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Belowground resource partitioning alone cannot explain the biodiversity-ecosystem function relationship: A field test using multiple tracers

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Abstract

1. Belowground resource partitioning is among the most prominent hypotheses for driving the positive biodiversity-ecosystem function relationship. However, experimental tests of this hypothesis in biodiversity experiments are scarce, and the available evidence is not consistent.
2. We tested the hypothesis that resource partitioning in space, in time, or in both space and time combined drives the positive effect of diversity on both plant productivity and total community resource uptake. At the community level, we predicted that total community resource uptake and biomass production above- and belowground will increase with increased species richness or functional group richness. We predicted that at the species level resource partition breadth will become narrower, and that overlap between the resource partitions of different species will become smaller with increasing species richness or functional group richness.
3. We applied multiple resource tracers (Li and Rb as potassium analogues, the water isotopologues - $H_2{}^{18}O$ and ^2H_2O, and ^{15}N) in three seasons at two depths across a species and functional group richness gradient at a grassland biodiversity experiment.

We used this multidimensional resource tracer study to test if plant species partition resources with increasing plant diversity across space, time, or both simultaneously.

4. At the community level, total community resource uptake of nitrogen and potassium and above- and belowground biomass increased significantly with increasing species richness but not with increasing functional group richness. However, we found no evidence that resource partition breadth or resource partition overlap decreased with increasing species richness for any resource in space, time, or both space and time combined.
5. **Synthesis:** These findings indicate that belowground resource partitioning may not drive the enhanced resource uptake or biomass production found here. Instead, other mechanisms such as facilitation, species specific biotic feedback, or aboveground resource partitioning are likely necessary for enhanced overall ecosystem function.

Keywords: ecosystem function, Jena Experiment, Levins B, resource uptake, water uptake, stable isotopes, rare element tracers, complementarity, proportional similarity

Introduction

There is increasing consensus that biodiversity enhances ecosystem function in grassland ecosystems (*e.g.*, Hooper *et al.* 2005; Balvanera *et al.* 2006; Cardinale 2011, Marquard *et al.* 2009). Many studies assert that belowground resource partitioning among different plant species is a likely cause for this ecosystem function enhancement (Loreau & Hector 2001, Cardinale *et al.* 2006, Hooper *et al.* 2012, Scherer-Lorenzen 2014, Tilman *et*

al. 2014). If species differentiate to take up different limiting resources in space or time or both (Fargione & Tilman 2005, Mueller *et al.* 2013) and use the available resources more efficiently then resource partitioning should allow for enhanced ecosystem function when biodiversity increases (Finke & Snyder 2008).

The hypothesis that belowground resource partitioning in chemical form, space and time drives enhanced ecosystem function in higher diversity systems builds on two components. First, ecosystem function is enhanced in mixture. This first proposition has been demonstrated numerous times when it comes to the production of biomass, both above and belowground (Balvanera *et al.* 2006, *e.g.* belowground see Ravenek *et al.* 2014). For resource uptake and use there is only limited evidence that either is enhanced (Fig. 1a, von Felten *et al.* 2009). For example, Roscher *et al.* (2008) and Oelmann *et al.* (2007, 2011) showed higher resource concentration in plant tissue in mixtures compared to monocultures. Several studies also showed lower soil resource levels in high diversity mixtures, implying that more resources are taken up in more diverse mixtures (*e.g.* Tilman *et al.* 1997, Scherer-Lorenzen *et al.* 2003, Palmborg *et al.* 2005, Roscher *et al.* 2008).

Second, species partition resources. Species may partition resources in several ways: 1. Species may use different chemical forms of resources (*e.g.* different inorganic N sources such as ammonium or nitrate). 2. Species may use the same resources in different locations (spatial resource partitioning). 3. Species may use the same resources at different times (temporal resource partitioning). These types of resource partitioning are not mutually exclusive. For clarity, we use the term resource partition as a multidimensional measure of the share of the total resource pool that a species uses (reviewed by Schoener 1974). This term is largely used as a description of prey-partitioning by consumers (Vance

1978, MacPherson 1981, Finke & Snyder 2008). Resource partition is also roughly synonymous with the term niche (see Finke & Snyder 2008) but is more specific so as to include only resources and not other potential niche dimensions (see Chase & Leibold 2003).

Published results differ in terms of experimental evidence for resource partitioning at the species-level. Two studies now demonstrate that species have deeper roots in more diverse systems, a strong indicator of spatial resource partitioning (Mueller *et al.* 2013; Oram *et al.* 2018). Alternatively, several studies show the opposite, that species are more likely to aggregate in the top soil (*i.e.,* Bessler *et al.* 2009, Frank *et al.* 2010, Mommer *et al.* 2010, Ravenek *et al.* 2014). However, stable isotope studies (*e.g.,* Kahmen *et al.* 2006, von Felten *et al.* 2009, Prechsl *et al.* 2015) may provide a better proxy for resource uptake, since root distributions across the soil profile are not always an accurate measure for actual root activity (Prechsl *et al.* 2015, Volkmann *et al.* 2016). From stable isotope tracers, von Felten *et al.* (2009) found that species used more narrow resource partitions and had less resource partition overlap among species when diversity was highest as would be predicted for resource partitioning.

There is also evidence that species partition resources in time (temporal resource partitioning; Husse *et al.* 2016). Veresoglou and Fitter (1984) found that coexisting grass species differ seasonally in growth and nutrient uptake. Similarly, Hooper & Vitousek (1998) showed that the maximum use of different resources differed with season across functional groups. Furthermore, spatial and temporal resource partitioning likely occur simultaneously (Fitter 1986, Mc Kane *et al.* 1990, Kulmatiski & Beard 2013). However, only a few studies combined both spatial and temporal axes of resource partitioning. Kahmen *et*

al. (2006) found no spatial, temporal, or chemical variability in N uptake in three semi-natural, temperate grasslands (see also Pornon *et al.* 2007, Ashton *et al.* 2010). Alternatively, McKane *et al.* (2002) found that arctic tundra species partitioned forms of nitrogen across seasons (see also Kulmatiski & Beard 2013 for savannas). Together, these experimental findings do not convincingly and unambiguously demonstrate spatial and/or temporal resource partitioning.

According to niche theory, if species partition resources in either chemical form, space, time, or any combination thereof, two qualities of their resource partition are likely to change with increasing diversity. First, the breadth of species' individual resource partitions will become smaller with increasing diversity. The resource partition breadth will decrease as plants compete heavily for resources and therefore specialize on a specific subsection of the available resources to avoid the costs of such heavy competition (Hutchinson 1959, Fig. 1b). Second, the extent to which these species' resource partitions overlap will also decrease in mixtures again as species alter their resource use to avoid strong interspecific competition over these resources (Hutchinson 1959, see von Felten *et al.* 2009, Kulmatiski & Beard 2013, Fig. 1c).

Here, we used water isotope tracers and nutrient analogues for the uptake of three essential resources: water, nitrogen, and potassium across three seasons and two soil depths (see Fig. 2) in a large grassland biodiversity experiment (the Jena Experiment). Our approach differs from the papers cited above in that we investigate resource partitioning simultaneously in space and time and across multiple limiting resources along a well-studied species richness and functional group richness gradient with demonstrated effects

on ecosystem function (see Marquard *et al.* 2009, Ravenek *et al.* 2014, Weisser *et al.* 2017,). We predict that:

1. Communities that are more diverse will have greater overall resource uptake and above- and belowground biomass, indicating enhanced ecosystem function (Fig. 1a).

2. As plant diversity increases, species will decrease the breadth of their spatial, temporal, or spatiotemporal resource partition, indicating increased resource partitioning (Fig. 1b).

3. As plant diversity increases, the amount of spatial, temporal, or spatiotemporal overlap in resource use between species will decrease, indicating increased resource partitioning (Fig. 1c).

