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running head: nitrogen partitioning between plants

Belowground nitrogen partitioning in experimental grassland plant communities of varying species richness

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Abstract

Partitioning of soil nitrogen (N) by niche separation among species may be an important mechanism explaining species coexistence and positive biodiversity–productivity relationships in terrestrial plant communities. However, there is little experimental evidence for such partitioning - in particular, as assessed across a gradient of species richness. In experimental communities of one, three, and six temperate grassland species in the field, we tested whether increasing species richness (1) decreases niche breadths of individual species, (2) decreases niche overlap among species, and (3) increases niche breadth of whole communities. Six N sources consisting of three different chemical forms of ^{15}N-labeled N ($^{15}NO_3^-$, $^{15}NH_4^+$, U-$^{13}C_2$-^{15}N-glycine) injected at two soil depths (3 and 12 cm) were applied to each community. The chemical form and the soil depth of N characterize the niches for which niche breadth (Levins' B) and overlap (Proportional Similarity) were measured. After 48 hours, aboveground plant material was harvested to measure ^{15}N enrichment. As expected, niche breadth of single species and niche overlap among species decreased with increased species richness, but community niche breadth did not increase. The decrease in niche breadth and niche overlap mostly occurred among subordinate species or pairs of subordinate and dominant species, rather than among dominant species. Species in the 6-species mixtures mostly preferred NO_3^- from shallow soil. This may be partly explained by the presence of legumes in all 6-species mixtures which allowed "N sparing" (i.e., increased availability of soil N since legumes rely more on atmospheric N_2 than on soil N). Niche separation with respect to N uptake from different chemical forms and soil depths neither contributed much to facilitating the coexistence of dominant species nor do our results suggest it as a major driver of positive diversity–ecosystem functioning relationships. However, partitioning of N may be important for the persistence of subordinate species.

Keywords complementarity, facilitation, Levins' B, ^{15}N uptake, niche breadth, niche overlap, niche separation, plant species richness, Proportional Similarity, resource

partitioning, temperate grasslands

Introduction

One central question in plant ecology is, how large numbers of plant species can coexist on a small area. A classical answer from resource-based competition theory focuses on species complementarity with respect to resource niches. Niches are a well established principle in animal communities (e.g., Hutchinson 1959). Plants, however, are sessile organisms that depend on a common set of resources (water, light, CO_2, mineral nutrients). Hence, little potential is left for the separation of resource niches in plant communities, and empirical support for their existence is scarce (but see reviews in Hutchinson 1978, Bazzaz 1996, Silvertown 2004). One way in which plant species within a community could differ in resource niches, is by partitioning the uptake of a common resource in space, time, or chemical form.

Species differences in vertical distribution of root biomass (Parrish and Bazzaz 1976, Yeaton et al. 1977) and activity (Mamolos et al. 1995, Veresoglou and Fitter 1984) have been suggested to promote species coexistence by reducing interspecific competition for soil resources. For example, the association of a deep-rooting herb species (*Plantago lanceolata*) with a shallow rooting grass with high competitive ability (*Anthoxanthum odoratum*) allowed nutrient uptake from deeper soil layers, which would otherwise remained unused (Berendse 1982).

Of all resources that plants generally take up from the soil, nitrogen (N) is likely to be most limiting to net primary production in temperate ecosystems (Vitousek and Howarth 1991). Apart from partitioning N by taking it from different depths of the soil, plants might partition N by using it in different chemical forms such as NO_3^- and NH_4^+. Even organic forms of N could matter, although evidence for plants to bypass microbial mineralization and directly take up dissolved organic N such as amino acids under field conditions mostly comes from studies in very nutrient-poor environments, such as arctic

tundra (Schimel and Chapin 1996), boreal forest (Näsholm et al. 1998), and low-productivity grassland (McKane et al. 1990, Bardgett et al. 2003). In arctic tundra, simultaneous partitioning of N in space, time, and chemical form (NO_3^-, NH_4^+, glycine) was demonstrated by McKane et al. (2002). Thereby, the most productive species used the most abundant N forms, and less productive species used less abundant forms. Such partitioning of N may not only facilitate coexistence of rare species, but also enhance the total N use of species-rich compared to species-poor communities. However, in temperate grasslands plants were shown to prefer inorganic N (Harrison et al. 2007) and NO_3^- in particular (Kahmen et al. 2006). The latter seems plausible since NO_3^- concentrations are usually higher than those of NH_4^+ in aerobic soils of neutral pH (Marschner 1995), as typically found in temperate grasslands. Hence, it is unclear whether plants under more nutrient-rich conditions show similar N partitioning as found in the arctic, and whether species richness would enhance it.

Within the last decade, many experiments have shown that species richness affects ecosystem functioning (as reviewed e.g., in Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2006 and 2007). In temperate grasslands, species richness typically increases productivity and mixtures yield more biomass than expected from averaging the monoculture yields of the constituent species. This "overyielding" has often been attributed to complementary resource use due to niche separation. Whereas some ecological theory (Tilman et al. 1997, Loreau 1998), as well as use of an additive partitioning method endorsed the role of species complementarity (Loreau and Hector 2001, Tilman et al. 2001, van Ruijven and Berendse 2003, Roscher et al. 2005, Cardinale et al. 2007), Hubbell (2001) formulated a Unified Neutral Theory, claiming that plant species are competitively equivalent, niche differences irrelevant, and diversity produced by random drift of species in and out of a community. These contrasting views have currently stimulated the debate on how important niches may be in structuring plant communities (see e.g., Fargione et al. 2003, Clark et al. 2007), particularly, since elucidating the underlying biological mechanisms (niche and neutral processes) is still

difficult.

In this study, we used ^{15}N-labeling techniques to test whether temperate grassland species partition soil N, and how this partitioning relates to species richness. We measured species niches characterized by two "niche axes", i.e., the chemical form and soil depth of N uptake. This is the operational definition of "niche" in this paper. We examined if species changed their niche when grown in communities of varying species richness, comparing species *fundamental niches* in monoculture with species *realized niches* in mixtures of three or six species (Hutchinson 1957). The niche breadth of each species at a particular richness level was calculated as Levins' B (Levins 1968), whereby the broadest niche results from even use of all N sources provided, the narrowest niche from exclusive use of one N source. Niche overlap was calculated as Proportional Similarity (Schoener 1970) between species. We hypothesized that with increasing species richness plants (1) narrow their niche breadths and (2) reduce their niche overlap with other species, allowing plants to partition N. Moreover, we hypothesized that (3) increased species richness would result in larger total niche space occupied by plant communities, and that mixtures would occupy a larger total niche space than individual monocultures (Fig. 1).

