for ecosystem functioning. Previous field studies conducted in 'The Jena Experiment' found that soil C storage significantly increased with plant diversity 4 years after the onset of the experiment, but uncertainty remained whether this was due to significantly higher C fixation or lower ecosystem respiration rates at higher diversity (Steinbeiss et al. 2008). This study found that ecosystem respiration (Reco) was not reduced, but marginally increased in lysimeters with 16 species, and that the increase in GPP during daytime more than com- pensated the increased respiration, leading to significantly higher NEE (+ 48.5 and + 35.1% for June and July, respectively), at least during the summer months.

We also document plant species and functional diversity effects on WUE and AQY, parameters generally overlooked by biodiversity–ecosystem functioning experiments. Although ecosystem evapotranspiration was higher in the lysimeters with 16 sown plant species relative to four species (not shown), the higher GPP at 16 species led to the WUE being 51.6% higher in June and 37.6% higher in July (Fig. 2a). This is in agreement with studies showing that photosynthetic processes are the dominant regulator of seasonal variations in WUE (Hu et al. 2008; Niu et al. 2011), but also with results of the only other study that specifically looked at plant diversity effects on water use efficiency (De Boeck et al. 2006).

Light response curves have been often used to model leaf and canopy photosynthetic responses (Cannell & Thornley 1998), but have been rarely used to investigate plant diversity effects on C fluxes. Using a similar approach, Stocker et al. (1999) found an increase in C uptake with plant diversity and elevated atmospheric CO_2 concentration. Here, in addition to fitting light response curves, we identified the relevant predic- tors for the parameters fitted by the non-rectangular hyperbola function, including AQY. Alongside biomass and LAI effects, we found that the functional diversity indices based

on leaf N concentrations (as measured by FD_Q-leafN% and FD_Q-SLN) are retained as relevant predictors for three of the four parameters (AQY, Rdark and maxNEE) describing the non-rectangular hyperbola function. To our knowledge this is the first study attempting to link ecosystem C light response curves to functional diversity indices; we identified predictors whose relevance needs to be further tested in order to extend our understanding from species-specific light response curves (Marino et al. 2010) to ecosystem-level responses. For example, the FD_Q-gs+CWM-height model predicted best the cur- vature of the light response curves (θ) (Table 2). At leaf level, θ is assumed to occur due to the transition between Rubisco-limited and RuP2-regeneration-limited rates of photosynthesis (Marshall & Biscoe 1980; Thornley 1998). However, at com- munity level, θ will depend on the community-aggregated light interception and CO_2 exposure. Hence, metrics related to the height of the species (CWM-height) and the diversity of stomatal conductance (FD_Q-gs) are likely relevant.

Our analyses identified the diversity of leaf nitrogen concentration in the canopy (as measured by FD_Q-leafN% and FD_Q-SLN) as a key functional predictor of C fluxes as it increased with species richness while outperforming species richness, either directly or indirectly via LAI and above- ground biomass effects (Fig. 3). This is in line with earlier findings from 'The Jena Experiment' that FD_Q-leafN% was one, although not the most important, predictor of community biomass over time (Roscher et al. 2012, 2013). Both FD_Q-leafN% and FD_Q-SLN indices can be viewed as indices of the unevenness of N allocation in the canopy. One mechanism through which a canopy with more diverse/uneven leaf N concentration can affect C uptake is through the formation of vertical N concentration profiles, with leaf N concentrations decreasing from top to bottom of the canopy following the decrease in light availability. In individual plants and monocultures a more optimal canopy N distribution has been found to lead to 10–30% higher C acquisition due to increased NUE (Field 1983; Hirose & Werger 1987; Anten et al. 1995), however the importance of the diversity of inter- and intraspecific leaf N to this mechanism has not been so far explicitly considered in biodiversity–ecosystem functioning experiments. Although we did not measure the light and canopy N profiles directly, we provide several indirect lines of evidence in support of the conjecture that, at least in our study, FD_Q-leafN% and FD_Q-SLN can be considered good proxies for the gradient of vertical leaf N distribution within the canopy. First, we found higher NUE, AQY and maxNEE in plots with larger interspecific differences in leaf N concentration

(Fig. 4b, c and Table 2). Second, path analyses show that FD_Q-leafN% is actually related to the diversity of the height of individual species in the different communities (FD_Q -height) as well as to specific leaf area (FD_Q -SLA) (Fig. 3). Both SLA and species height are good indicators of the likely position of a plant in a canopy (as both variables are often successfully used in describing plant strategies and identify subordinate and dominant species) (Wilson et al. 1999; Falster & Westoby 2003), with higher FD_Q values sug- gesting a canopy with individuals occupying more space/light niches in the canopy. Third, the slopes of the relationship between height and leaf N concentration or SLA calculated for each community showed a tendency of being higher and consistently positive in the 16 species compared to four species mixtures (Fig. S6). In support of these findings, two studies conducted in the Jena Experiment document plastic adjustments of leaf traits as an adaptive response to the different light conditions experienced in a plant diversity gradient; a decrease in leaf N per unit area in subordinate species was found with increasing species richness (Daßler et al. 2008; Roscher et al. 2011).

Broadly, our results are in line with the findings of Vojtech et al. (2008), emphasising the role of aboveground complementary use of canopy space for biomass production, but also with the findings of Wacker et al. (2009) hinting that more diverse communities might be better at assembling canopy N profiles to increase C gain. However, with the available data we cannot entirely rule out a different causation for the observed correlations between the diversity of leaf N concentrations and C fluxes. For instance, we found that both leaf N-based diversity indices were strongly correlated with the functional diversity index derived from all traits (FD_Q-all), with Pearson's $r_{FDQ\text{-}leafN\%,\ FDQ\text{-}leaf} = 0.71$ and $r_{FDQ\text{-}SLN,\ FDQ\text{-}\ leaf} = 0.70$. This suggests that the various light and C acquisition strategies of grassland species are well represented by functional diversity indices capturing the distribution of leaf N concentration in the canopy.

In conclusion, our results provide strong support for the hypothesis that ecosystems harbouring more plant species achieve higher C uptake (higher GPP and NEE) and increased nitrogen, light and water use efficiencies. Furthermore, we found that ecosystem C fluxes are better predicted by functional trait diversity indices based on leaf N concentration than by species richness alone. More generally, the results emphasise the importance of functional trait-based and Ecotron approaches to bridge the gap between biodiversity–ecosystem functioning and carbon flux research areas.

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Tables

Table 1 Results of path analyses for the most parsimonious adequate models for gross primary production (GPP), ecosystem respiration (Reco), net ecosystem exchange (NEE), water use efficiency (WUE), nitrogen use efficiency (NUE), apparent quantum yield (AQY), functional diversity based on leaf N concentrations (FD_Q -leafN%) and functional diversity based specific leaf nitrogen (FD_Q-SLN). See Fig. 3 for the idependence model; iAIC; Akaike's information criterion for most parsimonious model; RMSEA: root mean squared error approximation; CFI: comparative fit index. Adequate model fits are indicated by non-significant χ^2 tests ($P > 0.05$), lower AIC, lower RMSEA (<0.05) and a CFI close to one.

Model	χ^2	*df*	P	iAIC	mAIC	RMSEA	CFI
GPP	6.83	8	0.555	79.83	32.83	0	1
Reco	2.57	5	0.766	51.88	22.56	0	1
NEE	4.79	5	0.441	42.29	24.79	0	1
WUE	5.17	9	0.824	67.77	29.12	0	1
NUE	2.02	2	0.364	35.59	18.02	0.03	0.99
AQY	5.39	6	0.495	41.85	23.39	0	1
FD_Q -leafN%	3.86	4	0.425	44.58	25.86	0	1
FD_Q-SLN	1.14	1	0.285	23.73	11.14	0.11	0.99

Table 2 Results of ANOVAs testing the effect of sown species richness (Sdiv) alongside the most parsimonious multiple regression models predicting the parameters fitted by the non-rectangular hyperbola function to the NEE light response curves. Canopy structure-related (LegBM = biomass of legume shoots, ShootBM = biomass of all shoots, LAI = leaf area index, Leaf-to-ShootBM-ratio = the ratio of leaf biomass to shoot biomass) and functional trait-based predictors (FD_Q-leafN% = diversity of leaf N concentration, FD_Q-SLN = diversity of specific leaf nitrogen, FD_Q-gs= diversity of stomatal conductance, CWM-height = community weighted means of plant individual height, CWM-rootdepth = community weighted means of individual rooting depth) were separately analysed. See Table S2 for the list of all canopy structure and functional-trait based predictors included in the initial models before AICc-based model simplification was performed.

Parameter	**Sdiv (ANOVA)**	**Canopy structure predictors**	**Functional-trait based predictors**
Reco-night	ns	LegBM+ShootBM ($p = 0.009$, $r^2 = 0.50$)	FD_Q -leafN% ($p = 0.071$, $r^2 = 0.29$)
AQY (ϕ)	$p = 0.082$	LegBM ($p = 0.042$, $r^2 = 0.35$)	FD_Q-SLN + CWM-rootdepth ($p = 0.005$, $r^2 = 0.79$)
Curvature (Θ)	ns	Leaf-to-ShootBM-ratio ($p = 0.006$, $r^2 = 0.54$)	FD_Q-gs+CWM-height ($p = 0.003$, $r^2 = 0.73$)
maxNEE	$p < 0.001$	LAI ($p = 0.004$, $r^2 = 0.58$)	FD_Q -leafN% ($p = 0.044$, $r^2 = 0.34$)

Figure legends

Figure 1 Temporal dynamics of (a) gross primary production (GPP), (b) ecosystem respiration (Reco) and (c) net ecosystem exchange (NEE) as affected by sown plant species richness (4 vs. 16 species) with data pooled from four days with undisturbed C fluxes per month. Bars represent ± SEM and 'ns' represents non-significant *P*-values with $P > 0.05$. Note the different y-axis scales of the figures.