Materials & Methods

Study site

We conducted this experiment within the Jena Experiment, a large grassland biodiversity experiment (Roscher *et al.* 2004). The Jena Experiment was established at an arable field site in the floodplain of the Saale river near Jena in 2002 (Thuringia, Germany, 50°55`N, 11°35`E, 130 m a.s.l). Mean annual air temperature is 9.9 °C and annual precipitation is 610 mm (1980–2010; Hoffman *et al.* 2014) in the region. Based on the species composition of Central European mesophilic grasslands (Arrhenatherion; Ellenberg, 1996), a pool of 60 species was chosen. Species were assigned to four functional groups (16 grasses, 12 legumes, 12 small herbs and 20 tall herbs), according to a cluster analysis of a trait matrix including morphological, physiological and phenological traits (Weisser et al. 2017). The design of the main experiment combines a gradient of species

richness (1, 2, 4, 8, and 16 species) nearly-orthogonally to different levels of functional group richness (1, 2, 3 and 4 functional groups) reducing the extent to which both experimental factors confound each other as much as possible. Species composition for each species richness × functional group richness combination was chosen randomly. The plots were arranged in four blocks, following a gradient in soil texture perpendicular to the river. All plots were regularly weeded to remove species not included in the particular mixture composition. Plots were mown twice per year (early June and September) to mimic the usual management of hay meadows in this region.

Experimental design

We used 40 plots of the Jena Experiment; 10 plots of each sown number of 2, 4, 8 or 16 species. We included 9-11 plots of each functional group number (1, 2, 3 or 4). We did not include monocultures for several reasons. First, monoculture plots had significantly lower aboveground cover than other species richness levels. In this study, we universally removed plots that were significantly lower performing. We did this to provide the most conservative estimate of resource partitioning. This low performance criterion excluded all monocultures. Second, due to sampling limitations, 40 was the maximum number of plots that could be processed in the 48-hour sampling period during every season. Thus, because the monocultures did not have good comparative power without representing a disproportionate amount of our 40-plot sample, we chose to use only 2, 4, 8, and 16 species plots. The chosen plots were equally distributed among the four experimental blocks. In each of the 40 plots, three subplots were established, measuring 0.56 m x 0.44 m (0.246 m^2).

Tracer application

To exclude between plot variation in environmental conditions, we adopted a tracer application design that allows the assessment of both spatial and temporal resource uptake within the same plot (Fig. 2). We therefore selected different nutrient analogues or isotopologues for the soil resources of interest. Based on extensive testing (see Gockele *et al.* 2014), we used Rb and Li as potassium analogues. Both Rb and Li are taken up by the same ion transporters as potassium and their uptake is highly correlated (Läuchli & Epstein 1970, Fitter 1986, Doyle et al. 1998, Rodriguez et al. 2007, Gockele et al. 2014). In addition, Sr has previously been suggested as potassium analogue. In a pre-experiment to this large experiment we tested which of the three elements best serve as analogues for potassium where Sr was clearly worse compared to Li and Rb (Gockele et al. 2014). We also used the water isotopologues $H_2{}^{18}O$ and ^2H_2O to track water uptake and ^{15}N to trace nitrogen uptake. We could not test nitrogen uptake in two different depths because of the lack of a second field-compatible nitrogen isotope.

We pre-drilled 32 5-cm deep and 31 25-cm deep holes in a hexagonal grid for tracer application in each plot. The distance between holes of the same depth was 10 cm. We applied tracers to all 40 plots on two consecutive days. Half of the plots were labeled on the first day and half on the second, shallow and deep labels were always applied simultaneously in a single plot. One subplot in each plot was labeled in spring (April 18th-19th, start of the growing season), one in summer (June 27th-28th, during the regrowth after the first mowing May 30th-June 1st) and one in autumn (September 26th-27th, during the regrowth after the second mowing August 29th-30th) 2011 (Fig. 2). Tracer application,

harvest and sampling followed a very strict time schedule within comparable weather conditions and without precipitation.

We prepared two tracer solutions using deionized water; one with LiCl (Roth, Karsruhe, Germany) and $^{2}H_2O$ (Euriso-top) to label the deeper soil layer and one with RbCl (Roth, Karsruhe, Germany), $H_2{}^{18}O$ (Sigma-Aldrich, St. Louis, MS USA), and $^{15}NH_4{}^{15}NO_3$ (Cambridge Isotope laboratories, Tewksbury, MA USA) for the shallow soil layer (Fig. 2). Li and Rb were added with a concentration of 0.3 mol/L each and ^{15}N with 0.02 mol/L. The amount of ^{15}N used was estimated to result in plant target $\delta^{15}N$ values of approximately 100‰ and also chosen to be low enough to not induce fertilization effects (based on calculations by von Felten *et al.* 2009). Based on the average soil water content, $^{2}H_2O$ was added to result in an enrichment of 800‰ and $H_2{}^{18}O$ to reach 400‰ (for more details on water labeling, see Bachmann *et al.* 2015).

To apply the tracer solutions, we used a needle with four lateral holes at the tip, which was connected to a bottle-top dispenser (Socorex) through a silicon tube. The adjacent vegetation was protected with a plastic funnel. We released 2 mL tracer solution in each of the deep or shallow holes 2 cm above the drilling depths, to provide space for the tracer solution to slowly infiltrate the soil. Preceding dye applications and water isotope analysis in 3cm layers (Bachmann *et al.* 2015) showed that the actual labeling depth was between 0-10 and 20-30 cm, assuring a clear division of layers across a soil depth gradient where roots are present at both depths (Ravenek *et al.* 2014).

Sampling

During each sampling period, forty-eight hours after tracer application, we collected three to five individuals per species and plot and separated roots crowns from aboveground plant material. These root crowns were frozen for water isotope analysis (Barnard *et al.* 2006). We then cut all aboveground vegetation in a subplot just above ground level and sorted the biomass by species to quantify species specific biomass in the plot and community biomass. Aboveground biomass from the individuals cut for root crowns was added to species specific shoot biomass prior to milling and homogenizing shoot material. Weeds contributed on average 7.1% (min. 0.07%, max. 27.4%) to community biomass. In addition, there were unidentified samples, *i.e.* a mixture of target species and weeds, from 14 sampled plots which were not sorted to the species level. These unidentified samples contributed on average 18.2% (min 3.4%, max 57.4%) to the plot biomass in the 14 plots where they were found. Weeds and unidentified samples were included as additional species in the corresponding plot to calculate community biomass and tracer uptake.

We took three 3.5 cm-diameter soil cores in each plot during each sampling period and separated the cores into 0-10 cm and 20-30 cm layers. Corresponding layers were pooled per plot. The soil cores were stored at 4°C, and were later washed in tap water using a 200 μm mesh size sieve for root extraction. We pooled these three samples at each depth for our measure of belowground biomass, calculated as g/m^2. We then dried all above- and belowground plant samples at 70°C for 48 hours and weighed them.

Prior to each labeling event (spring, summer, autumn), we sampled root crowns and shoots of each species within the plot but adjacent to the designated sampling area to

determine the natural abundance of Rb and Li. We also collected root crowns from each functional group in every species richness level to measure water isotope background levels. To reduce sample load, we used ^{15}N background concentrations measured previously for all species in the plots (Gubsch *et al.* 2011).

Chemical analyses

For Li and Rb analysis, we performed a microwave accelerated (MARS 5, CEM Corporation) acid digestion with 3 mL H_2O, 5 mL 65% HNO_3 and 3 mL 30% H_2O_2, using approx. 0.3 g of dried and milled homogenized shoot material. We measured the diluted solution with a graphite furnace atomic absorption spectrometer (AAS 5 EA, Carl Zeiss Jena GmbH) to give the rare element concentrations.

For ^{15}N and %N analyses, we transferred approximately 1 mg of milled homogenized shoot material into tin capsules (IVA Analysentechnik, Meerbusch, Germany). We then processed samples with a Flash HT elemental analyser (Thermo-Scientific, Bremen, Germany). We flushed sample gas containing the analyte N_2 via a ConFlo III to an isotope ratio mass spectrometer (Delta V Advantage, Thermo-Scientific, Bremen). The isotopic values are expressed in delta notation (in ‰ units), relative to N_2 in air as standard.

When uptake could not be measured due to insufficient sample size, it was assigned an NA. Out of a total of 730 samples, only 6 total samples were too small to measure any uptake (0.8%). When insufficient plant material was present, we prioritized measuring nitrogen over rubidium and lithium. In addition to the 6 samples with no chemical analysis, 4 samples had measurements for lithium and rubidium but no nitrogen analysis (0.5%). 29

samples were too small to measure lithium and rubidium in addition to nitrogen. An additional 10 samples were too small to measure lithium in addition to nitrogen and rubidium. In total, 3.9% of our samples were too small to measure lithium and rubidium (5.3% for lithium). We assigned samples with sufficient sample size but no measured uptake a zero.