Methods

Experimental Design

N partitioning was tested using ^{15}N tracers, as part of a larger biodiversity experiment (Wacker et al. 2008), at a grassland site near Zurich (Switzerland, 8 ° 54 ' E/47 ° 38 ' N, 443 m a.s.l.). The site has a sandy-loamy soil with a pH of 7.6±2. Here, we used a subset of 24 plots of 1.5 m × 2 m that contained one, three, or six plant species (Table 1). Species were randomly assembled from two pools of six species, to avoid results restricted to a particular species pool. Each pool contained two grasses, three forbs and one legume, whereof nine experimental communities were formed: monocultures of all six species, two

3-species mixtures, and the full 6-species mixture. The 3-species mixtures were obtained by randomly splitting each pool in two non-overlapping groups of three species, one of them containing the legume. Mixtures were replicated once (2×2×3=12 plots), monocultures were not replicated (2×6=12 plots). In mid April and at the end of June 2004, each plot received 4 g N·m^{-2} and 2 g P·m^{-2} (granular fertilizers, Agroline, Lonza). The plots were constantly weeded throughout the growing season.

The ^{15}N tracer experiment presented here was organized in two sets. The plant communities of the first pool (n=12 plots) were ^{15}N labeled between 26–28 May (Set 1), those of the second (n=12 plots) between 19–21 July 2004 (Set 2). Six ^{15}N treatments were randomly allocated and applied to six 0.5 m × 0.5 m subplots within plots (Appendix Fig. 1). The treatments were three chemical forms of ^{15}N-labeled N (NO_3^-, NH_4^+, glycine) factorially crossed with two soil depths of application (3 and 12 cm). We used the amino acid glycine to represent organic forms of N, since it is one of the most abundant amino acids in the soil solution of grasslands (Streeter et al. 2000, Bol et al. 2002).

^{15}N tracer application

Each subplot received 6.95 mg ^{15}N (27.8 mg ^{15}N m^{-2}) homogeneously spread over 52 injection points receiving 2 ml tracer solution (4.4 mmol l^{-1} ^{15}N) each. Injection points were spaced by 7.5 cm in a hexagonal grid. Tracer solutions for the three chemical forms of N were K^{15}NO$_3$, ^{15}NH$_4$Cl (98 % ^{15}N), and U-^{13}C$_2$-^{15}N-glycine (98 % ^{13}C, 98 % ^{15}N). Dispensers were used for the injections (Eppendorf Multipette 4780 with Combitips plus 50 ml, Eppendorf, Germany) fitted with a 3 mm thick four-sideport needle. To avoid clogging of the needle, holes with 3 and 12 cm depth were drilled into the soil with a 5.5 mm thick screwdriver prior to labeling. We used funnels around the injection needle to prevent wet contamination of aboveground plant parts with ^{15}N. Since tracer solutions adsorbed to the soil rather slowly, they were spread from 0–3 cm and 7–12 cm depth, referred to as shallow and deep treatment, respectively.

Plant harvests and measurements

Two days after ^{15}N tracer application, 5 individual shoots per species were collected from each subplot. By individual shoots we mean tillers in the case of grasses, modules of a single upright stem for *G. mollugo* and *T. pratense*, modules with 2 leaves and (if present) a flower for *T. repens*, and individual rosettes for all other species. Whenever possible, shoots were collected from different genets (Harper 1977). One to two weeks after labeling, aboveground plant biomass was clipped at 5 cm height on an area of 0.5 m × 0.5 m in each plot (Set 1: June 7–16, data from Wacker et al. (2008), Set 2: July 27/28), sorted to species and dried (48 h at 80°C). The site management included two complete mowings, one directly after the first biomass harvest (between Set 1 and 2) and one in early September (after Set 2).

Plant $\delta^{15}N$ and $\delta^{13}C$ (glycine treatments) were analyzed with an isotope ratio mass spectrometer (Delta^{plus} XP, Finnigan MAT, Germany) that was coupled to an elemental analyzer (Flash EA 1112 NC, CE Instruments, Italy). Natural background concentrations were measured in plants harvested one day before ^{15}N tracer application (two individual shoots per species from each monoculture and from one replicate of each mixture).

Soil nitrogen

To determine plant available NO_3^- and NH_4^+ concentrations (N_{min}), four soil cores (12 cm deep, 1.3 cm in diameter) were taken from each plot one day before ^{15}N tracer application. Cores were cut in layers of 1–6 and 6–12 cm, pooled per plot and layer, and stored at –18 °C until analysis. Soil samples were sieved through a 2 mm sieve, and an aliquot of 5 g was extracted in 50 ml of 1 M KCl solution. NO_3^- and NH_4^+ concentrations were measured with a Flow Injection Analyzer (San++, Skalar, Netherlands). Unfortunately, plant available glycine concentrations could not be measured.

Calculations

Since δ^{15}N values refer to ^{15}N enrichment relative to standard atmospheric air N_2, we used excess ^{15}N ($[^{15}N_{ex}]$, in μmol $g_{dw}{}^{-1}$) to analyze plant ^{15}N tracer uptake (Table 2 and Appendix Table 1). For each labeled plant sample, $[^{15}N_{ex}]$ was calculated from the ^{15}N concentration in excess atom percent ^{15}N:

$$at\%\,^{15}N_{ex} = (F_{labeled} - F_{background}) \cdot 100 \tag{1}$$

Hereby, $F = R/(R+1)$ is the fractional abundance of ^{15}N of a sample, and R is the measured $^{15}N/^{14}N$ ratio. $F_{background}$ is the natural fractional abundance of ^{15}N of the respective plant species.

Likewise, $[^{13}C_{ex}]$ was calculated for samples from the glycine treated subplots.

As a measure of niche breadth for all species at all levels of species richness, we calculated Levins' normalized B (B_n, Levins 1968):

$$B_n = \frac{1}{6 \sum_{i=1}^{6} p_i^2} \tag{2}$$

Here, based on $[^{15}N_{ex}]$, p_i is the fraction of ^{15}N taken up from one out of six N sources (treatments) offered, by a species in a particular plot in two days, whereby ^{15}N taken up from all N sources sums up to 1 ($\sum_{i=1}^{6} p_i = 1$). Thus, B_n varies from $\frac{1}{6}$ to 1, indicating N use from one source exclusively to use from all sources in equal proportions. In addition, we calculated B_n for each community, using the average p_i's of the constituent species, weighted by their abundance.