Figure 2 (a) Temporal dynamics of water use efficiency (WUE) as affected by sown plant species richness. (b) Effects of sown species richness on canopy nitrogen use efficiency (NUE) and (c) apparent quantum yield (AQY), both measured in July. (d) Relationship between the diversity of specific leaf nitrogen (FD_Q-SLN) and WUE. (e) Relationship between the diversity of leaf nitrogen concentration (FD_Q-leafN%) and NUE. (f) Relationship between FD_Q-SLN and AQY. Bars represent ± SEM and 'ns' represents non-significant *P*-values with $P > 0.05$.

Figure 3 Minimal adequate path diagrams depicting the direct and indirect effects of realised species richness (Rdiv) on gross primary production (GPP), net ecosystem CO_2 exchange (NEE), ecosystem respiration (Reco), water use efficiency (WUE), nitrogen use efficiency (NUE), apparent quantum yield (AQY), diversity of leaf N concentration (FD_Q-leafN%) and diversity of specific leaf area (FD_Q-SLN) from July. Full and dashed arrows indicate significant and non-significant relationships, respectively. Numbers next to arrows show standardised regression weights. Squared multiple correlations (r^2) for endogenous variables are given above the variable box. Further abbreviations: biomass of shoots (ShootBM), biomass of legume shoots (LegBM), leaf area index (LAI), diversity of all leaf-related traits (FD_Q-leaf), diversity of leaf N concentration (FD_Q-leafN%), diversity of plant height (FD_Q-height), community weighted means of plant height (CWM-height), community weighted means of rooting depth (CWM-rootdepth), diversity of specific leaf nitrogen (FD_Q-SLN).

Figure 4 (a) Effects of sown species richness (4 vs. 16 species, n = 6) on the net ecosystem CO_2 exchange (NEE) response curves to quantum flux density of photosynthetic active radiation (PAR) during a whole day with clear sky (embedded the variation in PAR at vegetation level during the day). Clear symbols represent afternoon

values and grey-filled symbols represent morning values. Bars represent ± SEM. (b) NEE light response curves in communities with contrasting FD_Q-SLN% (low vs. high, n = 3). (c) NEE light response curves in communities with contrasting FD_Q-leafN% (low vs. high, n = 3). Light response curves from (b) and (c) were fitted on data pooled from 3 days with clear sky from sunrise to midday.

Figure 1

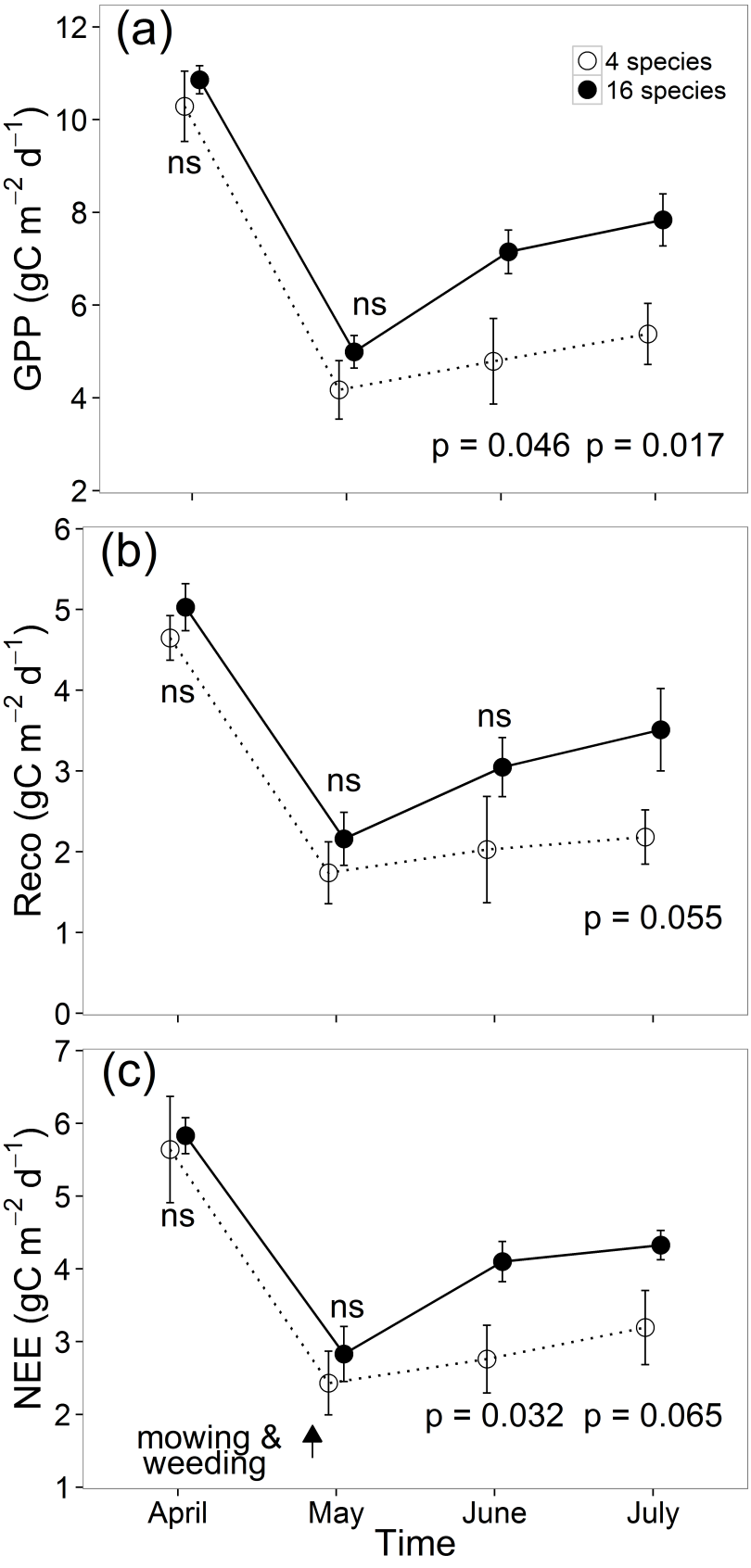

(a)
4 species
16 species
GPP (gC m^{-2} d^{-1})
ns
ns
p = 0.046 p = 0.017
(b)
Reco (gC m^{-2} d^{-1})
ns
ns
ns
p = 0.055
(c)
NEE (gC m^{-2} d^{-1})
ns
ns
p = 0.032 p = 0.065
mowing & weeding
April
May
June
July
Time