For ^{2}H and ^{18}O analyses, we extracted xylem water from the root crowns with a cryogenic water extraction line. The water samples were vaporized with a high-temperature conversion elemental analyzer and the isotopic composition was measured with an isotope ratio mass spectrometer via a ConFlo III interface (Deltaplus XP, Thermo-Scientific, Bremen, Germany). Isotopic values are expressed in delta notation (in ‰ units), relative to Vienna Standard Mean Ocean Water (VSMOW).

Calculations

Uptake rate

Excess tracer uptake rates ($exTR_{sp}$ per 48 hours) were calculated with the measured tracer values of each species in every community (TR_{sp}) and the natural abundance of each tracer (naTR) as follows:

$$exTR_{sp} = TR_{sp} - naTR \qquad \text{Eqn. 1}$$

The potassium analogues Rb and Li are expressed as molar shoot concentrations on biomass basis (µmol/ g dry weight per 48 hour time period, we refer to grams dry weight as g_{dw}). For water isotopologues and ^{15}N the units for $exTR_{sp}$ (the tracer uptake rate of a species) are given as isotope ratios. $exTR_{sp}$ for ^{15}N was additionally calculated on an aboveground biomass basis (in µmol ^{15}N / g_{dw}) and is later referred to as nitrogen uptake

rate. $\delta^{15}N$ was converted into an isotope ratio (^{15}N R, Eqn. 2), and then into atom fraction ^{15}N (Eqn. 3) and then related to the N concentration in the plant tissue (cN; in µmol N / $g_{dw,}$ Eqn. 4):

$$^{15}N\ R = \left(\frac{\delta^{15}\mathrm{N}}{1000} + 1\right) * \ 0.0036765 \qquad \text{Eqn. 2}$$

$$atom\ fraction^{15}N = \left(\frac{^{15}N\ R}{1+^{15}N\ R}\right) \qquad \text{Eqn. 3}$$

$$exTR_{sp}\left(^{15}N\right) = atom\ \ fraction^{15}N * \ cN \qquad \text{Eqn. 4}$$

We analyzed species specific uptake rates on a per biomass basis because root activity does not depend on species size and abundance. This measure enables us to compare species specific uptake efficiencies, which are not primarily based on differences in growth rates. We used molar concentrations to account for the number of molecules and not their weight. However, for water uptake rates we used isotope ratios because we do not know the plants´ water contents and transpiration rates.

Total community resource uptake

In addition to belowground biomass in 0-10 cm and 20-30 cm soil depth (g_{dw} m^{-2}), aboveground community biomass was calculated as the summed aboveground biomass of all species that constituted the community (g_{dw} m^{-2}). The community tracer uptake ($exTR_{comm}$) is expressed as the amount of tracer found in a plot minus the background level of the element as found in a plot without tracer injections (mmol m^2). We call this excess tracer and it is defined as the sum of tracer taken up and present in aboveground biomass of all plants of the community.

$$exTR_{comm} = \sum_{i=1}^{S} exTR_{sp\ i} \times BM_{sp\ i} \qquad \text{Eqn. 5}$$

where S is the number of species in a community, BM_i is the biomass of species i in g m^2, and $exTR_{sp\,i}$ is the tracer uptake rate (µmol g_{dw}, Eqn. 1 and 4) of species i. Missing tracer concentrations from samples with insufficient material (on average <0.5% of plot biomass) for chemical analysis were replaced by the mean of the same species across all plots and seasons. Community water uptake could not be calculated due to insufficient extraction of water for isotope measurements from several dominant species.

Resource partition breadth

To allow for comparisons among the different resources with different range (Fig. 2), species uptake rates were standardized according to:

$$sTr_{sp} = \frac{exTR_{sp} - mean(exTR_{sp})}{\max(exTR_{sp}) - \min(exTR_{sp})} \quad \text{Eqn. 6}$$

We then used these standardized species uptake rates to calculate Levins normalized B. Levins normalized B (Levins 1968) is a measure of resource partition breadth. Levins normalized B is the reciprocal of the Simpson-Index D, normalized by the number of sources.

$$B = \frac{1}{n \sum_{i=1}^{n} {p_i}^2} \quad \text{Eqn. 7}$$

where p_i is the fraction found in one source and n is the number of resource sources included (Fig. 4). p_i in all sources sums up to 1. Resource partition breadth is largest when all sources are used equally and lowest when one source is used exclusively. We calculated Levins B in three different ways to account for the different ways that plants could partition resources in our experiment: 1. in space, 2. in time, and 3. in both space and time. To measure uptake in space, we used the standardized water and potassium rates at the two

depths (*i.e.*, spatial partitioning only, 2 resources x 2 depths = 4 sources, for each water and potassium uptake). To measure uptake in time, we used the standardized water, potassium, and nitrogen uptake rates across the three seasons (*i.e.*, temporal partitioning only, 1 resource x 3 seasons = 3 sources for each tracer separately). To measure uptake in both space and time, we used the standardized water and potassium uptake rates from two depths and three seasons (*i.e.* spatiotemporal partitioning, 2 resources x 2 depths x 3 seasons = 12 sources). Only species present with a measured uptake rate in all sources of interest were included.

Resource partition overlap

We used proportional similarity to measure the amount of overlap between resource partitions among species (Schoener 1970, Colwell & Futuyma 1971):

$$PS = 1 - 0.5 \sum_{i=1}^{n} |p_{1i} - p_{2i}| \qquad \text{Eqn. 8}$$

where p_i is the fraction of a resource taken up such that p_i sums up to one for one species. Proportional similarity measures the intersecting area of the frequency distributions of resources used by two different species and ranges from 0 to 1 with 0 meaning no overlap and 1 meaning complete overlap. We calculated proportional similarity for all species pairs co-occurring in the same mixture plots.

Statistical analyses

We used R version 3.0.2 for calculations, statistical analyses, and graphics (R Development Core Team 2013). We used linear mixed-effects models (Bates *et al.* 2015, Kuznetsova *et al.* 2015) to examine the effect of plant diversity on resource partition breadth, proportional similarity, and community tracer uptake. To account for multiple

species measured at the same plots and measurement of the same communities at different times, we used plot nested in block as a random factor in all analyses (Table 1). We ran all models in two different ways. First, we ran two separate models, one containing species richness as a fixed effect and one containing functional group richness as a fixed effect (Tables 1 & 2). Second, in spite of the near-orthogonality in our experiment of species richness and functional group richness, we also assessed the degree to which species richness influenced our results for functional group richness by running a model with both species richness and functional group richness fitted sequentially. For this model, we fitted species richness first and functional group richness second (Supplementary statistical methods, Figure 3, Figure 4, Table S1, Table S2, Table S3). For all analyses, when results were significant, we used a Tukey's HSD test performed in the package "lsmeans" to differentiate the effects of different tracers and combinations of tracers (Lenth 2016).

For analyses of community tracer uptake, we used a nested random effect of tracer within season to control for differences due to stoichiometry of nutrient uptake and seasonal differences. We also used a separate random effect of plot to control for between plot differences (Final model – community tracer uptake ~ Fixed Effect + (1|Tracer/Season) + (1|Plot), where the fixed effect is as described above). For analyses of Levins B, we used a random effect of species identity nested within tracer type nested within axis type, where axis type refers to spatial, temporal or spatiotemporal effects (final model – Levins B ~ Fixed Effect + (1|Species/Tracer/Axis Type) + (1|Plot), where the fixed effect is as described above). For analyses of proportional similarity, we used a nested random effect of species pair identity nested within tracer nested within axis type (spatial,

temporal, spatiotemporal, final model – Proportional Similarity ~ Fixed Effect + (1|Species Pair/Tracer/Axis Type) + (1|Plot), where the fixed effect is as described above).

In addition to these models that calculated the general effect of species richness and functional group richness on our response variables, we also isolated these axis types (spatial, temporal, and spatiotemporal) in individual models using fixed effects for both sown species richness and functional group richness as well as the interactions between sown species richness/functional group richness and analogue/isotopologue (potassium, nitrogen, or water). The final model for this individual level statistical analysis was: Response variable$_{\text{axis type}}$~Species richness*Analogue+Functional Group Richness*Analogue+(1|Sample/Tracer)+(1|Plot).