As a measure of niche overlap, we calculated Proportional Similarity (PS) between pairs of species (Schoener 1970, Colwell and Futuyma 1971):

$$PS = 1 - 0.5 \sum_{i=1}^{6} |p_{1i} - p_{2i}| \tag{3}$$

PS defines the area of intersection between the frequency distributions of resources used by two different species. Values of PS range from 0–1 for no overlap to complete overlap (resources used in equal proportions). For each labeling, PS was calculated between pairs

of species: either two species grown in monoculture (n=1 per pair), or two species grown in the same mixture plot (n=2 per pair and mixture type), representing fundamental and realized niche overlap, respectively. For the 3-species mixtures, PS was calculated only for species pairs actually occurring together; for monocultures and 6-species mixtures, PS was calculated for all pairs (3 combinations for 3-species mixtures, 15 for monocultures and 6-species mixtures).

Note that due to missing plants in some of the subplots (although present in the plot), B_n and PS could not be calculated for each population or all pairs. This led to some values missing in the data analysis and missing bars or points in Fig. 2 and 3, respectively.

Data Analysis

For the analyses of excess ^{15}N ($[^{15}N_{ex}]$) and plant available soil N (N_{min}), we used general linear models and analysis of variance. For $[^{15}N_{ex}]$ at the level of populations (species×plot, Table 1), we fitted the following terms in sequential order: (1) set, (2) legume presence, (3) species richness (linear term), (4) set×legume presence, (5) set×species richness, (6) functional group, (7) legume presence×functional group, and (8) species richness×functional group (Table 2). According to the mixed-model structure with the random effects of plots, we tested the fixed terms 1–5 against the between-plot variation (plot residuals) and the fixed terms 6–8 against the residual variation. To test for species-specific ^{15}N uptake from different N sources, we analyzed $[^{15}N_{ex}]$ at the species×subplot level in the 6-species mixtures (see Appendix Table 1).

For the analysis of N_{min}, we fitted (1) set, (2) legume presence, (3) species richness, (4) set×legume presence, (5) set×species richness (1–5 tested against plot residuals), (6) soil depth, (7) chemical N form, (8) soil depth×chemical N form, and (9) all two-way interactions of set, legume presence and species richness with soil depth, and chemical N form (6–9 tested against the residual variation).

Since glycine was applied as a dual-labeled tracer (one ^{15}N and two ^{13}C-atoms), we

could test for uptake of intact glycine molecules using linear regressions of shoot [$^{13}C_{ex}$] on [$^{15}N_{ex}$] for each species (Näsholm et al. 1998). Thereby, a regression slope of 2 corresponds to 100 % intact uptake.

For the analysis of B_n at the level of populations (species×plot, Table 1) and PS (for pairwise combinations of species), we also used general linear models and analysis of variance. B_n and PS were arcsine square root transformed to meet the assumption of normal errors. Although all species in mixtures were originally sown in equal proportions, in the 6-species mixtures *T. pratense* and *A. elatius* together accounted for 76 % of the aboveground biomass in Set 1, *T. repens* and *T. flavescens* for 96 % in Set 2, whereby each of these species individually accounted for >20 %. Accordingly, we classified these four species as dominant (subordinate the others) and used the term "dominance" for this two-level contrast within "species" in the linear models for B_n and PS. The species pairs used for the calculation of PS were classified into three levels of dominance: pairs of two dominant species, pairs of a dominant and a subordinate species, and pairs of subordinate species. We fitted (1) set, (2) species richness, (3) legume presence, (4) dominance, (5) species richness×dominance, and (6) legume presence×dominance (Table 3). For B_n, terms 1–3 were tested against the between-plot variation, terms 4–6 against the residual variation. For PS, all terms were tested against the residual variation. In the linear model for B_n of whole communities, (1) set, (2) legume presence, (3) species richness were fitted.

Note that species richness and legume presence were partly confounded factors, as there was a legume species in all 6-species mixtures but in only half of the 3-species mixtures, and in one out of six monocultures. In all analyses, we therefore fitted both species richness before legume presence and vice versa, finally fitting first whatever term explained more variation in the first position (and the other term after).

Results

^{15}N tracer uptake

^{15}N tracer application led to highly increased plant δ^{15}N, relative to natural abundance values. Across the whole tracer experiment, δ^{15}N varied between –2.3 and 846.2 ‰ with mean±SE of 157.7±9.1 ‰.

Plant ^{15}N tracer uptake ($[^{15}N_{ex}]$, in μmol $g_{dw}{}^{-1}$) was larger for Set 2 than for Set 1, probably because plants were smaller at Set 2 (only about 5 weeks after mowing) and the ^{15}N was less diluted within plants (Table 2). Legumes always took up less ^{15}N than forbs or grasses. The presence of legumes in a plot also decreased $[^{15}N_{ex}]$ of grasses and forbs—most likely due to the delivery of unlabeled, symbiotically fixed atmospheric N_2—and explained more variation in $[^{15}N_{ex}]$ than did species richness (therefore legume presence was fitted first). The decrease in $[^{15}N_{ex}]$ due to legume presence was particularly strong for Set 2 (set×legume presence interaction), and stronger for forbs than for grasses (legume presence×grasses vs. forbs interaction). Moreover, legumes had lower $[^{15}N_{ex}]$ in mixture than in monoculture (separate analysis on legumes only, 31.6 % sums of squares [SS], $P<0.05$). Altogether, this means that legumes fixed more atmospheric N_2 under competition with non-legumes (Marschner 1995, Hartwig 1998), and that part of the fixed N_2 was passed on to non-legumes.