Figure 2

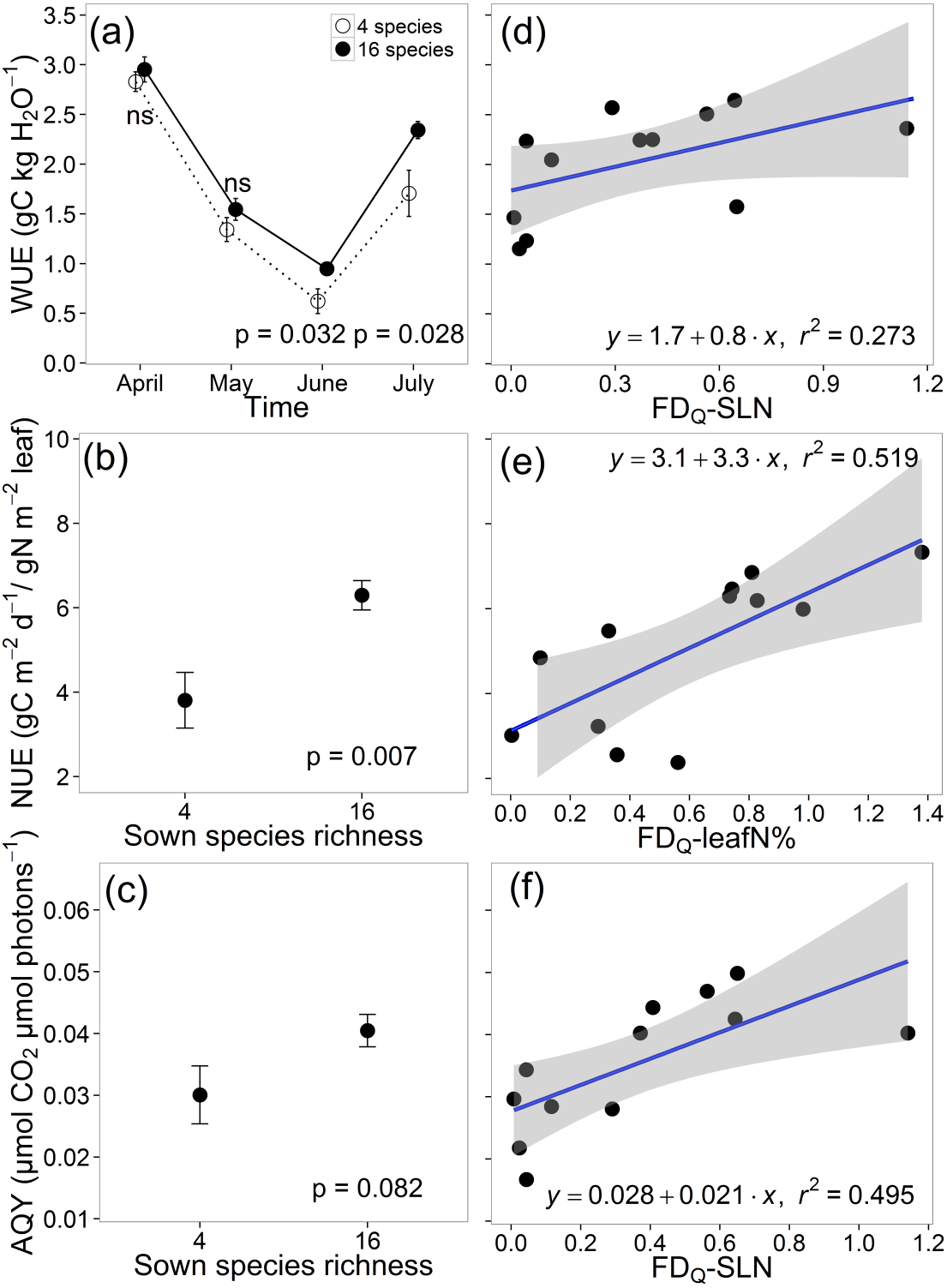

(a)
4 species
16 species
ns
ns
p = 0.032 p = 0.028
WUE (gC kg H2O−1)
April
May
June
July
Time
(b)
p = 0.007
NUE (gC m−2 d−1 / gN m−2 leaf)
Sown species richness
(c)
p = 0.082
AQY (µmol CO2 µmol photons−1)
Sown species richness
(d)
y = 1.7 + 0.8 · x, r2 = 0.273
FDQ-SLN
(e)
y = 3.1 + 3.3 · x, r2 = 0.519
FDQ-leafN%
(f)
y = 0.028 + 0.021 · x, r2 = 0.495
FDQ-SLN

Figure 3

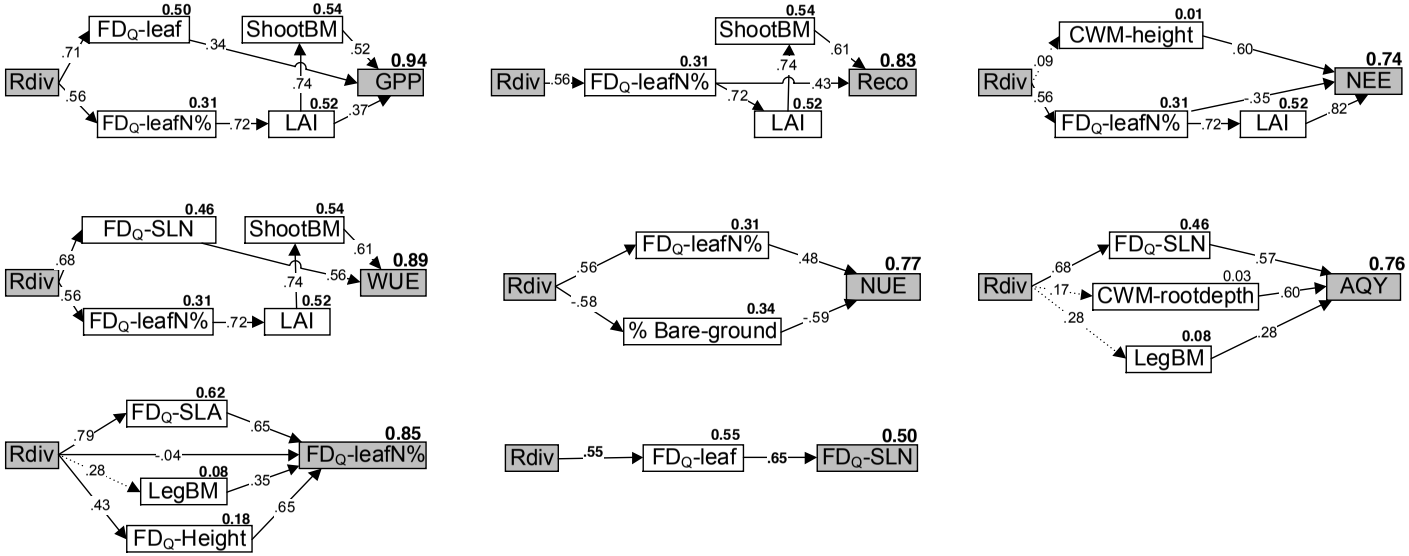
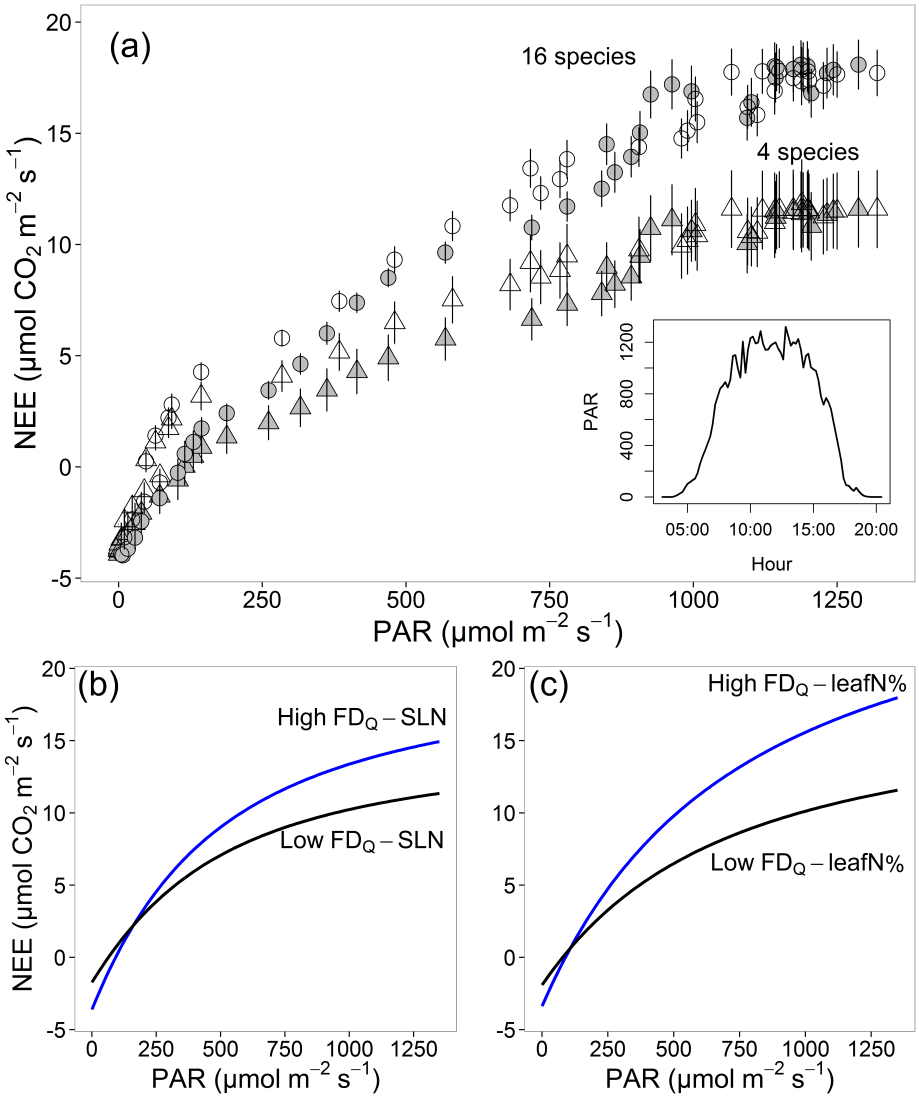

Rdiv
FDQ-leaf
0.50
ShootBM
0.54
GPP
0.94
FDQ-leafN%
0.31
LAI
0.52
Rdiv
ShootBM
0.54
FDQ-leafN%
0.31
Reco
0.83
LAI
0.52
Rdiv
CWM-height
0.01
NEE
0.74
FDQ-leafN%
0.31
LAI
0.52
Rdiv
FDQ-SLN
0.46
ShootBM
0.54
WUE
0.89
FDQ-leafN%
0.31
LAI
0.52
Rdiv
FDQ-leafN%
0.31
NUE
0.77
% Bare-ground
0.34
Rdiv
FDQ-SLN
0.46
AQY
0.76
CWM-rootdepth
0.03
LegBM
0.08
Rdiv
FDQ-SLA
0.62
FDQ-leafN%
0.85
LegBM
0.08
FDQ-Height
0.18
Rdiv
FDQ-leaf
0.55
FDQ-SLN
0.50

Figure 4


(a)
16 species
4 species
NEE (µmol CO2 m−2 s−1)
PAR (µmol m−2 s−1)
PAR
Hour
05:00
10:00
15:00
20:00
(b)
High FDQ – SLN
Low FDQ – SLN
(c)
High FDQ – leafN%
Low FDQ – leafN%

Supporting Information

Supplementary Methods

Extraction of soil monoliths

Soil monoliths were extracted by UMS GMBH (München, Germany) following a proprietary non-compacting extraction method. Using a hydraulic press, steel cylinders with cutting edges were pressed down to 2 m depth (to include the average depth of the water table in summer known from previous hydrological field investigations) while simultaneously digging out the soil 60 cm around the cylinder. The created space around the cylinders allowed to cut the bottom of the soil monolith and to insert a sealing plate in order to be able to lift the monolith and to create a water tight container/ lysimeter. The lysimeters where than extracted with a crane, and after inspection, were buried the experimental field until they were transported to the Ecotron facility at the end of March 2012.