Results

General results of tracer application

At the community level, total root biomass in both soil depths and shoot biomass increased significantly with plant species richness (Figure 3, Table S1). Total Rb (used as a potassium analogue in shallow soil) and community ^{15}N uptake (Eqn. 5) also increased significantly with increasing species richness (Figure 3, Table S1). Furthermore, aboveground biomass, root biomass (0-10 cm) and community nitrogen uptake significantly varied among seasons (Figure 3, Table S1). Plants produced significantly more aboveground biomass in spring and significantly more roots in autumn (Figure 3). Interestingly, root biomass in the 0-10 cm soil horizon significantly decreased with increasing species richness in spring and increased with increasing species richness in

autumn (Figure 3, Table S1). Total community resource uptake of ^{15}N was significantly higher in spring. In contrast, increasing functional group richness did not result in increased biomass above- or belowground or increased community level tracer uptake for any tracer (Table S1).

At the species level, increased species richness resulted in lower average uptake of $H_2{}^{18}O$ and ^{15}N (Figure 4, Table S2). That is, each species was able to obtain a smaller portion of the resources available but the sum of all resource uptake increased with species richness. Furthermore, the tracer uptake rates of all tracers varied significantly with season. The uptake of potassium analogues and water isotopologues (Rb, Li, $H_2{}^{18}O$, and ^2H_2O) was significantly higher in autumn than in spring (Figure 4). In contrast, uptake of nitrogen (in the form of ^{15}N) was significantly higher in spring than in autumn. However, these seasonal differences were not significantly affected by species richness (Table S2). Increased functional group richness did not significantly affect the species-level uptake of any tracer (Table S2). Uptake of water isotopologues and potassium analogues was higher in topsoil compared to deeper soil (Figure 4). However, species richness and depth did not interact for any resource (Table S3) while the interaction between season and depth was significant for both potassium analogues and water isotopologues (Table S3).

Prediction 1: Communities that are more diverse will have greater overall resource uptake and above- and belowground biomass (Fig. 1a).

Overall, we found that community tracer uptake (Eqn. 5) increased with increasing species richness but not with functional group richness (Table 1, Figure 5a). This increasing community tracer uptake appeared to be driven by increased uptake of Rb and

^{15}N (Figure 3, Table S1). Both above- and belowground biomass increased with increasing species richness but not with functional group richness (Figure 3, Table S1).

Prediction 2: As plant diversity increases, species will decrease the breadth of their spatial, temporal, or spatiotemporal resource partition (Fig. 1b).

Contrary to our prediction, we found that resource partition breadth, which is the relative distribution of resources used by plants (in terms of Levins B, Eqn. 7), did not significantly decrease with increasing species richness (Figure 5b, Table 1). Indeed, species did not specialize on different resources when in mixture neither spatially, temporally, nor across the multidimensional spatio-temporal resource partition (Figure 5b, Table 2). Species had uniformly broad spatial (for water and potassium) and temporal (for water, potassium, and nitrogen) resource partitions. When both space and time were used to calculate Levins B, resource partition breadth of the combined potassium analogues and water isotopologues was significantly smaller than both potassium analogues and water isotopologues alone (Tukey's HSD test: $p<0.0001$, t ratio= 9.152 and t ratio=9.152, respectively). We also found that resource partition breadth did not decrease significantly with increasing functional group richness when Levins B was calculated across either space, time, or both (Figure 6, Table 1).

Prediction 3: As plant diversity increases, the amount of spatial, temporal, or spatiotemporal overlap in resource use between species will decrease (Fig. 1c).

Contrary to our prediction, we found that the amount of general overlap (in terms of proportional similarity, Eqn 8) in resource use across space, time, and both did not

decrease significantly with increasing species richness (Figure 5c, Table 1). Species did not change in their pairwise overlap with each other. When proportional similarity was calculated across a temporal axis, species were more similar in their nitrogen uptake than their water uptake (Figure 5c, Table 2, Tukey`s HSD test, p=0.005, t test statistic=3.109). When proportional similarity was calculated across both space and time, species were less similar in their uptake of water and potassium together than of both potassium and water alone (Figure 5c, Table 2, Tukey's HSD test – $p<0.05$ and t ratio=3.54 and t ratio=4.322, respectively). This pattern supports the value of calculating multidimensional resource partitions. Furthermore, there was a significant interaction between the tracer type and species richness (Figure 5c, Table 2), indicating that the relationship between proportional similarity and species richness differed significantly between the three resource types (potassium, water, and nitrogen).

In general, resource partition breadth and overlap did not decrease as predicted with increasing functional group richness for any of our three calculations of Levins B and proportional similarity (Figure 6c, Table 2). However, we did find a significant interaction between functional group richness and the resource types, temporally. Across time, species were uniformly similar in their use of nitrogen across functional group richness while species slightly (though not significantly) increased in their similarity of water uptake with increasing functional group richness (Figure 6c, Table 2).

Discussion

Belowground resource partitioning is one of the most commonly invoked hypotheses as the explanation for the positive relationship between biodiversity and ecosystem function. Using the most comprehensive resource tracer experiment in a biodiversity-ecosystem function experiment to date, we tested the two criteria that should be met if resource partitioning is the major driver of biodiversity-ecosystem function relationships. First, we assert that total community resource uptake and above- and belowground biomass should increase with increasing diversity, demonstrating enhanced ecosystem function. Second, we assert that species that partition resources will occupy smaller and less overlapping resource partitions (synonymous with the realized "resource niche" sensu Tilman 1982) in higher diversity communities. We confirm here using nutrient and water resource uptake that community belowground resource acquisition increases with increasing diversity (see also Tilman *et al.* 1997, Roscher *et al.* 2008, Bessler *et al.* 2012 for evidence of resource partitioning based on other methods). We also found enhanced biomass production both above and belowground as in other studies (*e.g.* Tilman *et al.* 2001, Ravenek *et al.* 2014). However, while ecosystem function in terms of total community resource uptake and biomass increased with species richness, we found no evidence that resource partitions become smaller or less overlapping with either increasing species richness or with functional group richness. These changes in resource partitions are prerequisites if resource partitioning drives this enhanced ecosystem function.

These results add to a burgeoning majority of papers that do not find strong evidence for belowground resource partitioning in grasslands (Kahmen *et al.* 2006,

Mommer *et al.* 2010, Ravenek *et al.* 2014, Bachmann *et al.* 2015, Siebenkäs & Roscher 2016). In the Jena Experiment, Ravenek *et al.* (2014) found that low diversity communities and high diversity communities alike had significantly more roots in the top soil, providing evidence that species diversity does not increase root spatial segregation, a measure assumed to be indicative of resource partitioning (see also Siebenkäs & Roscher 2016). Tracer experiments, which overcome the problem that root presence is not necessarily directly related to resource uptake, came to similar conclusions (*i.e.* von Felten *et al.* 2009; Prechsl *et al.* 2015). In semi-natural managed grasslands on sites similar to the Jena Experiment, Kahmen *et al.* (2006) found that plants did not partition nitrogen across either space or time. Also at the Jena Experiment, Bachmann *et al.* (2015) found no evidence for plant partitioning of water resources across a biodiversity gradient. Given the enhanced ecosystem function with increasing species richness in the Jena Experiment, combined with the elevated total community resource uptake demonstrated here, our results strongly argue that belowground resource partitioning is not the sole driver of enhanced function in this experiment.

However, in spite of lack of support for our predictions there are three reasons why belowground resource partitioning may yet drive the positive relationship between biodiversity and plant productivity. First, our predictions are only valid if the resources we examined (potassium, nitrogen, and water) are limiting in either space or time. If, in this system, none of these three resources is limiting, then a higher demand for soil resources due to the increase in community biomass production with diversity may be met by higher resource uptake rates. Species need not partition these resources. However, there is ample evidence that both nitrogen and potassium are limiting in this system (Oelmann *et al.*

2007) and other temperate grassland systems (Hoekstra *et al.* 2014, Harpole *et al.* 2016). Indeed, nitrogen is cited as the most crucial limiting resource in temperate grassland ecosystems in humid climates like the Jena Experiment (Klapp 1971, Ellenberg 1977, Vitousek & Howarth 1991). Furthermore, Oelmann *et al.* (2007) demonstrated that the availability of nitrogen decreases with increasing species richness, suggesting that nitrogen is likely limiting at the Jena Experiment in spite of the site's long agricultural history. Further, Harpole *et al.* (2016) confirmed the limiting nature of both nitrogen and potassium using similarly composed grassland sites world-wide. Additionally, total biomass production decreased with time, indicating nutrient limitation (Ravenek *et al.* 2014). Alternatively, water limitation may only be relevant when longer periods of drought occur during the growing season (Ciais *et al.* 2005). Further, resource limitation may differ across seasons. We found strong seasonal differences in resource uptake. In particular, nitrogen uptake was highest during the spring when we expect growth to also be highest. These results imply that resource limitation (and thus resource partitioning) may be most important during the spring when the majority of growth is actively occurring (Figure 3, 4, Table S2). Other mechanisms may become more important during other parts of the year.

Second, our predictions for Levins B and proportional similarity are only indicative of belowground resource partitioning if resource partitioning occurs via a plastic response to interactions among species. Differences in resource use may be fundamental, *i.e.* species occupy different fundamental resource partitions/niches. Alternatively, differences in resource partitioning may be a plastic response to the resource use of their neighbors, *i.e.* species occupy different realized resource partitions/niches. If species occupy fundamentally different resource partitions then we do not expect their resource partition

breadth or overlap to change with increasing diversity. However, only the plastic response of a neighbor to its neighbors' resource use results in an increase in function relative to the monoculture of the individual component species. That is, in order for ecosystem function to increase above and beyond the expectation based on their monoculture performance resource partitioning must be plastic (Yachi & Loreau 2007, Naeem *et al.* 1996, Figure S1, Fiegna et al. 2015). When species with fundamentally different resource partitions in monoculture are combined in mixture, resource uptake increases for the total community in an "additive" manner (Figure S1). While it is theoretically possible that resource partitioning can be fundamental in this fashion, we do not think that this is solely the case in our experiment. Proportional similarity between our species' resource partitions was relatively high in low diversity mixtures and high diversity mixtures alike (Figure 5c, 6c). If species had non-overlapping resource partitions, we would expect their proportional similarity to be close to zero regardless of diversity.

Third, and potentially most likely, the resolution at which we are currently able to measure resource uptake is relative rough. Plants, however, may partition resources at very fine temporal and spatial resolutions. In space, groups that are significantly more different than the groups of plants examined here (trees and grasses) were shown by Kulmatiski et al. (2010) to partition water at a finer spatial scale than expected. Furthermore, in time, species may alter their uptake strategies often and at small temporal scales which current research methods are difficult to capture (Scanlon & Albertson 2003, Williams & Albertson 2004, Kulmatiski & Beard 2013).

Several alternative mechanisms may be more likely than belowground resource partitioning to explain overall increased ecosystem function in diverse communities. First,

if belowground resources are not limiting, species may partition resources aboveground and this may in turn lead to increased uptake belowground (van Ruijven & Berendse 2005, Bessler *et al.* 2012). In grasslands, light is considered a strongly limiting resource and a likely driver of resource partitioning (Naeem *et al.* 1994, Spehn *et al.* 2000, 2005). However, Bachmann *et al.* (2017) found that resource partitioning for light did not increase with increasing diversity at the Jena Experiment. Furthermore, at the Jena Experiment, these belowground resources are likely limiting (see above).

In addition to resource partitioning aboveground, facilitation - positive interactions between species - or negative biotic feedback from species specific pests and pathogens (negative density- and frequency-dependent effects) may drive the enhanced total community resource uptake of each tracer and biomass production found here (Loreau & Hector 2001, Spehn *et al.* 2002, Temperton *et al.* 2007, Schnitzer *et al.* 2011, Maron *et al.* 2011, de Kroon *et al.* 2012, Kulmatiski *et al.* 2012, Wright *et al.* 2013, Wright *et al.* 2017). In fact, facilitation by N fixing legumes may be likely at the Jena Experiment (Temperton *et al.* 2007, Gubsch *et al.* 2011, Roscher *et al.* 2011). Negative soil feedbacks also explain lower performance of low species richness mixtures when compared to high species richness mixtures at the Jena Experiment (Zuppinger-Dingley *et al.* 2014).

Conclusions

Overall, our results demonstrate that species-rich communities utilize more resources than species-poor communities without a significant decrease in the resource partition breadth of individual species in space or time. Furthermore, we found similar resource partition overlap between species across diversity. These results do not match

our predictions based on belowground resource partitioning. Furthermore, these results imply that other mechanisms must be co-occurring with resource partitioning either simultaneously or alternating throughout the year. Indeed, our results suggest that other mechanisms are potentially stronger drivers of enhanced ecosystem function than belowground resource partitioning. In combination, the effects of multiple mechanisms on resource uptake may cancel each other out at the species level. At the community level, however, each/all of these mechanisms may result in overall higher ecosystem function.

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Table 1. ***Summary of the mixed-effects models for community level tracer uptake, Levins B, and proportional similarity.*** Nested effects are denoted with a "/". For example "Tracer/Season" indicates that "tracer" was nested within "season" for the analysis. Significant results ($p<0.05$) are indicated with bold. Degrees of freedom are reported with the model degrees of freedom first followed by the residual degrees of freedom and are estimated via a Satterthwaite approximation.

Dependent variable	**Fixed effect**	**Random effects**	**P**	**Degrees of Freedom**	**T**	**Standard Error**	**R^2 (direction of effect)**	**Prediction**
Community tracer uptake	***Species richness***	***Tracer/Season + Plot***	***1.28 x e^{11}***	***4.23, 38.16***	***9.514***	***0.059***	***0.097 (↑)***	↑
Community tracer uptake	*Functional group richness*	*Tracer/Season + Plot*	*0.071*	*5.9, 38.07*	*1.856*	*0.498*	*0.011 (↑)*	↑
Levins B	Species richness	Species/Tracer/ Axis Type + Plot	0.174	61.05, 1.32	1.320	0.001	0.002 (↑)	↓
Levins B	Functional group richness	Species/Tracer/ Axis Type + Plot	0.240	49.403, 1.148	1.148	0.005	0.002 (↑)	↓
Proportional similarity	Species richness	Species Pair /Tracer/Axis Type + Plot	0.206	46.51,30.89	1.292	0.0019	0.003(↑)	↓
Proportional similarity	Functional group richness	Species Pair /Tracer/Axis Type + Plot	0.118	27.2, 1.616	1.616	0.008	0.005(↑)	↓

Table 2: Summary of individual mixed effect for each axis type separately with species richness and functional group richness as continuous variables, and the tracer analogue (*i.e.* potassium, water, and nitrogen) as factors as well as the interactions between species richness and analogue and functional group richness and analogue. Significant results ($p<0.05$) are presented in bold. Marginally significant results ($p<0.10$) presented in italics. These results are indicative of models that only include the possible levels (*i.e.* Nitrogen is not present in the spatial analysis because it was only injected at a single depth).

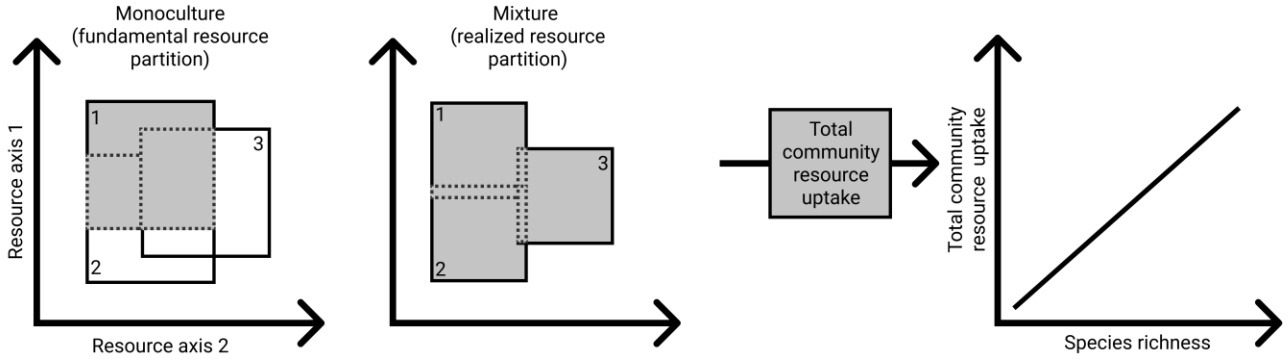
Dependent Variable	Axis Type	Fixed effect	P Value	Degrees of freedom	T	Standard error
Levins B	Spatial	Species richness	0.953	136.9	-0.059	0.0014
		Functional group richness	*0.084*	*126.0*	*-1.492*	*0.0069*
		Analogue	0.137	461.7	-1.49	0.0292
		Species richness * Analogue	0.509	651.6	-0.661	0.0085
		Functional group richness * Analogue	0.439	877.5	-0.774	0.0090
Levins B	Temporal	Species richness	0.246	207.0	1.162	0.0022
		Functional group richness	0.951	206.2	-0.061	0.0107
		Analogue (K)	0.774	613.9	0.288	0.0406
		Analogue (N)	0.181	614.0	1.338	0.0474
		Species richness * Analogue (K)	0.832	660.0	-0.212	0.0027
		Species richness * Analogue (N)	0.182	663.4	-1.336	0.0039
		Functional group richness * Analogue (K)	0.572	662.7	0.655	0.0130
		Functional group richness * Analogue (K)	0.864	659.2	0.171	0.0149
Levins B	Spatiotemporal	Species richness	0.150	164.1	1.45	0.00140
		Functional group richness	0.425	102.1	0.801	0.00674

		Analogue (K)	0.371	122.2	-0.894	0.0237
		Analogue (K +H_2O)	**2.49 x e^{-6}**	**1721**	**-4.725**	**0.0492**
		Analogue (N)	0.286	1669	1.068	0.0401
		Species richness * Analogue (K)	0.270	1721	-1.104	0.0015
		Species richness * Analogue (K + H_2O)	0.421	1946	0.804	0.0032
		Species richness * Analogue (N)	0.201	1930	-1.279	0.0026
		Functional group richness * Analogue (K)	0.912	1934	-0.111	0.0072
		Functional group richness * Analogue (K + H_2O)	0.864	1989	-0.172	0.0158
		Functional group richness * Analogue (N)	0.691	1995	-0.397	0.0124
Proportional similarity	Spatial	Species richness	0.898	57.20	-0.129	0.0023
		Functional group richness	0.323	45.80	0.999	0.0105
		Analogue	0.837	1205	0.205	0.0345
		Species richness * Analogue	0.282	1280	1.076	0.0021
		Functional group richness * Analogue	0.990	1438	0.012	0.0084
Proportional similarity	Temporal	Species richness	0.349	15.90	0.939	0.0029
		Functional group richness	0.224	83.90	1.220	0.0013
		Analogue (K)	0.135	1318	1.496	0.0446
		Analogue (N)	**6.52 x e^{-6}**	**1298**	**4.527**	**0.0493**
		Species richness * Analogue (K)	0.407	1320	-0.83	0.0028
		Species richness * Analogue (N)	**0.008**	**1318**	**-2.643**	**0.0030**
		Functional group richness * Analogue (K)	0.955	1283	0.056	0.0163
		Functional group richness * Analogue (N)	**0.047**	**1300**	**-1.986**	**0.0123**

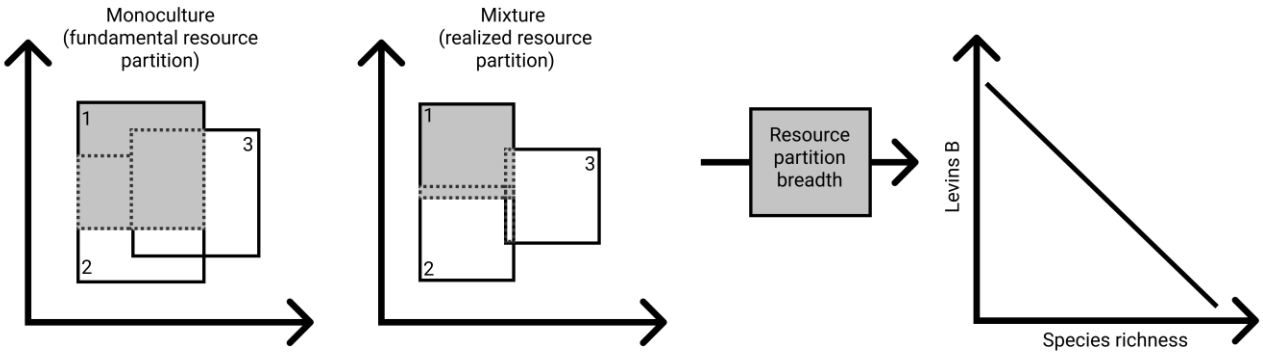
Proportional similarity	Spatiotemporal	Species richness	0.326	46.0	0.992	0.0021
		Functional group richness	0.287	40.0	1.079	0.0099
		Analogue (K)	0.739	1665	0.333	0.0254
		Analogue (K +H_2O)	**0.006**	**3628**	**-2.735**	**0.0635**
		Analogue (N)	**8.49 x e^{-4}**	**3297**	**3.339**	**0.0407**
		Species richness * Analogue (K)	0.846	1653	0.193	0.0015
		Species richness * Analogue (K + H_2O)	0.394	3587	0.853	0.0039
		Species richness * Analogue (N)	**0.002**	**3469**	**-2.985**	**0.0025**
		Functional group richness * Analogue (K)	0.450	2007	0.756	0.0063
		Functional group richness * Analogue (K + H_2O)	0.350	3671	0.935	0.0166
		Functional group richness * Analogue (N)	0.102	3464	-1.632	0.0099

Figure 1: ***Theoretical expectations for resource partitioning in grassland systems (after von Felten et al. 2009).*** In the figure below, each box labeled 1, 2, and 3 represents an individual species' resource partition across two resource axes (i.e., nitrogen and water or nitrogen and potassium). Areas in grey highlight a given comparison. All comparisons are from populations of one species (monoculture) to three species (mixture). If plants partition water, nitrogen, and potassium (the three resources we measured in terms of uptake rates in this study) then we expect (A) total community resource uptake of each tracer to increase with increasing plant diversity. We also expect that the breadth of the resource partition in terms of Levins B (B) will decrease with increasing plant diversity. That is, the size of the individual species resource boxes in the figure below will be smaller in mixture than in monoculture. We also expect that the overlap between species' resource partitions (boxes below) will decrease with increasing diversity (C). All predictions are made in terms of monocultures vs. mixtures, though our results do not include monocultures.

A)



B)



C)

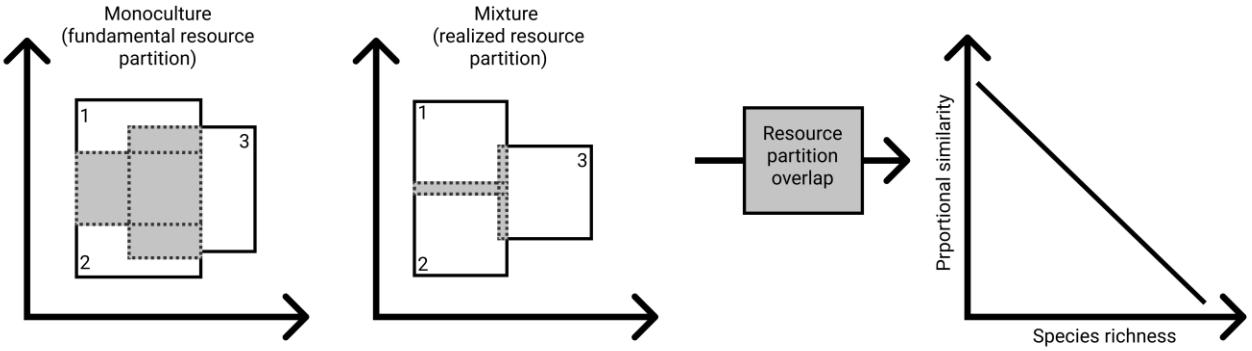


Figure 2. Experimental design. The experiment was conducted in spring (April), summer (June; regrowth after first mowing) and autumn (September; regrowth after second mowing). Tracers for potassium (triangles below, Rb, Li) and water (squares below, ^{18}O, ^{2}H) were applied in two soil depths at each time point. A nitrogen tracer (circles below, ^{15}N) was applied in the shallow soil layer only. The background drawing was modified from Ellenberg & Leuschner (2010).

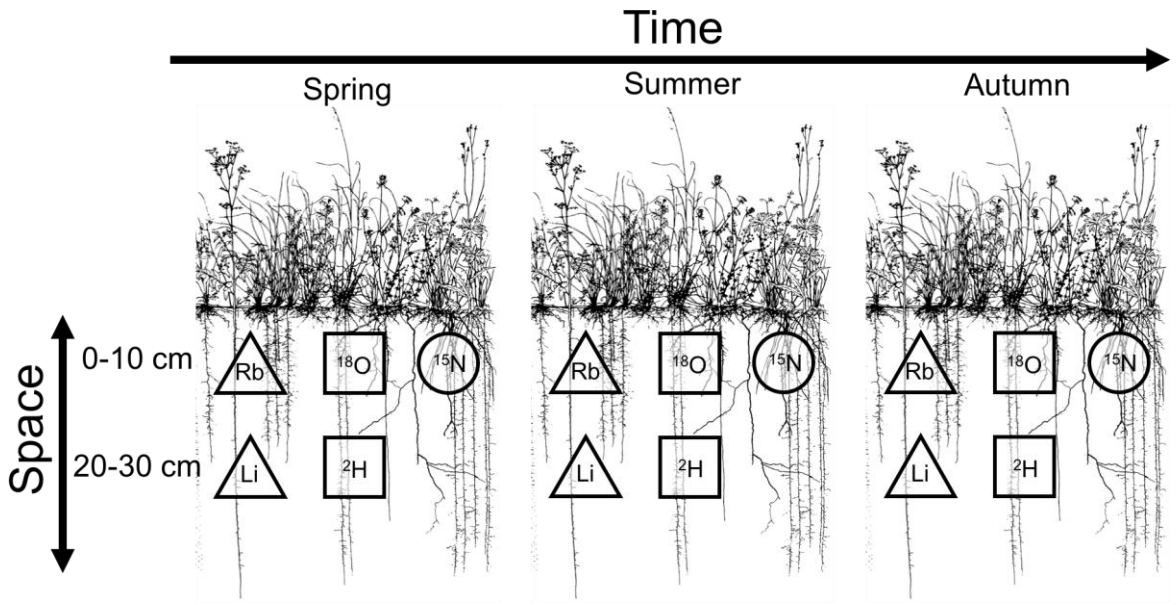


Figure 3: Community tracer uptake and above- and belowground biomass at different species richness levels, overall and in different seasons separately. Rb and ^{15}N were applied in shallow soil (0-10 cm), Li in deep soil (20-30 cm). Data are log transformed and means ± 1SE are presented (n= 40 for each tracer and season – 120 overall). See table S1 for full statistical results. Methods for these models can be found in the supplementary statistical methods.

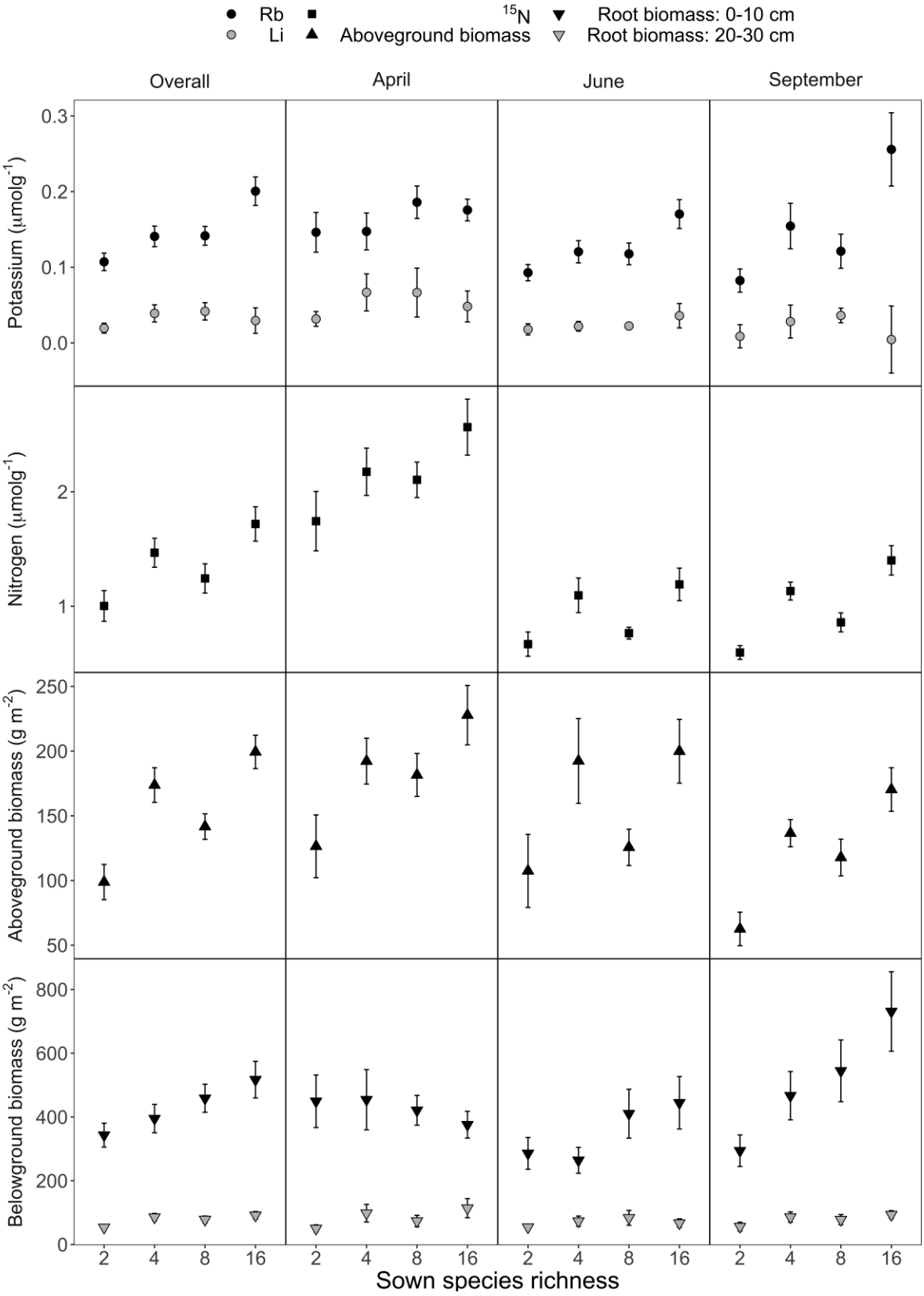


Figure 4: Species tracer uptake rate over 48 hours at different species richness levels and in different seasons. Black symbols indicate tracer application in shallow soil (0-10 cm), grey symbols in deeper soil (20-30 cm). Data are log transformed and means ± 1SE are presented (n: Rb=580, Li=571, ^{18}O=427, ^{2}H=427, ^{15}N= 601). See table S3 for full statistical results. Methods for these models can be found in the supplementary statistical methods.

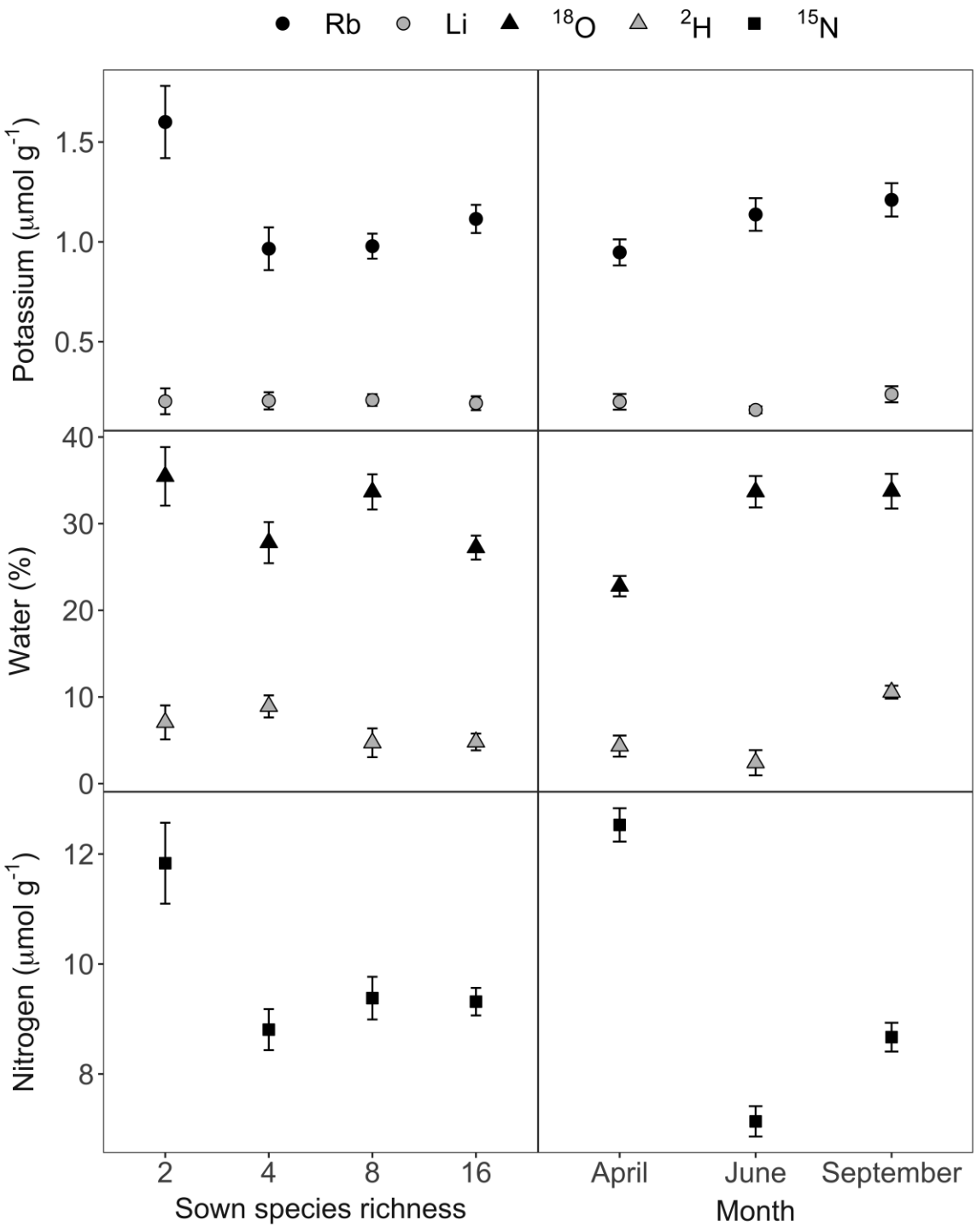


Figure 5: ***Results of water, nitrogen, and potassium tracer uptake at the Jena Experiment with increasing species richness.*** In agreement with our predictions, we found that community tracer uptake increased with increasing species richness. Shown are means (± 1 SE) per species-richness level for nitrogen (circles) and potassium (squares) (A). Contrary to our predictions, at the species level, we found that the breadth of the resource partition in terms of Levin's B (B) and the overlap between partitions in terms of proportional similarity (C) did not significantly decrease with increasing species richness when calculated by depth (spatial), across season (temporal), or both (spatiotemporal). Each figure shows regression lines from linear model approximations. Grey lines indicate non-significant linear models. Black lines represent significant linear models. *Results presented for "potassium and water" are the full multidimensional resource partition calculations, excluding nitrogen because this was not applied to two depths. See table 1 for full model statistics and table 2 for individual models.

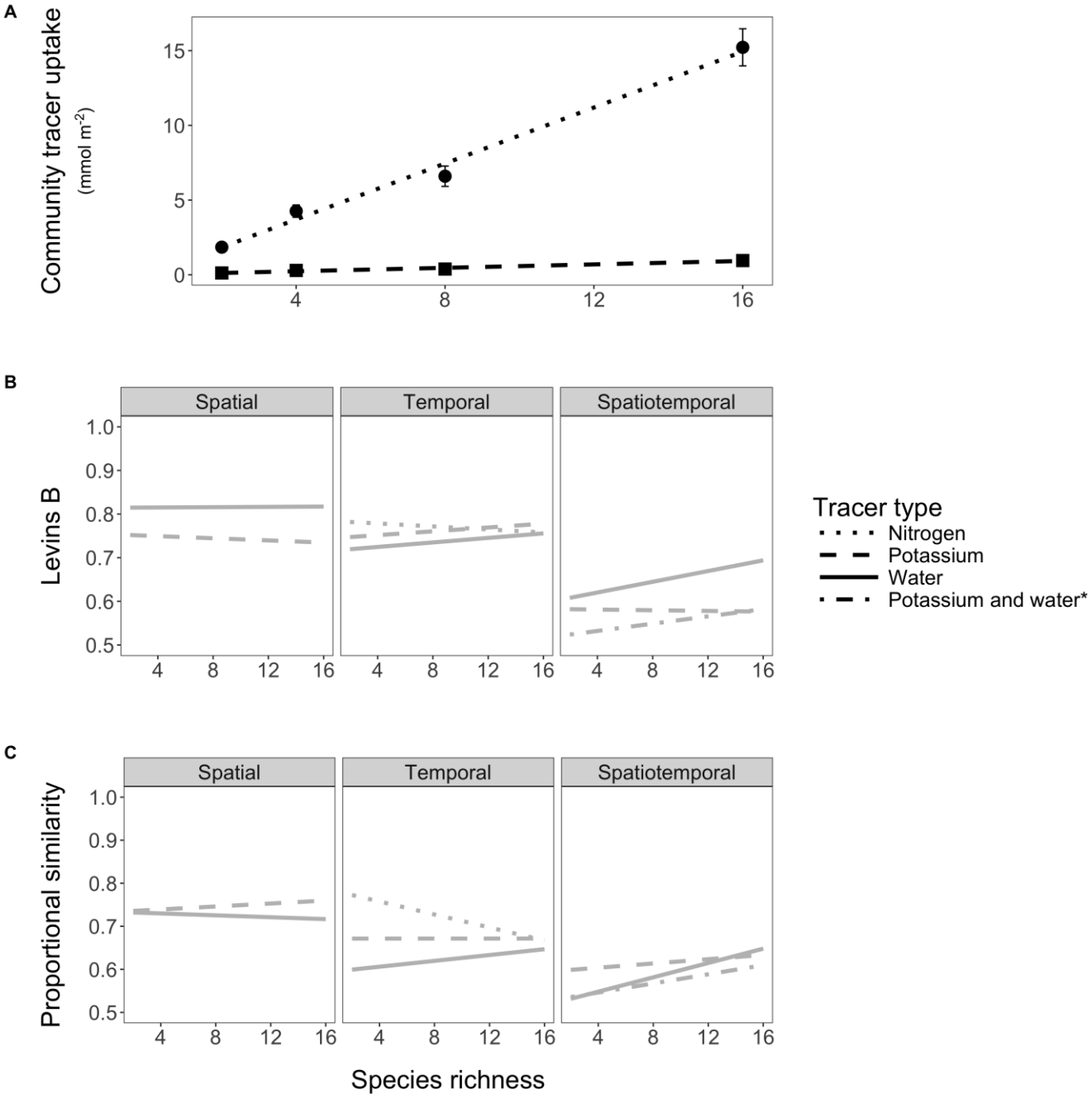


Figure 6: ***Results of water, nitrogen, and potassium tracer uptake at the Jena Experiment with increasing functional group richness.*** We found that contrary to our predictions community tracer uptake (A) did not increase significantly with increasing species richness. Shown are means (± 1 SE) per species-richness level for nitrogen (circles) and potassium (squares) (A). Contrary to our predictions, at the species level, we found that the breadth of the resource partition in terms of Levin's B (B) and the overlap between partitions in terms of proportional similarity (C) significantly increased with increasing functional group richness when calculated by depth (spatial), across season (temporal), or both (spatiotemporal). Each figure shows regression lines from linear model approximations. Grey lines indicate non-significant linear models. Black lines represent significant linear models. *Results presented for potassium and water are the full multidimensional resource partition calculations. See table 1 for full model statistics and table 2 for individual models.