In monoculture, most species (nine out of twelve) took up more ^{15}N from the NO_3^- source than from NH_4^+ and glycine, and (again nine out of twelve) more from shallow than from deep soil (Fig. 2). With increasing species richness, four species (*F. rubra*, *G. mollugo*, *L. vulgare*, *T. pratense*, all from Set 1) consistently increased ^{15}N uptake from shallow soil relative to deep soil, indicating niche narrowing in mixtures in line with Hypothesis 1 (Fig. 1, top). Three plant species switched their preferences: *T. officinale* took up slightly more ^{15}N from shallow than from deep soil in monoculture (as all other species in Set 1), but increased uptake from deep soil when grown in mixture, while *H. lanatus* and *L. flos-cuculi* (Set 2) increased uptake from shallow soil in the 6-species

mixture compared to monoculture and 3-species mixture. However, with only five populations per species (one in monoculture, two in the 3- and 6-species mixture each), only the increase in shallow uptake for *T. pratense* and *G. mollugo* were statistically significant (77.5 % SS, $P<0.05$) and marginally significant (70.5 % SS,$P<0.1$), respectively. Moreover, these changes in the behavior of single species did not result in clear patterns of resource partitioning in the mixtures. Similar to monocultures, NO_3^- was the preferred chemical form by eight species and shallow soil the preferred soil depth by nine species in the 6-species mixtures (Fig. 2).

Enrichment with ^{13}C of plants from the glycine treated subplots, indicating uptake of ^{13}C from the glycine tracer, was very small. Mean background $\delta^{13}C$ was –29.25 ‰ for both Set 1 and 2. Mean $\delta^{13}C$ of labeled plants was not different from background for Set 1 (–29.27 ‰) but increased for Set 2 (–28.45 ‰, $t_{55} = 7.55$, $P<0.001$). The test for intact uptake of glycine molecules, implied by a significant relationship between shoot $[^{13}C_{ex}]$ and $[^{15}N_{ex}]$, was not significant for any of the 12 plant species. Thus, glycine was either not taken up as an intact molecule, or not transferred as such from roots into shoots—at least not in detectable amounts (e.g., due to much stronger dilution of ^{13}C compared to ^{15}N in plants, see Näsholm and Persson (2001)). In spite of this caveat, we decided to include the glycine treatments for the calculations of niche breadth and niche overlap for two reasons: (1) one cannot test either whether ^{15}N from NO_3^- and NH_4^+ was taken up and transferred to shoots in the chemical form added (i.e., transformation in the soil prior to uptake cannot be ruled out), and (2) the processes involved between mineralization and translocation of glycine from soil into plants may be different from those involved for inorganic N uptake, e.g., with regard to soil microbes.

Niche breadth and niche overlap for N uptake

Species-specific niche breadth, assessed by Levins' B, decreased significantly with species richness (Table 3; Fig. 3, top panel), implying that plant species occupied narrower niches when grown in competition with other species than when grown in monoculture.

This is in line with hypothesis (1).

Niche overlap, assessed by Proportional Similarity between pairs of species, also decreased with species richness (Table 3; Fig. 3, bottom panel), consistent with hypothesis (2). Nevertheless, although the sharing of N sources was reduced in relative terms, most plant species still showed a preference for N from shallow rather than deep soil, and for NO_3^- rather than NH_4^+ or glycine (Fig. 2). In particular in the 6-species mixtures, species primarily took up N from the same source, NO_3^- from shallow soil (soil depth×chemical N form interaction, see Appendix Table 1). Exceptions preferring a different N form than NO_3^- are *T. officinale* and *T. pratense* in Set 1 (species×chemical N form interaction), whereas in Set 2, all species preferred NO_3^- from shallow soil (n.s. species×chemical N form interaction, Appendix Table 1).

The niche breadth of whole communities remained constant across all levels of species richness; hypothesis (3) is therefore not confirmed. Also, community niche breadth was unaffected by legume presence.

Species richness explained more variance than legume presence in the analyses of Levins' B and Proportional Similarity, and was therefore fitted first in the models. Since both measures were based on relative ^{15}N uptake within communities, between community differences in absolute ^{15}N uptake due to legume presence were eliminated. Dominant species (*A. elatius*, *T. flavescens*, *T. pratense*, *T. repens*) had larger values of Levins' B, indicating wider niches than subordinate species (Table 3; Fig. 3). There was no effect of dominance on Proportional Similarity, indicating similar niche overlap between pairs of only dominant, only subordinate, or pairs of a dominant and a subordinate species. In a separate analysis, dominant species alone showed no decrease in niche breadth with increasing species richness, whereas subordinate species did (34.2% SS, $F_{1,9}$=16.7, P<0.01). The pattern for niche overlap was similar, i.e., no decrease with increasing species richness for pairs of dominant species, but a decrease for pairs of a dominant and a subordinate, and pairs of subordinate species. However, without an overall effect of dominance on niche overlap this result is only exploratory.

Soil mineral N

Legume presence increased plant available NO_3^- and NH_4^+ (N_{min}) concentrations in the soil (see Appendix Fig. 2). This effect was stronger in Set 2 (set×legume presence interaction, $P<0.05$) and in shallow soil (depth×legume presence interaction, $P<0.05$). N_{min} concentrations were generally higher in shallow than in deep soil ($P<0.01$). In Set 1, concentrations of NO_3^- were higher than those of NH_4^+ whereas in Set 2, concentrations of NH_4^+ were slightly higher (set×chemical N form interaction, $P<0.001$).

Discussion

Niche breadth and niche overlap among species

When plants grew with interspecific competition in mixtures, species occupied smaller niches for N uptake (realized niches, Hutchinson 1957), overlapping less in soil depth and chemical N form than when grown in monoculture with intraspecific competition only (fundamental niches). These findings support the first two of our hypotheses (see Fig. 1) as well as Hutchinson's niche theory, because it is expected that the realized niche of a species should be smaller than its fundamental niche.

We expected that plants in monoculture would rely on the most accessible N source, i.e., NO_3^- out of the three chemical forms available (for temperate grasslands with neutral pH, Marschner 1995), and on shallow rather than on deep N, which we could confirm with our data. We further expected that some species would increasingly take up N from other sources when grown in mixture. However, despite the relative adjustment of the realized niches resulting in reduced niche overlap, only in a few cases did we observe an absolute switch of preferences. The general pattern showed no clear divergence in N uptake of species when grown in mixture. In fact, eight out of ten species preferred the same N source in the 6-species mixture: they took up most of their N as NO_3^- from shallow soil depths, in line with McKane et al. (1990) and Kahmen et al. (2006). Comparing N

uptake from shallow versus deep soil (pooled across chemical N forms) we found that all species except *T. officinale* preferred N from shallow soil. This finding corroborates the results of a pot experiment (von Felten and Schmid 2008), where mixtures of four temperate grassland species were more productive and had higher complementarity effects (sensu Loreau and Hector 2001) when grown on shallow soil compared to deep soil of the same volume, suggesting nutrient uptake from deeper soil being rather costly.