Sampling methodology

Shoot biomass was estimated by clipping the vegetation at ground level in a rectangle of 0.8 x 1.0 m per plot and drying at 65°C for three days. Root biomass was estimated from three cores of 3.5 cm diameter and 60 cm depth. The soil cores were separated into six layers (0-5, 5-10, 10-20, 20-30 and 40-60cm) before they were pooled per layer, washed with tap water and dried following the same procedure as for shoot biomass. Leaf area index (LAI) was estimated using a portable LAI-2000 plant canopy analyzer (LI-COR, Lincoln, USA). LAI was measured in the evening under diffused light conditions with one measurement above the canopy as a reference and the average of five measurements near ground level positioned at different places in the center of each lysimeter. The zenith angle was restricted to 0-43° in order to minimize the edge effect (Hyer & Goetz, 2004) inherent to a canopy with a surface of $2m^2$. In lysimeters with a high proportion of one rosulate species (*Plantago media*) with leaves laying very close to the ground, the values measured with the LAI-2000 were replaced with the average value of two leaf area measurements using a LI-3100 leaf area meter (LI-COR, Lincoln, USA) leaf area meter) from two rectangles of 0.2 × 0.5 m per plot. Percentage vegetation cover and bare ground as well species specific percentage cover were visually estimated for the whole lysimeter

(2 m^2). Stomatal conductance was measured using a portable porometer (SC-1 Leaf porometer, Decagon Devices, Pullman, USA). Leaf greenness was estimated with a hand-held chlorophyll meter (SPAD-502, Konica-Minolta, Osaka, Japan), which enables non-destructive assessment of leaf greenness by measuring the absorbance by the leaf of two different wavelengths (650 nm and 940 nm). The SPAD values were calibrated (r^2 =0.69) against spectrophotometrically determined chlorophyll concentrations from leaf extracts following the method of Moran (1982). Leaf dry mater content (LDMC) is defined as the ratio of leaf dry mass to saturated fresh mass according to (Vile et al. 2005). LDMC was determined with the partial rehydration method following the protocol of Wilson et al. (1999). Samples were put sealed plastic bags promoting rehydration by storing leaves overnight between sheets of moistened tissue paper. Then, samples were blotted dry using tissue paper to remove any surface water and immediately weighed. Samples were oven-dried (65°C, 3 days) and reweighed to get values for dry mass. This procedure gives a good approximation in comparison to the complete rehydration method (Vaieretti et al. 2007).

Test of species identity effects

We tested any potential species identity/ sampling effects (Aarssen, 1997) by independently analysing the effect of the ten species appearing in more than two plots using the measured species-specific shoot biomass per plot as predictor for our response variables. Of the ten species tested, we only found a trend effect of *Vicia cracca*, a legume appearing in five plots, on water use efficiency ($F_{1/10}$ = 3.56, P = 0.088) and nitrogen use efficiency ($F_{1/10}$ = 3.70 P = 0.073). Subsequently, we included the shoot biomass of *Vicia cracca* in the analyses for determining the most parsimonious predictors, but it did not alter the original results.

Estimation of ecosystem respiration (Reco)

Ecosystem respiration over 24h was calculated as the sum of the day- and night-time ecosystem respiration (Reco = Reco-night + Reco-day). While we have direct, continuous measurements of night-time ecosystem respiration (as NEE-night = Reco-night), we estimated the daytime ecosystem respiration (Reco-day) based on the night-time temperature to night-time ecosystem respiration (Reco-night) relationship using the flux partitioning algorithm of Reichstein et al. (2005). This is done in two steps. First, the temperature

sensitivity of respiration to temperature is estimated by relating night-time respiration measured in the previous 15 days to night-time air temperature (T) following the exponentialregression model of Lloyd & Taylor (1994):

$$\text{Reco-night} = R_{ref}\, e^{E0(1/(Tref-T0) - 1/(Tnight-T0))}$$

where R_{ref} is the respiration rate at reference temperature (Tref =10°C), T0 is kept constant at – 46.02 °C as in Lloyd & Taylor (1994), T is expressed in absolute temperature (K) and the activation energy parameter (E0), which determines the temperature sensitivity, as free/ estimable parameter. After the temperature sensitivities have been estimated for each community, the temperature independent level of respiration (i.e. the R_{ref} parameter) is estimated. Since this parameter is also temporally varying in an ecosystem, it is estimated for consecutive 4-day periods by nonlinear regression using the same equation as above but fixing all parameters except R_{ref}. Consequently, for each point in time, an estimate of Reco- day can be provided according to the above equation but with time dependent parameters (E0 and R_{ref}). Estimates for Reco-day were calculated using the online flux-partitioning tool available at http:// www.bgc-jena.mpg.de/~MDIwork/eddyproc/index.php [see Reichstein et al. (2005) for further details on the method and the implementation of the algorithm]. The modelled Reco-day values were also compared to the Kok method (Sharp et al. 1984), which can be applied in our case to estimate Reco-day by extrapolating the NEE response curve at light levels below the compensation curves to intersect the x-axis. We found a very good agreement between the values derived with the Lloyd and Taylor model and the Kok method (Pearson's $r = 0.95$). Furthermore, we tested the predictive power of the Lloyd & Taylor (1994) model vs. measured Reco-night and found a reasonably good fit ($r^2 = 0.73$).

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Supplementary tables

Table S1. Sown species richness (Sdiv) and functional group (G = grasses, H = herbs and L = legumes) composition of the twelve selected plots from the Jena Experiment. The species present in the lysimeters at the final harvest are marked in bold in the table.

Plot ID	Sdiv	G	H	L	Species composition	Ecotron dome
B2A22	16	5	5	6	*Cc*, ***Fp***, ***Tf***, ***Pp***, ***Pt***, ***Cj***, ***Ra***, *So*, ***Am***, *CAp , Th*, ***Lc***, ***VIc***, ***Tr***, ***Lp***, ***Ov***	1
B4A04	4	1	2	1	***Ae***, ***Pl***, ***As***, *Tc*	2
B1A01	16	4	8	4	***AVp***, ***Pp***, ***Ao***, ***Bh***, ***Pl***, ***To***, ***Ar***, ***Rr***, *As*, ***Gp***, *TRp*, ***CAc***, *Tc*, ***VIc***, ***Lp***, ***Lc***	3
B1A04	4	1	2	1	***Fp***, ***Pl***, *CAp*, *Ov*	4
B3A23	4	1	2	1	*Bh*, ***Rr***, ***Lv***, ***TRf***	5
B2A18	16	4	8	4	***Ap***, *Bh*, ***Pp***, ***Cc***, ***Rr***, ***Pm***, ***Ar***, *Pv*, ***CAp***, ***Gp***, *As*, *Cp*, *Ml*, ***Tr***, ***Td***, *Tc*	6
B4A18	16	4	8	4	*Cc*, *LUc*, ***Ap***, *Bh*, *La*, ***Pm***, ***Vc***, ***To***, ***Cb***, *CAc*, ***PIm***, ***Hs***, *Th*, *Tc*, ***Lp***, ***Ov***	7
B2A01	4	1	2	1	***Ao***, ***Pv***, ***Ka***, ***Tp***	8
B3A22	16	4	8	4	***PHp***, ***Fr***, *Ao*, ***Be***, ***Rr***, ***Ar***, ***Bp***, ***Vc***, ***Gp***, ***Cb***, ***Ra***, ***Gm***, ***VIc***, ***Ov***, *TRf*, *Td*	9
B2A16	4	*0*	*3*	*1*	***Pm***, *La*, ***Ka***, ***VIc***	10
B3A24	16	6	5	5	***Fp***, *Bh*, ***Ap***, *Ao*, ***Pt***, ***Ae***, ***To***, ***Rr***, ***Ar***, *Pv*, ***Gh***, ***Lc***, ***Tp***, *Tr*, ***VIc***, ***Ms***	11
B4A11	4	1	2	1	***Tf***, ***TRp***, ***Hs***, ***Ms***	12

Grasses (G): Ae = Arrhenatherum elatius L. (J. et C. PRESL), Ao= Anthoxantum odoratum L., Ap= Alopecurus pratensis L., AVp = Avenula pubescens HUDS. (DUM.), Be= Bromus erectus HUDS., Bh= Bromus hordeaceus L., Cc= Cynosurus cristatus L., Dg= Dactylis glomerata L., Fp= Festuca pratensis HUDS., Fr= Festuca rubra L., Hl= Holcus lanatus L., LUc = Luzula campestris L (DC.), PHp= Phleum pratense L., Pp= Poa pratensis L., Pt= Poa trivialis L., Tf= Trisetum flavescens L. (P. BEAUV.);

Herbs (H): Am= Achillea millefolium L., Bp= Bellis perennis L., Cb= Crepis benis L., CAc= Carum carvi L., CAp = Campanula patula L., Cj= Centaurea jacea L., Co= Cirsium oleraceum L., Cp= Cardamine pratensis L., Dc= Daucus carota L., Gm= Galium mollugo L., Gh= Glechoma hederacea L., Hs = Heracleum sphondylium L., Ka= Knautia arvensis L., La= Leontodon autumnalis L., Lh= Leontodon hispidus L., Lv= Leucanthemum vulgare Lam., PIm = Pimpinella major L. (HUDS.), Pl= Plantago lancelolata L., Pm= Plantago media L., PRv = Primula veris L., Pv= Prunella vulgaris L., Ra= Rumex acetosa L., To= Taraxacum officinale WEBER, TRp= Tragopogon pratensis L., Vc= Veronica chamaedrys L;

Legumes (L): Lp= Lathyrus pratensis L., Lc= Lotus corniculatus L., Ml= Medicago lupulina L., Ms=Medicago x varia MARTYN, Ov= Onobrychis viciifolia SCOP., Td= Trifolium dubium SIBTH., TRf = Trifolium fragiferum L., Th= Trifolium hybridum L., Tr= Trifolium repens L., Tp= Trifolium pratense L., VIc= Vicia cracca L.

Table S2. Overview table with descriptions and abbreviations of all predictors, covariates and response variables. FD_Q abbreviation indicates a functional diversity index estimated using Rao's quadratic entropy index (Botta Dukát 2005). CMW indicates a community weighted means estimated for the respective functional trait.

Functional trait-based predictors	**Description**	**Type of trait / source**
FD_Q-leafN, CWM-leafN	Based on leaf nitrogen concentration in the canopy	Continuous / measured in situ
FD_Q-SLN, CWM-SLN	Based on nitrogen specific leaf area in the canopy	Continuous / measured in situ
FD_Q-SLA, CWM-SLA	Based on specific leaf area in the canopy	Continuous / measured in situ
FD_Q-GS, CWM-GS	Based on stomatal conductance in the canopy	Continuous / measured in situ
FD_Q-greenness, CWM-greenness	Based on leaf greenness	Continuous / measured in situ
FD_Q-LDMC, CWM-LDMC	Based on leaf dry matter content	Continuous / measured in situ
FD_Q-height, CWM-height	Based on recorded species height	Continuous / measured in situ
FD_Q-rootdepth, CWM-rootdepth	Based on rooting depth	Continuous / literature
FD_Q-typeroot, CWM-typeroot	Based on rooting type	Ordinal / literature
FD_Q-seasonality, CWM-seasonality	Based on the seasonality of foliage	Ordinal / literature
FD_Q-leaf	Based on all six leaf related traits	Continuous / measured in situ
FD_Q-all	Based on all ten functional traits	Continuous and ordinal / in situ and literature
Vegetation structure predictors	**Description**	**Type of variable / source**
Sdiv	Sown species richness	Categorical (4 and 16 species) / in situ
Rdiv	Realised species richness at July harvest	Counts / measured in situ
LAI	Leaf area index	Continuous / measured in situ
Bare-ground	Percentage bare ground	Percentage / measured in situ
ShootBM	Shoot biomass	Continuous / measured in situ
RootBM	Root biomass	Continuous / measured in situ
TotalBM	Total biomass (shoot + root biomass)	Continuous / measured in situ
LegBM	Biomass of legumes	Continuous / measured in situ
Leaf-to-ShootBM-ratio	Ratio of leaf to shoot biomass ratio	Ratio / measured in situ
HerBM	Biomass of herbs	Continuous / measured in situ

GraBM	Biomass of grasses	Continuous / measured in situ
Covariates tested	**Description**	**Type of variable / source**
Extractable soil N	Extractable soil NH_4^+ and NO_3^- at 0-20 cm depth	Continuous / measured in situ
Microbial biomass	Substrate induced microbial C biomass	Continuous / measured in situ
Soil texture (sand %)	Soil sand content at 0-20 cm depth	Percentage / measured in situ
Air temperature	Achieved air temperature	Continuous / measured in situ
Air humidity	Achieved air humidity (RH %)	Percentage / measured in situ
Soil humidity	Volumetric water content (%) at 10, 30 and 60 cm depths	Percentage / measured in situ
Response variables	**Description**	**Type of variable / source**
GPP	Gross primary productivity	Continuous / measured in situ
Reco	Ecosystem respiration	Continuous / measured in situ night-time respiration with estimated daytime respiration (Lloyd & Taylor 1994)
NEE	CO_2 net ecosystem exchange	Continuous / calculated from measured in situ GPP and Reco (NEE = GPP– Reco)
WUE	Water use efficiency	Continuous / derived from measured in situ evapotranspiration
NUE	Nitrogen use efficiency	Continuous / estimated from in situ measurements of GPP and specific leaf nitrogen
AQY	Apparent quantum yield	Continuous / derived from light response curves measured in situ and fitted with non-rectangular hyperbola functions

Table S3. Overview table with mean and standard deviations (SD) of the vegetation structure-related variables.

Vegetation structure related variables	Within 4 species		Within 16 species		Between diversity levels	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
LAI	1.45	0.97	2.78	0.84	2.12	1.11
Bare ground (% cover)	26.83	20.50	6.25	9.23	16.54	18.58
Biomass of shoots (g m^{-2})	148.7	61.07	193.01	43.31	170.86	55.52
Biomass of roots (g dw m^{-2})	100.52	39.87	154.07	37.77	127.29	46.40
Total biomass (g dw m^{-2})	249.24	93.34	347.08	55.74	129.16	89.35
Legume shoot biomass (g dw m^{-2})	18.24	26.65	42.86	40.27	30.55	34.34
Grass shoot biomass (g dw m^{-2})	34.12	61.66	36.81	52.14	35.46	54.46
Herb shoot biomass (g dw m^{-2})	83.15	71.47	95.58	54.15	89.37	60.60
Weeds (g dw m^{-2})	9.47	8.54	9.78	5.55	9.62	6.87
Unidentified shoots (g dw m^{-2})	3.71	8.19	7.97	9.31	5.84	8.65
Leaf-to-ShootBM ratio	0.63	0.14	0.67	0.09	0.65	0.11

Supplementary Figures

Figure S1. Simplified schematic of one controlled environment unit of the CNRS Ecotron facility.

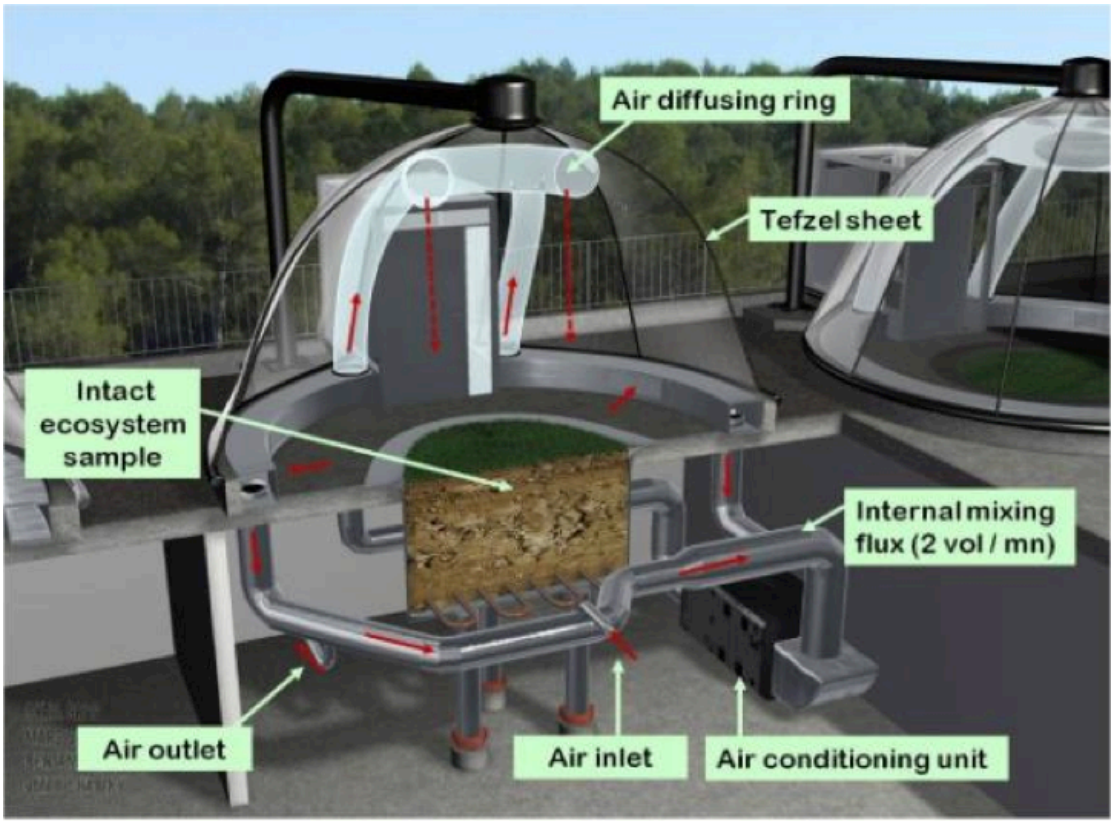


Figure S2. Comparison of cumulative precipitation (mm) recorded in the "Jena Experiment" in 2007 and simulated in the Ecotron.

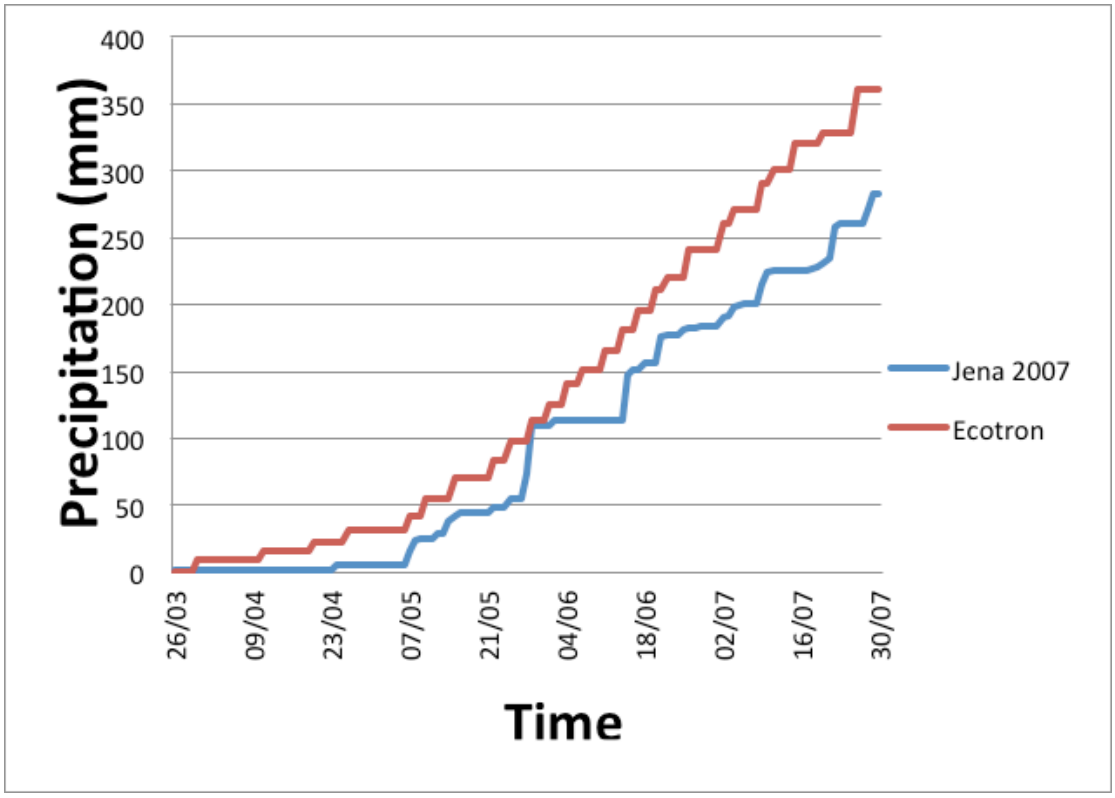


Figure S3. Comparison of the soil moisture achieved in the Ecotron and the "Jena Experiment" field site in 2007 at similar depths for the period with available Ecotron data.

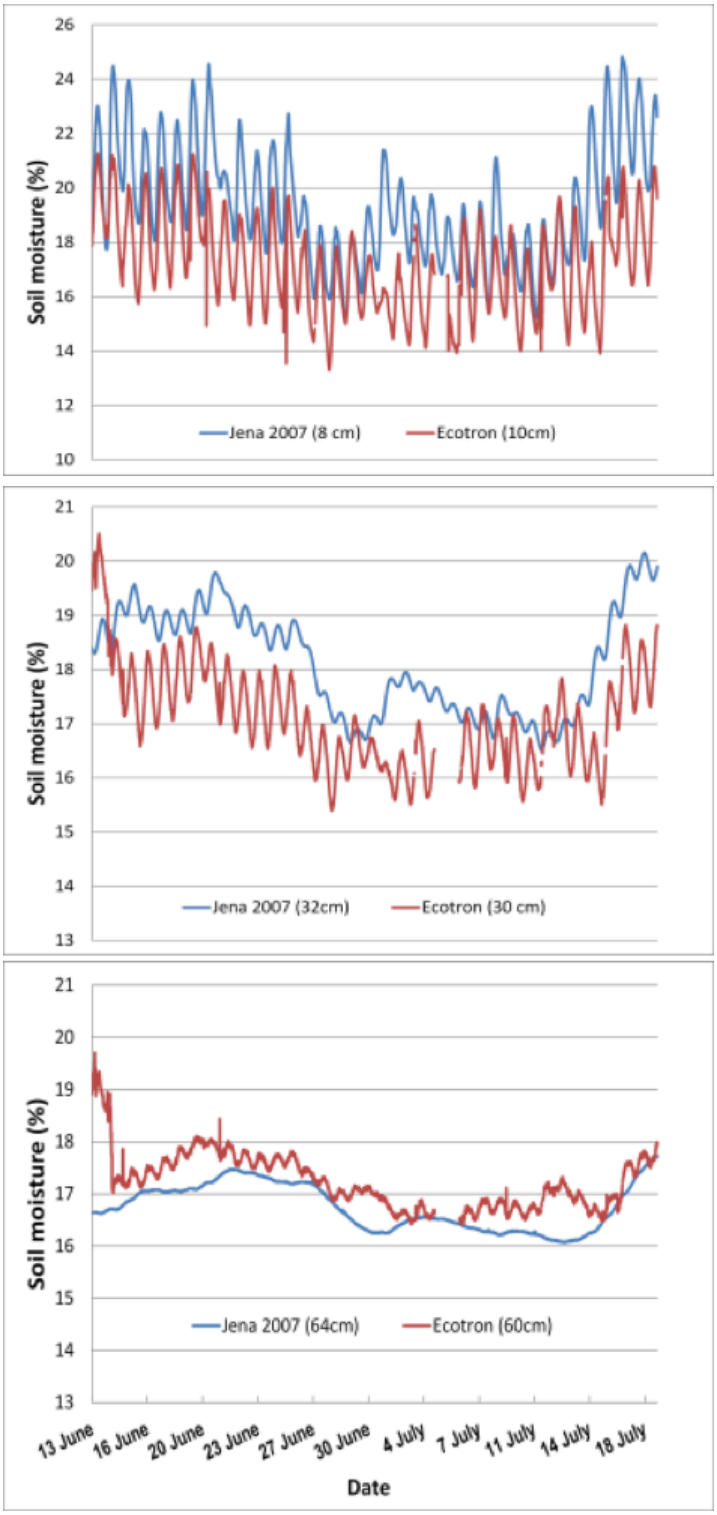


Figure S4. Schematic of the maximal model used in the path analysis.

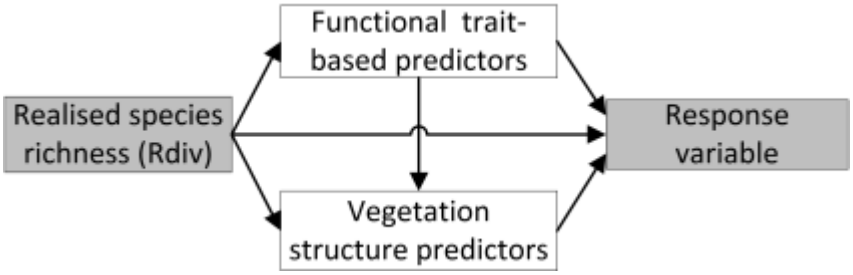
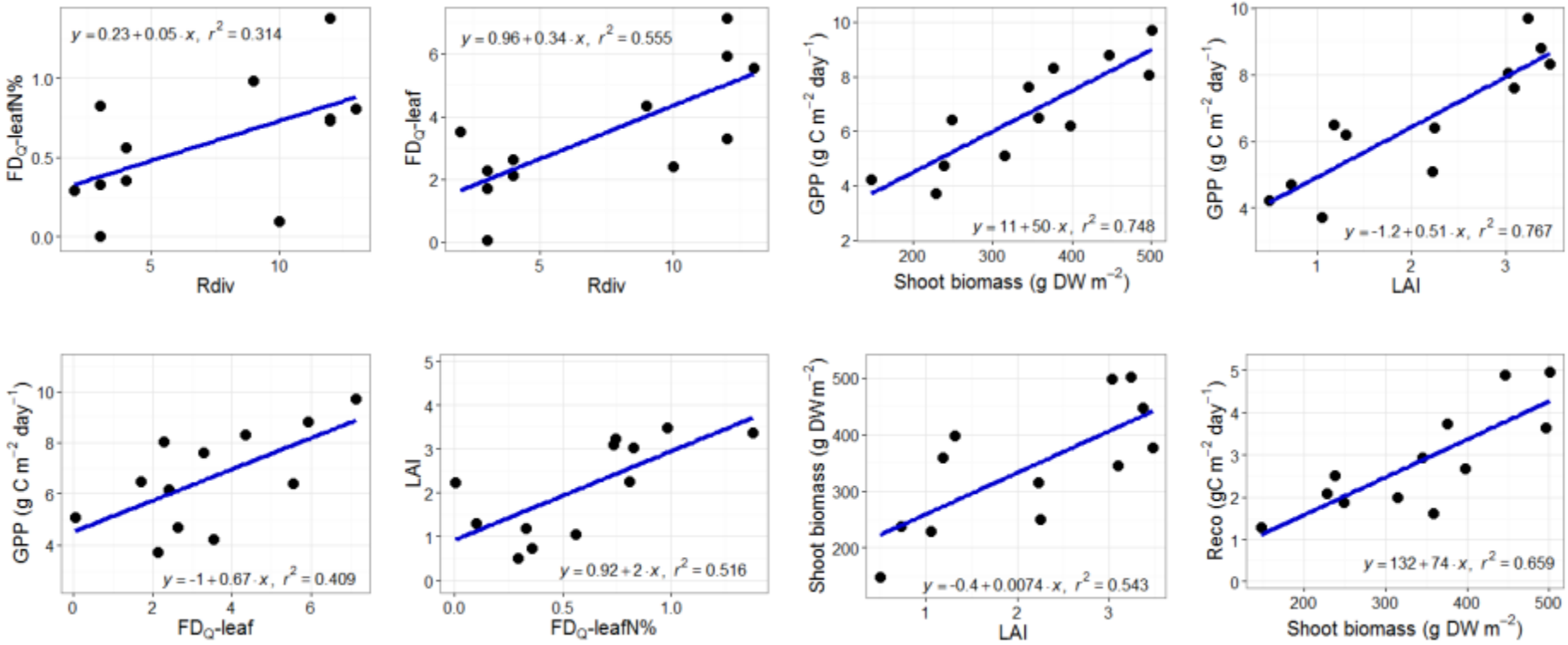
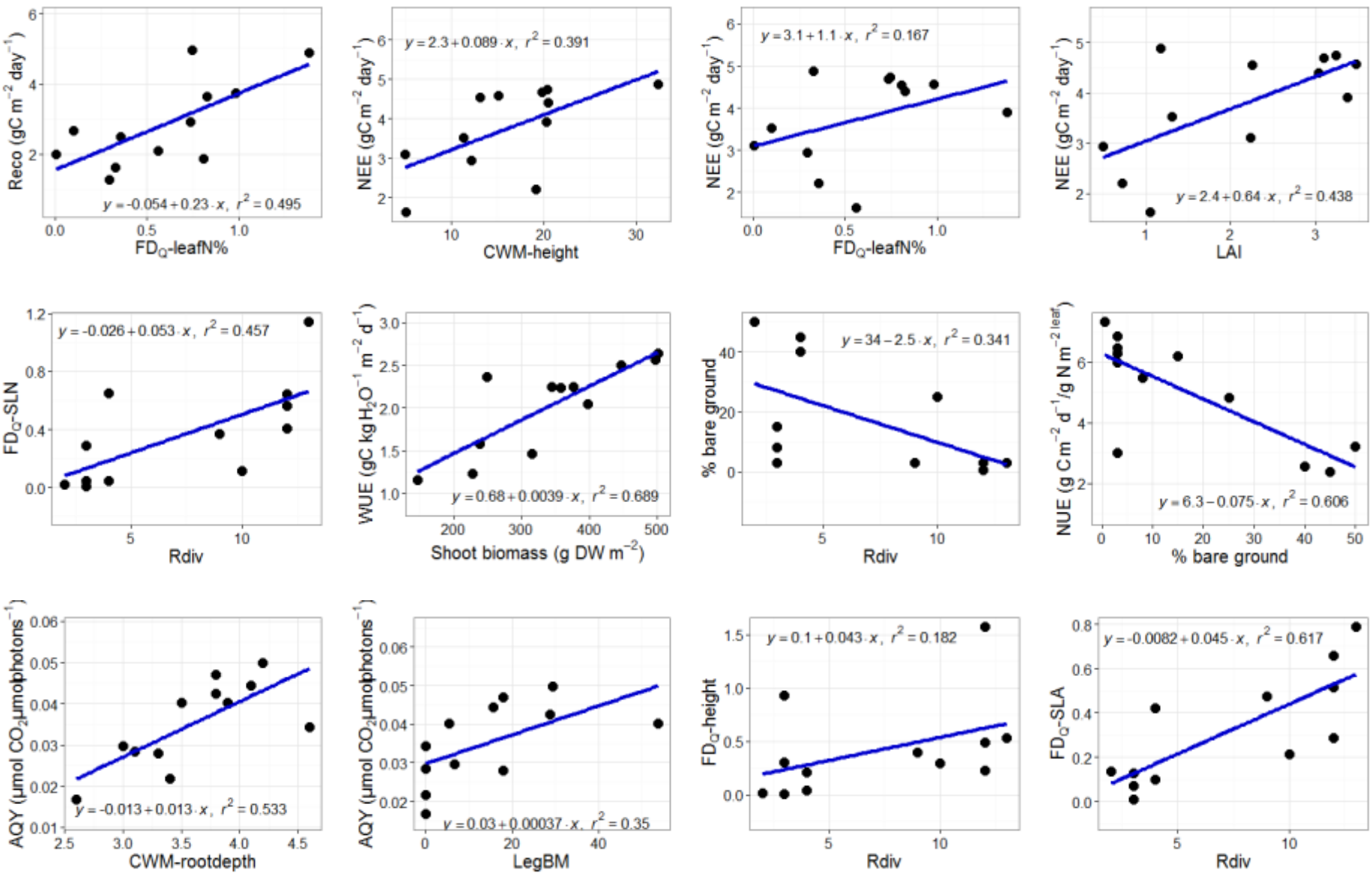
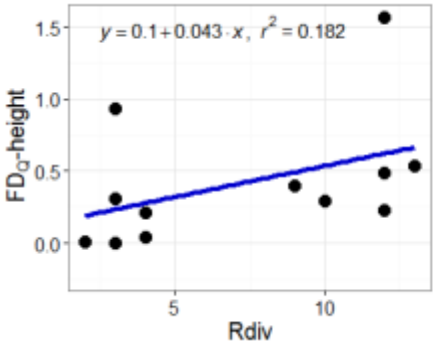
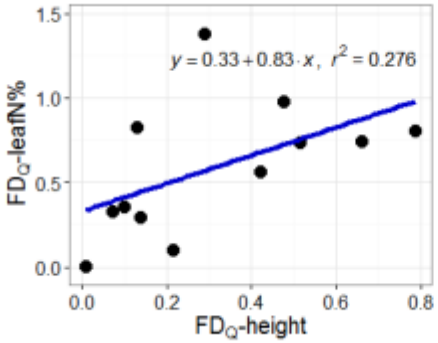


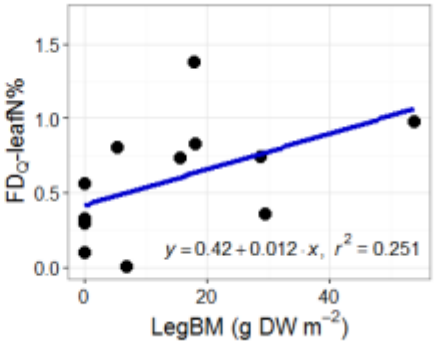
Figure S5. Scatterplots with linear regression lines for the significant relationships retained in the path analyses in addtion to those presented in Fig. 3.

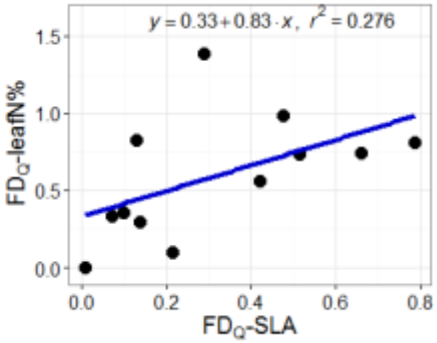



Reco (gC m^{-2} day^{-1})
$y = -0.054 + 0.23 \cdot x$, $r^2 = 0.495$
FD$_Q$-leafN%
NEE (gC m^{-2} day^{-1})
$y = 2.3 + 0.089 \cdot x$, $r^2 = 0.391$
CWM-height
NEE (gC m^{-2} day^{-1})
$y = 3.1 + 1.1 \cdot x$, $r^2 = 0.167$
FD$_Q$-leafN%
NEE (gC m^{-2} day^{-1})
$y = 2.4 + 0.64 \cdot x$, $r^2 = 0.438$
LAI
FD$_Q$-SLN
$y = -0.026 + 0.053 \cdot x$, $r^2 = 0.457$
Rdiv
WUE (gC kg H$_2$O^{-1} m^{-2} d^{-1})
$y = 0.68 + 0.0039 \cdot x$, $r^2 = 0.689$
Shoot biomass (g DW m^{-2})
% bare ground
$y = 34 - 2.5 \cdot x$, $r^2 = 0.341$
Rdiv
NUE (g C m^{-2} d^{-1}/g N m$^{-2\ leaf}$)
$y = 6.3 - 0.075 \cdot x$, $r^2 = 0.606$
% bare ground
AQY (µmol CO$_2$µmolphotons^{-1})
$y = -0.013 + 0.013 \cdot x$, $r^2 = 0.533$
CWM-rootdepth
AQY (µmol CO$_2$µmolphotons^{-1})
$y = 0.03 + 0.00037 \cdot x$, $r^2 = 0.35$
LegBM
FD$_Q$-height
$y = 0.1 + 0.043 \cdot x$, $r^2 = 0.182$
Rdiv
FD$_Q$-SLA
$y = -0.0082 + 0.045 \cdot x$, $r^2 = 0.617$
Rdiv


$y = 0.1 + 0.043 \cdot x,\ r^2 = 0.182$
FD_Q-height
Rdiv


$y = 0.33 + 0.83 \cdot x,\ r^2 = 0.276$
FD_Q-leafN%
FD_Q-height


$y = 0.42 + 0.012 \cdot x,\ r^2 = 0.251$
FD_Q-leafN%
LegBM (g DW m^{-2})


$y = 0.33 + 0.83 \cdot x,\ r^2 = 0.276$
FD_Q-leafN%
FD_Q-SLA

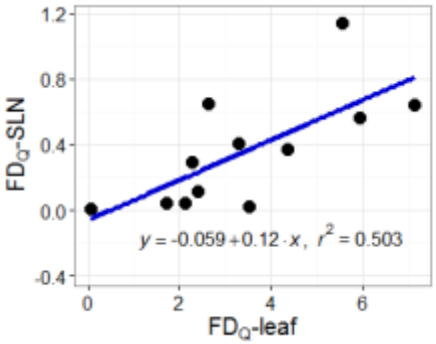
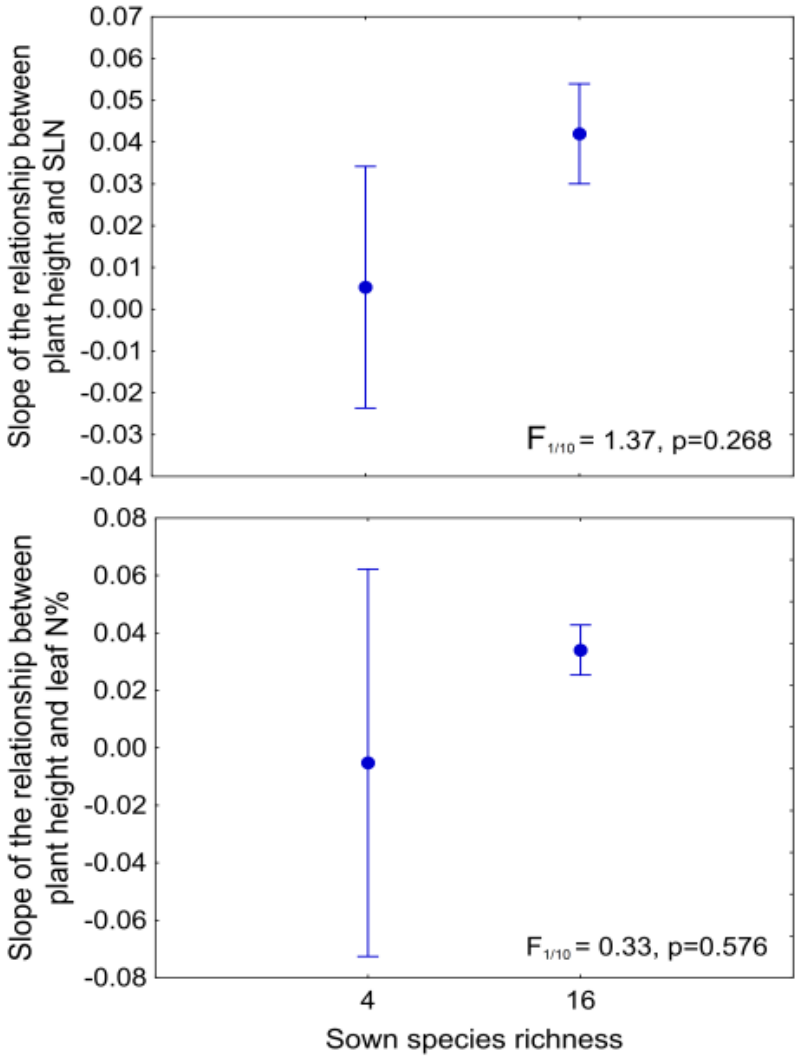

$y = -0.059 + 0.12 \cdot x,\ r^2 = 0.503$
FD_Q-SLN
FD_Q-leaf

Figure S6. Graphs showing the effect of sown species richness on the slope of the relationship between height and SLN (top) and leaf nitrogen concentration (leaf N%) (bottom). Error bars represent ± SEM.



General discussion

Previous studies revealed that high biodiversity promotes different aspects of ecosystem functioning such as productivity or invasion resistance (Cardinale et al. 2012). Despite increasing research, the underlying mechanisms for these positive effects are not well understood. This thesis aimed to investigate if complementarity in resource use provides a mechanistic explanation for the positive effects of increasing biodiversity on ecosystem functioning in grassland.

Chapter 1 described a study testing whether increased spatial or temporal complementary water use can explain positive effects of increasing species or functional group richness. A tracer experiment with two different stable water isotopes applied at two different soil depths in grassland communities of varying species and functional groups revealed that the main water uptake was from the upper soil layer in all mixtures and at each time. Water exploitation from deeper soil layers did not increase with increasing species richness or functional group number. Furthermore, functional groups did not differ in their spatial or temporal water acquisition. These results indicate that increased complementary water use in high diverse compared to low diverse communities is not a likely explanation for positive effects of high biodiversity on ecosystem functioning, at least under the conditions of the Jena Experiment. The outcome of this study is contrary to results of earlier studies that found increased complementary water use with increasing species richness by investigating, for instance, soil moisture patterns (Van Peer et al. 2004), canopy surface measurements to estimate evapotranspiration rate (Verheyen et al. 2008) or $\delta^{13}C$ ratios (Caldeira et al. 2001). However, these studies assessed water use indirectly, while the study at hand is presenting an unique approach by directly testing water uptake by using stable water isotopes. Nevertheless, other studies using stable water isotopes found evidence for differential water uptake of coexisting species but did not compare communities of varying plant diversity (e.g. Ehleringer et al. 1991, Dodd et al. 1998). Moreover, these studies were conducted in semi-arid ecosystems, where water in upper soil layers is scarce and spatial differentiation in water uptake is necessary for coexistence to access, for instance, groundwater and deeper moist soil layers. However, a lack of species richness effects on root biomass distribution (Ravenek et al., personal communication), complementary nitrogen use (Kahmen et al. 2006, von Felten et al.

2009) or on soil water balance (Leimer et al. 2014) supports our finding of main water uptake from the upper soil layer in all diversity levels.

Another approach to analyze the data would have been to compare the enrichment in ^{18}O and ^{2}H of the xylem water of single species along the diversity gradient and to explore whether the uptake depth changes depended on the number of species or the composition of the mixtures in which a species occur. However, the communities of the Jena Experiment had been randomly assembled out of a species pool of 60 species, which does not allow following single species along the diversity gradient, since each single species is not present in each species richness level. A follow-up experiment could be carried out in the newly established communities within the Jena Experiment (New trait-based experiment). These were assembled out of a smaller species pool to gain either high complementarity or high redundancy concerning above- and belowground traits among the containing species, and which would allow following species along the diversity gradient as each single species is present in all levels of species richness.

Further aspects to be considered are that niche separation might occur rather horizontally or that vertical niche differentiation occurs at smaller distances (de Kroon et al. 2012). Testing this went beyond our research design, but could be explored by varying the depth of tracer application in additional subplots or by applying a third tracer in an intermediate soil depth.

Furthermore, plant growth is often limited by multiple resources (Tilman et al. 1997). Therefore, the mechanism explaining positive effects of high plant diversity on ecosystem processes might be complementarity not only for a single resource, but for multiple resources (Harpole and Tilman 2007), e.g., differences in water uptake combined with different use of nitrogen forms and different adjustment to light availability in space and time. The presented study on differential water uptake was carried out in collaboration with the University of Freiburg, Germany, investigating spatial and temporal nitrogen uptake and root activity (via cation uptake (Rubidium, Strontium)) patterns. Combined analyses are planned, for instance calculations of niche breadth (von Felten et al. 2009) involving water, nitrogen and cations, that might reveal more insight in multidimensional resource uptake and improve the mechanistic understanding of positive diversity effects.

In a second study (*chapter 2*), we assessed whether species grown in high diverse mixtures use light more efficiently than species in low diverse mixtures, which might

contribute to explain positive biodiversity-ecosystem functioning relationships. We combined direct measurements of light intensity to describe the light niche within the canopy with measurements of leaf traits related to light acquisition. We aimed to identify if the adjustment of these traits to reduced light availability within the canopy of high diverse compared to low diverse mixtures might result in increased light exploitation. We found high temporal variation of the light niche with increasing light attenuation towards the ground with increasing species richness, but only at peak biomass times. Leaf trait expression also varied temporally, but not in parallel to the temporal pattern of changes in light availability. Furthermore, we found no effect of species richness on the trait expression except for one trait (SLA). However, functional groups showed significant differences as well as temporal variations in leaf trait expression, but not in parallel to the changing light conditions. Thus, we did not find evidence of increased complementary light use with increasing species or functional group richness. But our trait measurements reflected different resource use strategies of plant functional groups, supporting the importance of the functional composition of a community for optimal resource exploitation.

Strongest vertical profiles of light attenuation in the high diverse community might suggest a higher variation in light availability that might result in greater variation among species in leaf trait expressions with increasing diversity. Furthermore, Gubsch et al. (2011) and Roscher et al. (2011) found the expression of light acquisition traits of grasses and legumes to be largely species-dependent. A higher variety of light acquisition characteristics among species might increase the opportunities for increased complementary light use in high diverse communities. Thus, the next step would be to calculate the functional diversity of the used communities with the measured leaf traits. An increased dissimilarity among species regarding the leaf traits would lead to a higher functional diversity with increasing species richness, which might improve optimal light acquisition (Cadotte et al. 2009).

However, the measured traits might also be influenced by changes in nutrient availability. Thus, the response of leaf traits to light availability was probably coupled to the response to nutrient availability, which questions the use of leaf traits being appropriate to assess light acquisition and to use them to calculate functional diversity in order to assess complementary light acquisition. Nevertheless, a larger dissimilarity or higher functional diversity in traits are clearly beneficial for multidimensional complementary resource use.

In the last study (*chapter 3*), the effect of functional diversity on ecosystem carbon fluxes and different measures of carbon use efficiency was analyzed using lysimeters derived from the Jena Experiment with communities of four and 16 species. Continuous measurements of ecosystem carbon and water fluxes revealed higher gross and net ecosystem carbon uptake rates in high compared to low diverse communities as well as increased water use efficiency, nitrogen use efficiency and canopy apparent quantum yield. By including plant traits and structural characteristics of the vegetation into path analyses, this study additionally identified the functional diversity of leaf nitrogen concentration in the canopy as the best predictor for carbon fluxes and the derived efficiency measures. A higher diversity of leaf nitrogen concentration in the canopy of high compared to low diverse communities suggested an optimal vertical distribution of leaf nitrogen within the canopy in parallel to the vertical light attenuation. This might optimize canopy photosynthesis, and eventually canopy carbon gain in accordance with the optimal-N-distribution-hypothesis (Anten et al. 1995). However, although this study found strong support for the functional diversity of leaf nitrogen concentration as predictor for carbon fluxes, this assumption still requires to be tested directly by field measurements of leaf nitrogen concentration and leaf gas exchange at different height strata within the canopy.

This thesis investigated the underlying mechanism of positive plant diversity effects on ecosystem functioning. Moreover, it emphasized the need of experimental approaches using direct methods, but also the importance of functional diversity as measure of complementary resource use. The first two chapters suggested no or rather small evidence for complementarity in the use of single resources, while the third chapter found evidence for increased carbon gain potentially via an optimal distribution of leaf nitrogen concentrations within the canopy.

Considering the suggested improvements and complementarity towards multiple resources in combination, but also bearing in mind that other mechanisms (e.g. plant-soil fauna organism interactions; De Deyn et al. 2003) are additionally operating under field conditions could explain the beneficial effects of high biodiversity for several aspects of ecosystem functioning mankind relies on.

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Bachmann, D., Both, S., Bruelheide, H., Erfmeier, A.: Effects of light and nutrients on native and invasive herb species in subtropical China - Implications for forest ecosystem invasibility? BIOLIEF 27.-30. Oct 2009, Porto, Poster

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Publications

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