We could show that species richness reduced the niche overlap between species, calculated between single species pairs within the same mixture (or both species in monoculture). However, this result seems not to be mirrored by the mean N uptake patterns of species in the 6-species mixtures, as shown in Fig. 2, with n=2 replicates for each species per mixture. Thus, while plants of a certain species indeed decreased niche overlap with other species when grown in mixture, they did this in an opportunistic way, e.g., uptake patterns of individual species differed between mixture replicates. In a ^{15}N tracer study with NO_3^-, NH_4^+, and glycine, Miller et al. (2007) showed that neighbor identity influenced the capacity of plant species to take up different forms of N. Although in our study, each species occurred in only one specific mixture composition per level of species richness (e.g., *A. elatius* always grown with *F. rubra* and *T. pratense* in the 3-species mixture), the specific position of individuals and the direct neighbors, accordingly, may well have affected a species' N uptake pattern.

In our results, subordinate plant species had smaller niche breadths than dominant species. Also, niche breadth decreased with species richness for subordinate species, but was constant for dominant species. This suggests that spatio-chemical partitioning of N could be relevant for the persistence of subdominant species in mixtures (Fargione and Tilman 2005). This is in line with McKane et al. (1990), showing that subordinate species occupied peripheral spatio-temporal niches compared to dominant species in an old field community. In our study, *T. officinale*, shows the most peripheral pattern in 6-species mixture. However, niche breadth (and niche overlap between pairs) of dominant species did not decrease with species richness. Thus, spatio-chemical partitioning of N

may not be an important mechanism for the coexistence of dominant species used in this experiment.

Our third hypothesis, that the community niche breadth should increase with species richness (Fig. 1, bottom), was not supported, since it remained constant across levels of species richness. Indeed, species richness decreased niche overlap among individual species, which could lead to an increase in community niche breadth. However, this might have been compensated for by the simultaneous decrease in individual species' niche breadths, indicating that multiple species together shared a similar niche space in mixture, as single species in monoculture. Further, since no decrease in niche overlap was found for dominant species only (which accounted for more than 75 % of species abundances in the 6-species mixtures), the observed general decrease in niche overlap might be of no consequence for the community niche breadth, when accounting for species abundance.

Facilitation by legumes

The clear preference for NO_3^- and shallow soil N by most species—in particular in the 6-species mixtures—may be partly explained by legume facilitation.

We can exclude that the high ^{15}N uptake of plants from NO_3^- and shallow soil was an artifact due to lower pool dilution (by smaller pools) of the respective ^{15}N tracers. In fact, accounting for pool sizes of NO_3^- and NH_4^+, would result in similar or even more pronounced patterns. N_{min} concentration was higher in shallow than in deep soil, especially in the presence of legumes (thus in all 6-species mixtures), implying even stronger dilution of the ^{15}N signal and underestimation of N uptake from shallow soil. Likewise, NO_3^- levels—and thus pool dilution—as well as the NO_3^-/NH_4^+ ratio did not decrease with species richness. As a caveat of our study, we have no data on glycine pools in the soil. However, it is reasonable to assume that plant available glycine was the least abundant chemical N form used here (see e.g., Bardgett et al. 2003), and that thus ^{15}N uptake from glycine was overestimated.

Hence, we can say that the preferred N sources in our experiment were those that were available in high concentrations. The positive effect of legumes on N_{min} concentrations, is in line with Palmborg et al. (2005), Roscher et al. (2008); together with the simultaneous decrease in $[^{15}N_{ex}]$ of non-legumes, in line with Temperton et al. (2007), this suggests that "N sparing" (i.e., increased availability of soil N since the legumes relied more on atmospheric N sources than soil N) played a significant role for species' N uptake patterns in mixtures. Legumes were present in all 6-species mixtures, where other species' shifts in N uptake towards deeper soil layers or N sources other than NO_3^- might have been rendered unnecessary. While the N fixing property of legumes may be considered as facilitation of other species, it may as well be considered as a kind of complementary N use, counting N_2 as an additional N source. Anyway, legumes had a major impact on the N cycle in the plant communities studied here, and it is likely that "N sparing" significantly lowered competition for N and reduced the importance of complementary N use with respect to soil depth and chemical N form tested here.

Implications for biodiversity and ecosystem functioning

Resource partitioning due to niche separation of species was often claimed to be an important mechanism underlying positive diversity-ecosystem functioning relationships (e.g., Hooper et al. 2005). For example, resource partitioning could explain increased biomass production (e.g., Hector et al. 1999, Tilman et al. 2001, van Ruijven and Berendse 2003, Roscher et al. 2005) as well as larger nutrient pool sizes in plants (e.g., Roscher et al. 2008), or reduced nutrient pools in the soil (e.g., Tilman et al. 1996, Hooper and Vitousek 1998, Scherer-Lorenzen et al. 2003). Our study is to our knowledge the first that directly quantifies N partitioning in a biodiversity experiment. However, the species' N uptake patterns we observed in the mixtures were not as distinct as one might expect, and we also found no evidence for more diverse communities covering a larger niche space. Nevertheless, we found a general decrease in niche breadth and niche overlap, with testing for two niche axes only. Possibly, testing for a larger number of

niche axes, e.g., by additionally including timing of N uptake (McKane et al. 1990 and 2002, Fargione and Tilman 2005, Pornon et al. 2007) or other resources such as water (Caldeira et al. 2001, De Boeck et al. 2006) or light (Dassler et al. 2008, Vojtech et al. 2008), would result in stronger patterns. We could show that N uptake patterns of species were affected by the presence of interspecific competitors. This clearly contradicts the main premise of Hubbell's (2001) neutral theory, i.e., fitness equivalence and identical effects of species on one another. In summary, while our results provide limited evidence for partitioning of N, suggesting that it may not be the major driver of the biodiversity–productivity relationship, they fit with the recent resurgence of high-dimensional niches (Harpole and Tilman 2007, Clark et al. 2007).

Conclusions

In our study, niche breadth of single species and niche overlap between pairs of species with respect to chemical form (NO_3^-, NH_4^+, glycine) and soil depth (1–3 cm and 7–12 cm) decreased with increased species richness (Hypotheses 1 and 2, Fig. 1), but without resulting in increased niche breadth of mixtures compared to monocultures (Hypothesis 3, Fig. 1). We conclude that several species in mixture together occupy a similar niche space as one single species does in monoculture. There is evidence that the complementarity in N use tested here (soil depth and chemical form) was neither important as a mechanism to facilitate coexistence of dominant species since dominant species showed no decrease in niche breadth with increased species richness, nor that it is a major driver of positive diversity–ecosystem functioning relationships. However, complementary N use may be important for the subordinate species which could persist by reducing niche overlap with dominants and among themselves.

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Table 1: Experimental communities of species Pool 1 and 2, their species richness (SR), functional group composition (FG), replication (Repl), and the resulting number of plots and "populations" (Pop). Note that Pool 1 was ^{15}N labeled between 26–28 May (Set 1), Pool 2 between 19–21 July 2004 (Set 2). Functional groups are grasses (g), forbs (f), and legumes (l). Note that the populations (Pop) are species×plot combinations (obtained by multiplying the number of plots by the species richness in each row of the table), and are referred to as "populations" in the text.

	Community	SR	FG	Repl	Plots	Pop
Pool 1	each species in monoculture	1	g or f or l	1	6	6
	Arrhenatherum elatius (g), *Festuca rubra (g)*, *Trifolium pratense (l)*	3	g,g,l	2	2	6
	Galium mollugo (f), *Leucanthemum vulgare (f)*, *Taraxacum officinale (f)*	3	f,f,f	2	2	6
	all six species	6	g,g,l,f,f,f	2	2	12
Pool 2	each species in monoculture	1	g or f or l	1	6	6
	Trisetum flavescens (g), *Trifolium repens (l)*, *Lychnis flos-cuculi (f)*	3	g,l,f	2	2	6
	Holcus lanatus (g), *Silene nutans (f)*, *Tragopogon pratensis (f)*	3	g,f,f	2	2	6
	all six species	6	g,g,l,f,f,f	2	2	12
Total					24	60

Nomenclature follows Lauber and Wagner (1998).

Table 2: Mixed model analysis of variance of excess ^{15}N ($[^{15}N_{ex}]$, in μmol $g_{dw}{}^{-1}$ over natural background) for populations (n=60). Data are averaged per species over all ^{15}N treatments (three chemical N forms × two soil depths). This analysis shows the general patterns of ^{15}N uptake. See Appendix Table 1 for a more detailed analysis of the 6-species mixtures.

	$[^{15}N_{ex}]$			
Source of variation	d.f.	Error[a]	% SS[b]	
Set	1	P	39.9	***
Legume presence	1	P	13.9	***
Species richness	1	P	0.5	ns
Set×Legume presence	1	P	9.7	***
Set×Species richness	1	P	0.3	ns
Functional group	2	R	4.9	*
Legume vs. others	1	R	2.9	*
Grasses vs. Forbs	1	R	2.0	.
Legume presence×Grasses vs. Forbs	1	R	3.2	*
Species richness×Functional group	2	R	0.9	ns
Plot residuals (P)	18		10.1	
Residuals (R)	31		16.7	
MODEL	10		73.2	

[a] P refers to residuals at the plot level, R to residuals at the lowest (population) level.

[b] % sums of squares (SS) indicate increase in multiple R^2 (explained variance) due to the addition of a term to the model. Significant terms are indicated by asterisks (* P<0.05; ** P<0.01; *** P<0.001), marginally significant terms by a dot (. P<0.1), non-significant terms by ns.

Table 3: Analyses of variance of Levins' normalized B (B_n) and Proportional Similarity (PS) for species and species pairs, respectively.

	B_n[a]				PS[a]		
Source of variation	d.f	Error[b]	% SS[c]		d.f	% SS[c]	
Set	1	P	2.98	ns	1	0.27	ns
Species richness	1	P	20.69	***	1	9.98	**
Legume presence	1	P	1.26	ns	1	0.10	ns
Dominance	1	R	12.79	**	2[d]	0.22	ns
Species (within Dom.)	9	R	25.74	*			
Species richness×Dominance	1	R	2.98	.	2[d]	0.94	ns
Species richness×Species (within Dom.)	10	R	7.83	ns			
Plot residuals (P)	13		13.93				
Residuals (R)	14		11.82		75	88.48	
MODEL	24		74.27		7	11.51	

[a] B_n and PS were arcsine square root transformed to meet the assumption of normal errors.

[b] P refers to residuals at the plot level, R to residuals at the lowest (population) level.

[c] % sums of squares (SS) indicate increase in multiple R^2 (explained variance) due to the addition of a term to the model. Significant terms are indicated by asterisks (* $P<0.05$; ** $P<0.01$; *** $P<0.001$), marginally significant terms by a dot (. $P<0.1$), non-significant terms by ns.

[d] Dominance has 3 levels for PS: pairs of two dominant, a dominant and a subordinate, or two subordinate species.

Figure Legends

1 Hypotheses regarding niche breadth and niche overlap of species in monoculture vs. mixture (as indicated in gray): (1) The niche breadth of each individual species should be lower in mixture than in monoculture (compare niches of species A in top panels). (2) The niche overlap between species in mixture should be lower than between species in monoculture, allowing plants to partition N (compare overlap between species A, B, and C in mid panels). (3) Mixtures should cover a larger total niche breadth than individual monocultures (compare niche of species A with combined niche of species A, B, and C in bottom panels). 29

2 Patterns of plant ^{15}N uptake for all plant species (Set 1: left, Set 2: right) from all six N sources: NO_3^- (nit), NH_4^+ (amm), and glycine (gly), combined with two depths of application: shallow (s, 0–3 cm) and deep (d, 7–12 cm), at all levels of species richness (1, 3, and 6). Bars represent the fraction of ^{15}N taken up (p_i) from one out of six N sources offered by a species in a particular plot in two days (^{15}N taken up from all N sources, e.g., $\sum_{i=1}^{6} p_i = 1$). For each species the uptake from shallow (white bars) and deep soil (black bars) summed up across all chemical N forms is shown on the right. Note that the proportions are based on single values for the monocultures, but on means from two replicates for the mixtures. Error bars show standard errors of proportions ($SE = \sqrt{\frac{p(1-p)}{n}}$). The incomplete profile of *T. flavescens* (Tri fla) in monoculture is based on a total 0.67 instead of 1 (no data for glycine). 30

3 Niche breadth as Levins' normalized B (top) and niche overlap as Proportional Similarity (PS, bottom) for Set 1 (circles) and Set 2 (triangles) at all levels of plant species richness ($\frac{1}{6}$ < Levins' B < 1; 0 < PS < 1). Closed symbols: the six most dominant species (pairs of two dominant species for PS); open symbols: the six subordinate species (pairs of two subordinate/a subordinate and a dominant species). Bold lines: Overall linear regression lines (across both sets); for Levins' B separate lines are shown for dominant (thin line) and subordinate species (thin dashed line). See Table 3 for the ANOVA 31

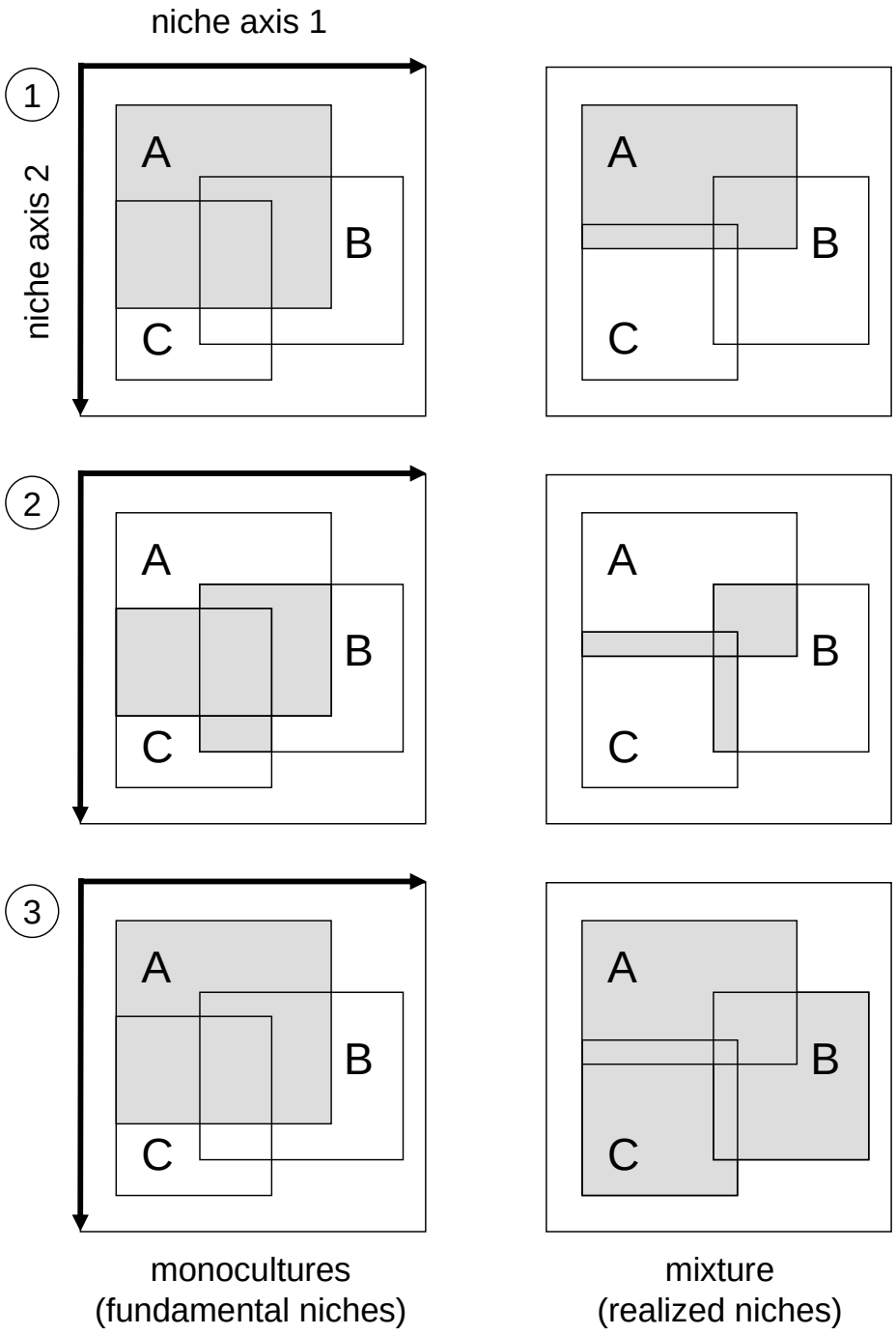
niche axis 1
niche axis 2
1
2
3
A
B
C
monocultures
(fundamental niches)
mixture
(realized niches)

Figure 1:

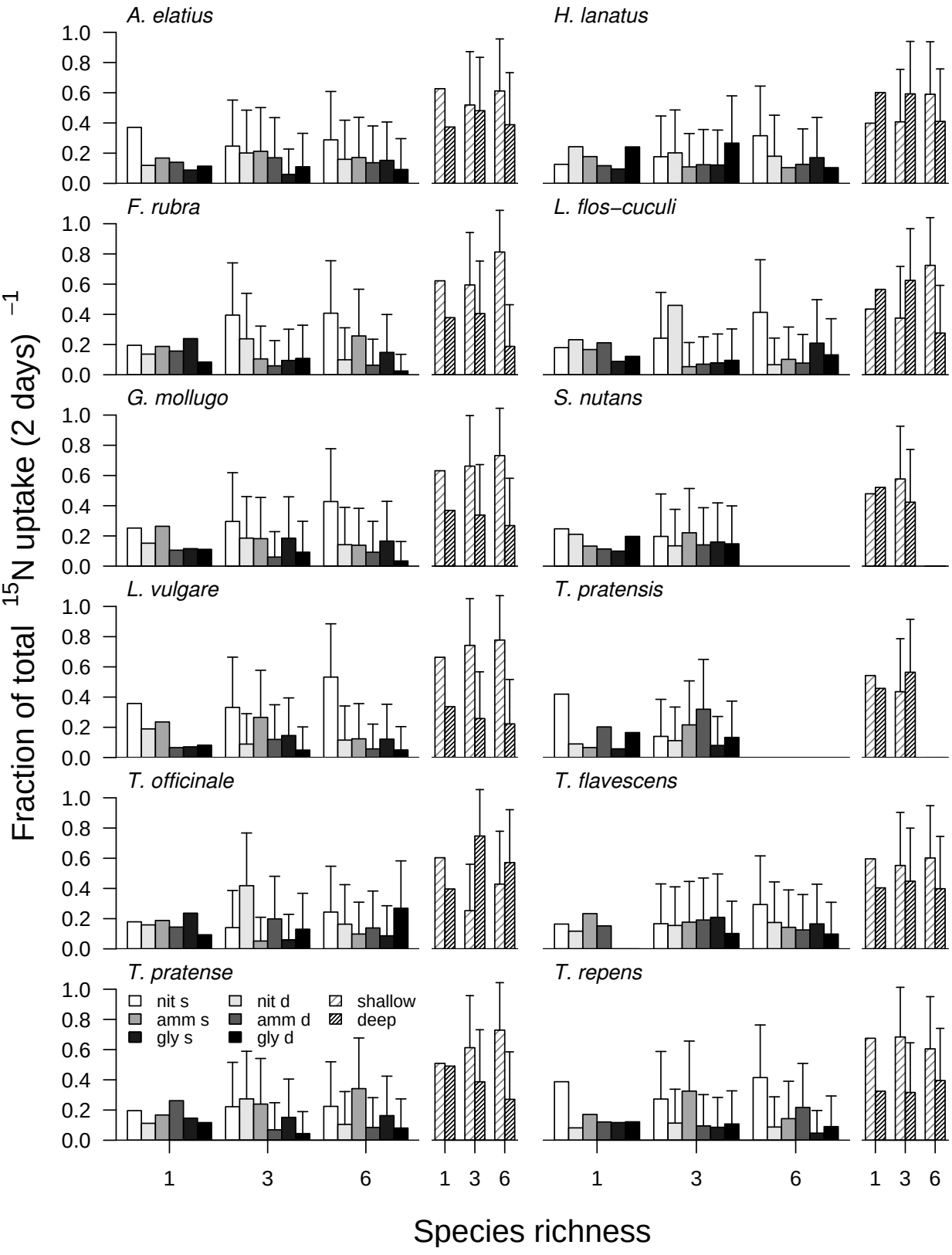
A. elatius
H. lanatus
F. rubra
L. flos–cuculi
G. mollugo
S. nutans
L. vulgare
T. pratensis
T. officinale
T. flavescens
T. pratense
T. repens
nit s
amm s
gly s
nit d
amm d
gly d
shallow
deep
Fraction of total 15N uptake (2 days) −1
Species richness
0.0
0.2
0.4
0.6
0.8
1.0
1
3
6

Figure 2:

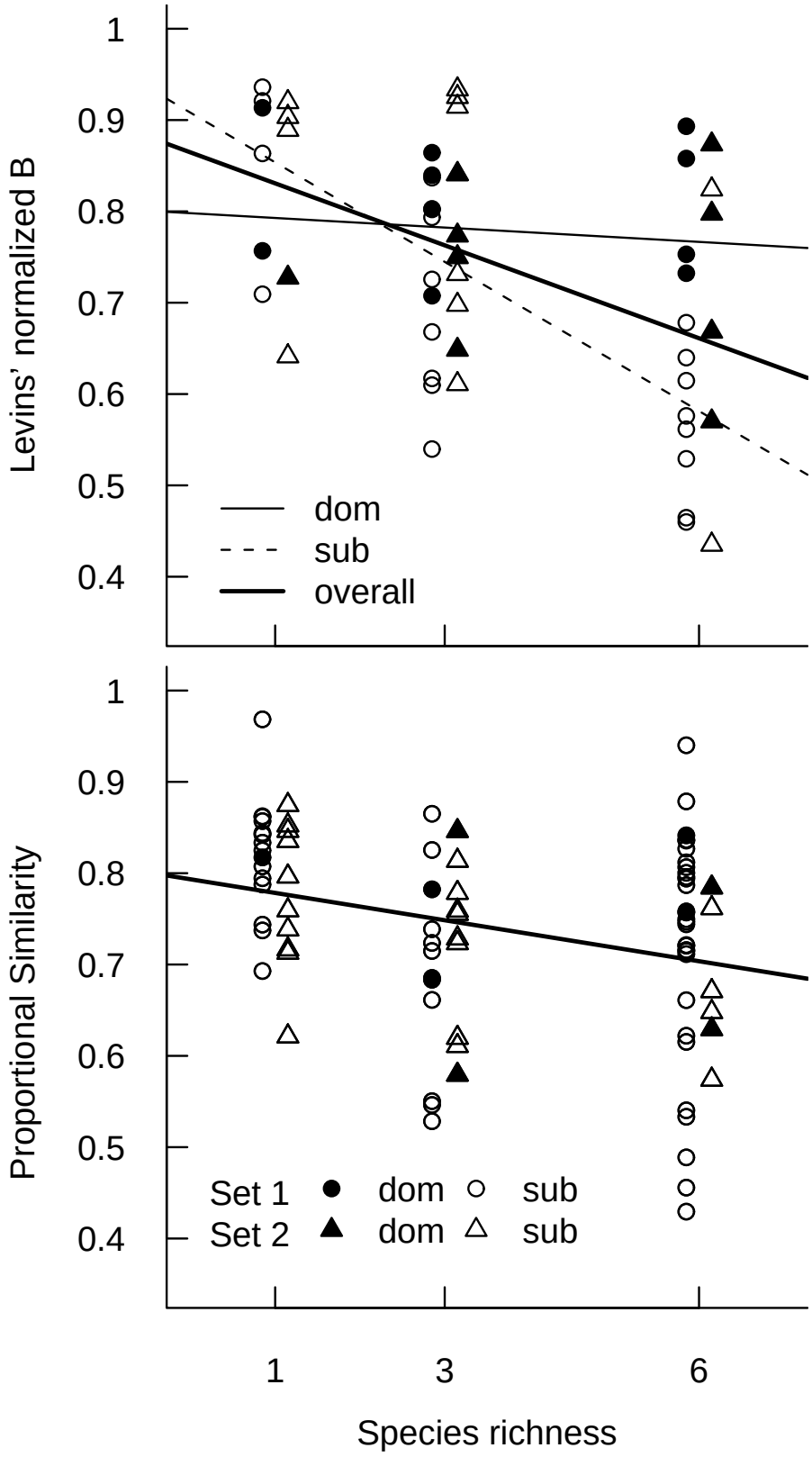
Levins' normalized B
1
0.9
0.8
0.7
0.6
0.5
0.4
dom
sub
overall
Proportional Similarity
1
0.9
0.8
0.7
0.6
0.5
0.4
Set 1 dom sub
Set 2 dom sub
1
3
6
Species richness

Figure 3: