NEUTRAL VS. NICHE-STRUCTURED COMMUNITIES:
TESTING FOR RESOURCE PARTITIONING BY PLANTS

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Summary
Experiments that manipulated plant species richness in temperate grasslands have generally found that species richness increased plant productivity. It has often been suggested that this is due to complementary use of resources—such as nitrogen—by plant species. Complementarity, e.g., in space, chemical form, or timing of resource uptake should result in better resource use of mixtures. In fact, such partitioning of single resources could not only explain positive biodiversity–productivity effects, but also how large numbers of plant species are able to coexist, given that they all depend on a rather small set of common resources. On the other hand, “neutral” models assuming that species distributions are random and that plant species are competitively equivalent have recently challenged this view. Moreover, rigorous experimental tests for resource partitioning are still lacking.

The aim of this thesis was to experimentally test for complementary resource use in temperate grassland plant communities, across gradients of experimentally manipulated species richness. We used a variety of approaches, a central one being $^{15}$N labeling techniques, focusing on partitioning of nitrogen (N) by soil depth and chemical form, and on changes of plant N uptake patterns along the species richness gradient.

In Chapter 1 we tested whether available soil depth and nutrients change the effect of plant species richness on productivity, through increased “niche space”. Soil volume was kept constant to avoid confounding soil depth and volume. Using monocultures and mixtures of four common grassland species in pots, we applied an additive partitioning method to separate net biodiversity effects on plant productivity into components due to species complementarity (niche separation or facilitative interactions) and dominance (of species with particular traits). Net biodiversity and complementarity effects were consistently higher at the low nutrient level and, unexpectedly, in shallow soils. These two results suggest that although belowground partitioning of resources was important, especially under low nutrient conditions, it was not due to differences in rooting depths.

In Chapter 2 we used experimental plant communities in the field to test whether increasing species richness decreases the niche breadths of individual species, and niche overlap among species with regard to N uptake from two soil depths and three chemical forms. N uptake from different sources was tracked with soluble $^{15}$N-tracers ($^{15}$NO$_3^-$, $^{15}$NH$_4^+$, $^{13}$C$_2$$^{15}$N-glycine), injected at two soil depths to assess niche breadths and niche overlap. As expected, niche breadth of single species and niche overlap among species decreased with increasing species richness. However, community niche breadth was con-
stant. The decrease in niche breadth and niche overlap mostly occurred among subordinate species or pairs of subordinate and dominant species, rather than among dominant species. Moreover, species in the 6-species mixtures differed less than expected in their N uptake patterns and mostly preferred NO$_3^-$ from shallow soil. Chapter 3 is a short comment on technical difficulties when testing for organic N uptake using dual $^{13}$C-$^{15}$N-labeled amino acids such as glycine.

The questions investigated in Chapter 4 were similar to those in Chapter 2, but focused on N partitioning by soil depth. Here, we used a solid organic $^{15}$N label directly mixed into the soil (either shallow or deep) in a pot experiment. The more controlled conditions allowed to quantify tracer uptake from shallow and deep soil for each species across two years. Further, we linked species’ N uptake patterns in mixtures to biodiversity effects, expecting larger complementarity effects in mixtures with low niche overlap between species. In the first year, niche overlap between species decreased with species richness, albeit not in the way expected: low niche overlap was most frequent in mixtures dominated by species with higher-than-average yield in monoculture. These took up a higher fraction of $^{15}$N from deep soil than subordinate species. Although results converged to our expectations in the second year, they did not clearly support them. The total $^{15}$N uptake was higher in mixtures vs. monocultures over all harvests, but there was no linear effect of species richness.

In the same experiment, we assessed competition for N between plants and soil microbes. In Chapter 5, we tested whether plant species richness alters N partitioning between plants and microbes, expecting diverse plant communities to compete more successfully. However, species richness of plant communities neither affected partitioning of N nor of the $^{15}$N label between plants and microbes. Instead, partitioning of N and microbial N depended on the plant species composition. Although plant biomass was significantly higher in mixtures than in monocultures, we found only a trend for increased plant N, due to higher biomass:N ratios.

This study was to our knowledge the first that investigated resource partitioning among plant species across gradients of plant species richness. In conclusion, we could clearly demonstrate that species respond to interspecific competition by changes in N uptake patterns. For example, we could show that niche overlap between species decreased with species richness. However, species N uptake patterns in mixtures were not as distinct as
expected, and our results provide limited evidence for complementary N use as being a main mechanism to explain positive effects of species richness on productivity or species coexistence. Furthermore, the potential for resource partitioning among plant species by soil depth may have been overestimated so far, since we observed larger complementarity effects in shallow soil than in deep soil. Also, although a small effect of plant species richness on total plant N uptake seems possible, higher biomass production in diverse plant communities does not necessarily require higher N uptake, but can be mediated by higher biomass:N ratios. Finally, although our results provide limited evidence for the existence of niches in plant communities, they neither support neutrality as a mechanism, because of species differences and interactions. We regard it likely that plant coexistence is mediated by a combination of mechanisms instead of a single most important one.
Zusammenfassung


Im selben Experiment analysierten wir den Konkurrenzkampf um N zwischen Pflanzen...

General Introduction
Scientific background

Functional significance of biodiversity

Human activities on Earth increasingly affect the environment in many ways. Worldwide loss of biodiversity in the form of genes, species, or biotopes is one aspect of this “global change” and has lately become a major issue in ecology. Loss of biodiversity—caused by overexploitation, eutrophication, habitat destruction and fragmentation—threatens human well-being, which heavily depends on ecosystem services such as food production, soil fertility, purification of air and water, pollination, or protection from natural hazards (Daily 1997, Millennium Ecosystem Assessment 2005). Within the last decade, the functional importance of biodiversity was therefore intensively studied (as reviewed e.g. in Kinzig et al. 2002, Loreau et al. 2002, Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2006 and 2007). In contrast to observational studies, where the most productive ecosystems typically exhibit low species diversity (Huston 1994, Grime 2001), diversity was experimentally manipulated and used as a predictor variable to assess its effects on various ecosystem processes (see Schmid 2002, for a distinction of both approaches). Thereby, factors such as site fertility, typically confounded with biodiversity, are kept constant. It is now widely accepted that biodiversity positively affects the functioning of ecosystems, by enhancing single functions such as productivity, ecosystem stability, ecosystem resilience, invasion resistance (Hooper et al. 2005, for a review), and the restoration process (Callaway et al. 2003, Piper et al. 2007). Moreover, biodiversity can simultaneously enhance multiple ecosystem processes (Hector and Bagchi 2007).

A lot of this research was and is still being done in temperate grasslands, the study object of this thesis. Here, a positive relationship between plant species richness and ecosystem functioning—productivity in particular—was typically found and has risen interest into the underlying mechanisms. However, with regard to the mechanisms, there is still considerable uncertainty: whereas some ecologists argue that more diverse communities are simply more likely to contain more productive species (known as the sampling effect, see Huston 1997, Wardle 1999, Grime 2002), others propose complementarity in species’ resource use (by niche separation) as the main mechanism for the observed diversity–productivity patterns (Tilman et al. 1997, Loreau 1998, Hector et al. 1999, Tilman et al. 2001).
A step forward in resolving this debate was the method of additive partitioning that allows to mathematically partition the net effect of biodiversity into effects of species complementarity and selection (Loreau and Hector 2001). Application of the method corroborated the role of species complementarity (Loreau and Hector 2001, van Ruijven and Berendse 2003, Roscher et al. 2005, Spehn et al. 2005). However, showing what biological factors account for species complementarity in plants, i.e., what particular niche axes create the relevant functional differences between species, remained difficult.

**Plant species coexistence and the niche**

While there has been a recent burst of research on the relationship between plant species richness and ecosystem functioning, the subject is not completely new. “As with many things in biology, Darwin got there first” (Hector and Hooper 2002). In The Origin of Species Darwin says (1985, Chap. 4, p. 185), “It has been experimentally proved that if a plot of ground be sown with one species of grass, and a similar plot be sown with several distinct genera of grasses, a greater number of plants and a greater weight of dry herbage can thus be raised.” He clearly identified that ecological differences between species can make communities both more diverse and more productive.

However, earlier work on functional differences between plant species was mostly related to species coexistence, as a mechanism creating or allowing for species diversity, rather than as mechanism behind positive diversity–ecosystem functioning relationships. Woodhead (1906) mentioned the term *complementary association*, referring to herbaceous species in the forest understory that differ in soil requirements (*edaphic complementarity*), modes of life, periods of active vegetative growth, and times of flowering and fruiting (*seasonal complementarity*).

According to the *Principle of competitive exclusion* (Gause 1934), based on the Lotka-Volterra competition model (Volterra 1926, Lotka 1927), two species can only co-exist when they occupy different niches. The niche concept was coined by zoologists: it was first introduced by Grinnell (1917), more clearly defined by Elton (1927), but both formalized and popularized in ecology by Hutchinson (1957). Hutchinson pictured a species’ niche as an n-dimensional hypervolume, each dimension defining the conditions along an environmental variable in which the species can survive and reproduce (fundamental niche). This niche hypervolume can then be restricted through competition with another
species (realized niche). “Why are there so many kinds of animals” was the question Hutchinson (1959) asked, and in fact the best examples of species niches and Gause’s principle are found in animal communities, where co-existing species often live on different diets or exhibit different foraging patterns. A famous example are the “Darwin finches” of the genus Geospiza: Where G. fortis and G. fuliginosa co-occur on an island, there is a significant separation in bill size, allowing them to use different food sources. But where either species exists alone, as on Crossman Island and Daphne Island, the bills are intermediate and presumably adapted to eating modal sized food (Lack 1947).

Since plants provide animals with food and shelter, as well as structure the habitat, they are frequently the source of niche separation in animal communities. In contrast, how a diversity of plant species can coexist on a small scale poses a puzzle in plant science given that plants all require the same small set of resources (i.e., light, space, carbon, water, mineral nutrients). Recent biodiversity–ecosystem functioning research has caused a need to resolve this paradox, all the more since “neutral models” are challenging the validity of the niche concept in plant communities (Bell 2001, Hubbell 2001). These models assume that plant communities are composed by a process of random drift of species (similar to random drift of genes) that are competitively equivalent (i.e., identical per capita demographic rates). While neutral models show surprising success in reproducing community patterns, i.e., distribution of plant species abundance, this does not necessarily imply neutral mechanisms at work (Harpole and Tilman 2006). Moreover, neutral models are incapable of explaining biodiversity effects such as increased productivity or stability (Zhou and Zhang 2008).

Another problem with the term “niche” in plant communities is the spatial scale it refers to, i.e., the scale at which species diversity is considered (see also Ackerly and Cornwell 2007). In a classic experiment for example, the famous German plant ecologist Ellenberg nicely demonstrated that different plant species segregate along a gradient of water table depth (Ellenberg 1953 and 1954). Also, Ellenberg is probably best known for the indicator values named after him (Ellenberg et al. 1992), which can be used to describe the conditions of a site where a particular set of plant species occurs, clearly indicating that plants do not occur at random, at least among sites. In a heterogeneous environment, high levels of $\beta$-diversity (species diversity measured along environmental gradients or between different habitats) are created if plant species differ in their habitat
requirements. However, the $\beta$-niche characteristics will tend to be shared among co-occurring species. Many niche studies, strictly speaking, investigated niche differentiation at the level of $\beta$-diversity, e.g. Silvertown et al. (1999) on water table depth or Sipe and Bazzaz (1994 and 1995) on gap partitioning among maples. Such niche studies, as well as Tilman’s niche model (1997), require a certain degree of spatial heterogeneity. In contrast, biodiversity experiments typically consider $\alpha$-diversity, with different species growing directly next to each other within homogeneous plots. From a niche perspective, species might be specialized on different resource forms and on where they obtain them in space and time. Such resource partitioning through $\alpha$-niche separation would allow different plant species to both coexist on a small scale and cause biodiversity effects on ecosystem functioning.

**Nitrogen and the resource niche**

For green plants, nitrogen (N) is quantitatively the most important mineral nutrient (Ellenberg 1977) and is therefore of central importance to all terrestrial ecosystems. Biologically available N is commonly believed to be the major limiting nutrient to primary production in most ecosystems, among them temperate grasslands (Vitousek and Howarth 1991, Aerts and Chapin 2000). In contrast, phosphorous is probably the most limiting in tropical systems. There, due to the lack of glaciation, soils are very old resulting in a “terminal steady state” of profound phosphorus limitation (Walker and Syers 1976). Indeed, this prevailing paradigm was challenged by a recent meta-analysis, suggesting that freshwater, marine, and terrestrial ecosystems are surprisingly similar in terms of N and P limitation (Elser et al. 2007). However, certainly, N is one of the two most limiting nutrients to plants.

Complementary resource use has been suggested as one mechanism of niche separation among plant species (see above), and N is a very important resource in temperate grasslands. Hence, differences in N uptake should promote species coexistence by reducing interspecific competition for soil N, resulting in increased productivity in plant mixtures.

Plants were long known to use inorganic forms of N ($\text{NO}_3^-$, $\text{NH}_4^+$), previously mineralized by soil microbes. Although plant uptake of organic N compounds, such as amino acids (Virtanen and Linkola 1946), and their transfer into plants through fungal mycelium (Melin and Nilsson 1953) were suggested much earlier, the topic just recently gained in-
Growing attention. Using $^{14}$C labeling techniques, plants were shown to directly take up dissolved organic N (DON) such as intact amino acids in solution culture (Chapin et al. 1993, Kielland 1994). Later, plant intact amino acid uptake was shown in the field, in competition with soil microbes, using dual-labeled $^{15}$N-$^{13}$C-amino acids (Näsholm et al. 1998). This technique allows to simultaneously assess the uptake of labeled $^{15}$N and $^{13}$C, and initiated a whole series of studies. Plant amino acid uptake even was shown repeatedly in arctic and alpine systems (Lipson and Monson 1998, Lipson et al. 1999, Nordin et al. 2001), but also in temperate grasslands (e.g., Näsholm et al. 2000, Bardgett et al. 2003, Wiegelt et al. 2003). While its quantitative relevance for plant nutrition is still doubted (Jones et al. 2005), the potential role of direct plant amino acid uptake along with NO$_3^-$, NH$_4^+$ has provoked the question of whether plants chemically partition N, through preferential uptake from different chemical forms.

Experiments testing species preferences for different chemical N forms were done in alpine or arctic environments (Miller and Bowman 2002, Miller et al. 2007, Pornon et al. 2007), including one that demonstrated simultaneous partitioning of N in space (soil depth), time (over the growing season), and chemical form (glycine, NO$_3^-$, NH$_4^+$, McKane et al. 2002). However, in aerobic soils of neutral pH—as typically found in temperate grasslands—concentrations of NO$_3^-$ are usually higher than those of NH$_4^+$ (Marschner 1995), and organic N may play a minor role. In fact, two recent field experiments showed that inorganic N (Harrison et al. 2007) and NO$_3^-$ in particular (Kahmen et al. 2006) were the preferred N forms by temperate grassland plants. Hence, it is unclear whether plants under conditions less harsh than in alpine and arctic ecosystems show similar N partitioning.

However, uptake of N from different soil depths may well be an important mechanism in temperate grasslands. 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 shown. Also, a nice series of studies by Berendse (1979 and 1981 and 1982) demonstrated that a deep-rooting herb species (*Plantago lanceolata*) was forced to use nutrients from deeper soil layers when grown with a shallow rooting grass with high competitive ability (*Anthoxanthum odoratum*), whereas this was not the case in monoculture. To our knowledge, only two studies using $^{15}$N labeling techniques investigated species differences in the depth of N uptake in temperate grasslands: McKane et al.
(1990) found species to be spatially and temporally differentiated, whereas Kahmen et al. (2006) found somewhat less clear patterns.

Many studies investigating species differences in N uptake were done with plants grown either individually (Miller and Bowman 2002), in monoculture (Weigelt et al. 2005), or in mixed communities in the field (McKane et al. 2002, Kahmen et al. 2006, Pornon et al. 2007, Harrison et al. 2007). However, with regard to biodiversity–ecosystem functioning relationships, there is a clear need to study N partitioning across experimentally manipulated gradients of species richness. Do species behave the same when growing in monoculture (in their fundamental niche) as when grown with interspecific competition in mixture (realized niche)? And does the diversity of the mixture affect the realized niche?

To our knowledge, only one study (Miller et al. 2007) investigated chemical N partitioning of plants both in monocultures and in two-species mixtures, finding that competition within interspecific neighbor pairs often caused reduced uptake of a particular form of N, as well as shifts to uptake of an alternative form of N. So far, nobody investigated partitioning of N from different soil depths when depending on species richness. In this thesis, the main aim was thus to investigate N partitioning between different soil depths along gradients of plant species richness. One experiment additionally includes different chemical forms of N.

Soil microbes

One often forgotten aspect are plant-microbial interactions. Microorganisms mineralize organic matter, thus, providing many essential plant resources. However, it is increasingly recognized that plants can directly compete with soil microbes for resources, resulting in a complex mixture of positive and negative feedbacks on plant growth (Hodge et al. 2000, Schimel and Bennett 2004). Also, plant biodiversity was shown to affect N cycling (e.g., Hooper and Vitousek 1998, Spehn et al. 2000, Niklaus et al. 2001, Zak et al. 2003, Niklaus et al. 2006, Oelmann et al. 2007), suggesting potential changes in plant–microbe competition. However, this has not been tested until now, despite the high relevance for ecosystem functioning and sustainable resource use. In the last chapter of this thesis, we therefore assess partitioning of N between plants and microbes, as modulated by plant species richness.
Thesis outline

In this thesis, we experimentally test whether plant species in temperate grasslands, known to commonly co-occur, occupy different resource-based niches. Focusing on soil nitrate (N), we investigate if species differ in N uptake with respect to soil depth, but also with respect to chemical forms of N. Further, we link species’ N uptake patterns to manipulated levels of biodiversity, testing whether plant species richness affects the behavior of individual species by changing the intensity of intra- vs. interspecific competition, and whether species in mixtures partition soil N, resulting in complementary N use. We conducted three experiments, two in pots and one in the field, using communities of common temperate grassland species and of varying species richness (Fig. 1 and 2, Table 2.1, and 4.1). To avoid results being restricted to a particular species pool, two pools were used in two of the experiments. N uptake was tracked by $^{15}$N labeling techniques (except for Chapter 1). Pot experiments (Chapter 1, Chapters 4 and 5) were conducted in the experimental garden of the Institute of Environmental sciences at the University of Zurich ($8^\circ 33'\ E/47^\circ 23'\ N,\ 546\ m\ a.s.l.$). The field experiment (Chapter 2) was part of a larger biodiversity experiment in Reckenholz near Zurich (Switzerland, $8^\circ 54'\ E/47^\circ 38'\ N,\ 443\ m\ a.s.l.$, Wacker et al. 2008).

Chapter 1  In a pot experiment using monocultures and mixtures of four species (two grasses and two forbs, Fig. 1), we tested whether soil depth and nutrient availability modify the effects of species richness on productivity. Hereby, we avoided confounding soil depth and soil volume, using pots of different depths but constant volumes. We expected both deeper soil and increased nutrient concentration to increase overyielding of the mixtures through increased complementarity effects (von Felten and Schmid 2008).

Chapter 2  In a field experiment with already established plant communities of one, three, or six species, we tested whether species richness affects species N uptake from two soil depths and three different chemical forms, including organic N. Plant communities were assembled from two different pools of six species (two grasses, three forbs, and one legume, Fig. 2). We used $^{15}$N tracer solutions of $\text{NO}_3^-,\ \text{NH}_4^+,\ \text{and dual-labeled}^{15}\text{N-}^{13}\text{C-glycine}$ to label the respective N forms in the soil. We expected that niche breadth of individual species (Levins’B, with respect to N use), as well as niche overlap (Proportional
Similarity) between species decrease with species richness. Further, we expected the niche breadth of whole communities to increase with species richness (von Felten et al. in press).

**Chapter 3** This chapter is a comment on a paper published in Ecology (Harrison et al. 2007). We point out methodological difficulties related to using dual-labeled $^{15}$N-$^{13}$C-amino acids with varying C:N ratios. This comment relates to Chapter 2 of this thesis, where we also test for plant uptake of organic N (von Felten et al. 2008).

**Chapter 4** In a pot experiment with plant communities of one, two, and four species, assembled from two pools of four species (Fig. 1), we tested whether species richness affects species N uptake from shallow vs. deep soil. Here we used a more slowly released, solid organic $^{15}$N label. The label was homogeneously mixed with the natural field soil, allowing microbial mineralization. Further, only grasses and forbs were used, since legumes dilute $^{15}$Ntracer signals due to atmospheric N$_2$ fixation. Similar to Chapter 2, we expected that niche overlap (similarity) with respect to N uptake between species decreases with increasing species richness. Further, we expected that smaller niche overlap is correlated with larger complementarity effects, and that total N uptake of communities increases with species richness, due to increased uptake from deep soil.

**Chapter 5** Within the same experiment, we also assessed the partitioning of N and the added $^{15}$N label between plants and microbes, in relation to plant species richness. Microbial N was extracted from shallow and deep soil using the chloroform fumigation extraction method to quantify both microbial biomass N and $^{15}$N. Then, for each community, we calculated the share of total biotic N and $^{15}$N contained in plant biomass. We expected plant species richness to increase the plant share of N and $^{15}$N, through more complete N exploitation of mixtures, and to increase total biotic N and $^{15}$N recovery. Further, we expected microbes to be better competitors for the labeled N source in the short-term but plants to win in the long-term.
Figure 1: Plant species used in pot experiments: *A. elatius*, *H. lanatus*, *L. vulgare*, and *P. lanceolata* (Pool AHLP, upper row) and *D. glomerata*, *L. perenne*, *R. acris*, *T. officinale* (Pool DLRT, lower row). Pool AHLP was used in Chapter 1, both AHLP and DLRT were used in Chapters 4 and 5. Plates are from Thomé (1885) and Lindman (1901-1905), www.BioLib.de.
Figure 2: Plant species used in the field experiment in Reckenholz (Chapter 2).

* A. elatius¹, F. rubra², L. vulgare³, G. mollugo⁴, T. officinale⁵, and T. pratense⁶ (Set 1, two uppermost rows), as well as T. flavescens⁷, H. lanatus⁴, L. flos-cuculi⁷, S. nutans⁸, T. pratensis⁹, and T. repens¹⁰ (Set 2, two lowermost rows).¹¹

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General Introduction


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General Introduction


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General Introduction


Chapter 1

Complementarity among species in horizontal vs. vertical rooting space

Abstract

**Aims** Many experiments have shown a positive effect of species richness on productivity in grassland plant communities. However, it is poorly understood how environmental conditions affect this relationship. We aimed to test whether deep soil and limiting nutrient conditions increase the complementarity effect of species richness due to enhanced potential for resource partitioning.

**Methods** We grew monocultures and mixtures of four common grassland species in pots on shallow and deep soil, factorially combined with two nutrient levels. Soil volume was kept constant to avoid confounding soil depth and volume. Using an additive partitioning method, we separated biodiversity effects on plant productivity into components due to species complementarity and dominance.

**Important findings** Net biodiversity and complementarity effects were consistently higher in shallow pots, which was unexpected, and at the low nutrient level. These two results suggest that although belowground partitioning of resources was important, especially under low nutrient conditions, it was not due to differences in rooting depths. We conclude that in our experiment (1) horizontal root segregation might have been more important than the partitioning of rooting depths and (2) that the positive effects of deep soil found in other studies were due to the combination of deeper soil with larger soil volume.

**Keywords:** biodiversity effects, nutrient limitation, resource partitioning, root competition, soil depth
Introduction

Evidence for positive effects of species richness on ecosystem functioning has rapidly accumulated in recent years (as reviewed e.g. in Kinzig et al. 2002, Loreau et al. 2002, Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2006). In particular, plant species richness was shown to increase productivity in temperate grassland communities (e.g. Tilman et al. 1996, Hector et al. 1999, van Ruijven and Berendse 2003, Roscher et al. 2005). However, little is still known about the precise mechanisms that create this relationship and how environmental conditions can alter it.

In line with resource-based competition theory (e.g. Tilman et al. 1997), the positive effect of species richness on productivity (i.e. overyielding of mixtures) has largely been attributed to complementarity of species with regard to resource use. Through differences among species in nutrient uptake in space or time, species richness is thought to improve the resource use of mixtures. It is possible to statistically assess the importance of species complementarity as opposed to selection or dominance by additive partitioning (Loreau and Hector 2001, Fox 2005). While application of the method has shown that complementarity is an essential mechanism behind the diversity–productivity relationship (Loreau and Hector 2001, Tilman et al. 2001, van Ruijven and Berendse 2003, Roscher et al. 2005, Cardinale et al. 2007), it remains difficult to demonstrate which plant traits are actually involved in complementary resource use leading to overyielding in mixtures.

It has long been known that grassland species differ in root morphology including rooting depth (e.g. Cole and Holch 1941, Weaver 1958, Parrish and Bazzaz 1976, Fitter 1986), suggesting consequences for inter-specific competition. McKane et al. (2002) found evidence for complementary use of nitrogen in arctic plant communities with respect to depth, chemical form and timing of uptake. Berendse (1981 and 1982) demonstrated that a deep-rooting temperate grassland species benefits from deeper soil when growing in mixture with a shallow-rooting species. The effect of increased soil depth was larger in unfertilised than in fertilised soil, indicating that different environmental aspects (soil depth and nutrients) interact, and that species complementarity may be more relevant under limiting conditions. However, in other studies, increased soil fertility enhanced the effect of diversity on productivity in experimental plant communities (Reich et al. 2001, He et al. 2002, Fridley 2002 and 2003).

A recent study showed that increased biotope space in terms of soil depth and volume
linearly increased biodiversity effects due to increased complementarity of species (Dimitrakopoulos and Schmid 2004). However, in this experiment as well as in Berendse’s (1981, 1982), soil volume and soil depth were confounded, and it remains unclear whether the result was due to increased soil depth or soil volume and the nutrients within. More precisely, did deep soil benefit mixtures by allowing differences in rooting depths per se, thereby relaxing inter-specific competition, or rather by providing a higher total amount of nutrients, explored by those species able to grow deeper roots?

In this study, we tested whether soil depth and nutrients — independent of soil volume — positively affect the diversity–productivity relationship in mixtures of four common grassland plant species. We hypothesised that (1) deep soil would allow for enhanced complementarity between plant species with respect to rooting depth, and (2) that this may be more pronounced under limiting nutrient conditions. As a consequence, we expected both increased productivity and increased complementarity in mixtures grown on deep soil compared with those on shallow soil.

**Materials and Methods**

**Experimental Design**

Our experiment was conducted in the experimental garden of the Institute of Environmental Sciences of the University of Zurich (Switzerland). We established monocultures and mixtures of four perennial species and grew these in four different soil environments. Two different soil depths (“shallow” and “deep”), were combined with two nutrient levels (“unfertilised” and “fertilised”). The plant species used were *Arrhenaterum elatius* (L.) P. Beauv. ex J. & C. Presl (tall grass, relatively deep roots), *Holcus lanatus* L. (shorter grass, shallower roots), *Leucanthemum vulgare* Lam. (tall rosette herb, rather shallow, mainly adventitious roots), and *Plantago lanceolata* L. (shorter rosette herb, deep tap root). We chose these plant species because of their high abundance in natural grasslands of the region, high germination rate, ability to form monocultures, and potential for belowground niche separation (Dimitrakopoulos and Schmid 2004). They represent different functional types, and show between-species variation in maximum rooting depth and both above- and belowground biomass distribution (Grime et al. 1988, Kutschera and Lichtenegger 1982). The mean depth of root biomass in a previous field study was
2.9, 1.6, 1.5 and 3.2 cm for *A. elatius*, *H. lanatus*, *L. vulgare*, and *P. lanceolata*, respectively (see Dimitrakopoulos and Schmid 2004). We did not include legumes because of their potential to alter nitrogen dynamics. Two replicates of each monoculture and eight replicates of the four-species mixture were grown for each environment in a full-factorial design \( n = (2 \text{ monocultures} \times 4 \text{ species} + 8 \text{ mixtures}) \times 4 \text{ environments} = 64 \).

Our pots were designed to allow different rooting depths with a constant soil volume to avoid confounding of depth and volume. Deep pots were 46.4 cm deep by 19.5 cm wide, while shallow pots were 18.8 cm deep by 31 cm wide. Pots were made from polyvinylchloride (PVC) tubes with a punched PVC bottom attached (with 5 holes of 1 cm diameter). To allow proper drainage of the soil, we added 1 l of gravel at the bottom of each pot, resulting in a gravel layer of 1.3 and 3.4 cm height in shallow and deep pots. The gravel was covered with a separating fleece to prevent the soil from clogging the gravel. Pots were then filled with a 1:1 mixture of natural grassland soil and sand (13 l), to which we evenly mixed 14.3 g Osmocote fertilizer (Osmocote mini, 18% N + 6% P + 12% K, Scotts, De Meern, Netherlands) for all fertilised soils, corresponding to 12 g N per m\(^2\) (assuming a light interception area of 0.215 m\(^2\) per pot based on the spacing of pots). The grassland soil here was relatively nutrient rich, so the combination of adding sand and fertilizer was chosen to work with two nutrient levels, with the lower one poorer than the original soil.

In early June 2004, we grew seedlings of each species in small pots (3 cm diameter, 4.6 cm depth) in the experimental garden. After 25 days, approximately 10 cm tall seedlings were transplanted to the experimental pots (rosettes of *L. vulgare* were shorter). To even out size differences and to prevent strong transpiration directly after transplantation, a leaf was cut or trimmed from large seedlings. To avoid confounding soil depth and planting density, we constrained the planted area in the shallow but wide pots to the size of that in deep but narrow pots by covering the outer ring of the upper surface with a flat PVC ring. Per pot, 12 seedlings (three per species in mixtures) were planted in an inner ring of four and an outer ring of eight seedlings. In the mixtures, seedlings were randomly assigned to planting positions with one individual of each species present in the inner ring and two in the outer ring. The 64 pots were randomly assigned to positions within two blocks, each containing half of the replicates. Pots were watered daily with a constant amount of water except on rainy days (automated irrigation system), and weeded regularly.
Measurements

Five weeks after transplanting, on 2–4 August 2004, we measured the “extended” height of all individuals (distance from soil surface to uppermost leaf tip or inflorescence tip, when stem and leaves were pulled up to form a straight, vertical line). On 4–5 August 2004, we cut plants at 5 cm height to mimic mowing, sorted the cut plant material to species and measured its biomass after drying at 80°C. In 5 pots, one individual of *H. lanatus* had died and was replaced. Twelve weeks after transplanting, we again measured individual plant height, and fully harvested aboveground biomass on 16–19 September 2004. Plants were cut at ground level, and the biomass of each plant individual was measured \((n=64\times12=768)\) after drying at 80°C. Thereafter, pots were kept in the garden and soil cores (4.8 cm diameter, extending to the bottom) were taken in the center of each pot on 13–15 December 2004. Cores were cut into horizontal slices of 5 cm, and after washing the roots in a 1 mm sieve, total root biomass in each slice was determined after drying at 80°C.

To avoid root development becoming pot bound, the duration of our experiment was kept relatively short. Inspection of soil volumes at the end of the experiment confirmed that roots reached all parts of the pots, but had not yet accumulated along the pot walls and bottoms.

Data analysis

We used general linear models to analyse shoot and root biomass (combined from both harvests) and summarised the results in analysis of variance (ANOVA) tables (according to Schmid et al. 2002). For the analysis of shoot biomass at the pot=community level \((n=64)\), we fitted the following terms: (1) block, (2) nutrients, (3) soil depth, (4) species richness, and (5) monoculture species (species composition term fitted after species richness, mixtures have equal composition), and (6) several interaction terms (Table 1.1). The same model was used for root biomass (Table 1.1), except that the term block was omitted, as root samples were taken only in one block \((n=32)\). To test whether vertical root distribution differed between monocultures and mixtures, we evaluated for each pot the proportion of roots present in each 5-cm soil layer. For the deep pots, the two adjacent soil layers were pooled, resulting in 4 layers per pot (as in shallow pots). The proportion of root biomass within each layer was analysed depending on (1) nutrients, (2) total soil
depth, (3) actual soil depth (layer), (4) species richness, (5) monoculture species, and (6) all interaction terms.

In addition, we analysed biomass at the population level (species within pots, \( n=160 \), Table 1.2). Because the biomass of a species was based on \( n=12 \) individuals in monoculture but only \( n=3 \) individuals in mixture, we used mean individual plant biomass as response (to avoid confounding of species richness and abundance). For the same reason, we used a weighing variable in the ANOVA with weight 1 and 0.25 for populations in monoculture and mixture, respectively. Terms that varied at the pot-level (block, nutrients, soil depth, species richness and species in monocultures) were tested against the between-pot variation, terms which varied at the population-level against the residual variation. We did not detect problems with autocorrelated residuals within pots (in mixtures, biomass values for all four species were used in the analysis). Residuals were never positively correlated between species, rather slightly negative autocorrelations lead to slightly inflated error terms and thus conservative tests for population-level effects.

To test whether effects of biodiversity on aboveground productivity in the mixtures changed with soil depth and nutrient level, we used the additive partitioning method of Loreau and Hector (2001). This method allows to partition the net effect (NE) of biodiversity into a complementarity effect (CE) due to niche separation or facilitative interaction of species, and a selection effect (SE) due to dominance of species with particular traits. For each of the four identical mixtures per environment in each block, the four monocultures from the same block and treatment were used in the calculations. In addition, we used the tripartite partitioning method of Fox (2005), to further subdivide SE into a dominance effect (DE), strictly analogous to natural selection, and a trait-dependent complementarity effect (TDCE), attributable to species complementarity. We also calculated the relative yield total (RYT) for each mixture, which is the sum of every species’ yield in mixture divided by its yield in monoculture (de Wit and van den Bergh 1965, Loreau 1998). For the different components of the biodiversity effects and the RYT of the mixtures \( (n=32) \), we fitted a general linear model including the terms (1) overall mean, (2) block, (3) nutrients, (4) soil depth, and (5) nutrient x soil depth interaction (Table 1.3). The net effect (NE) was also calculated for belowground productivity in all four environments. However, partitioning of this belowground NE was not possible, because roots of the different species in mixtures could not be separated.
Between-species variance components for plant height at both harvests were estimated, to measure morphological differentiation (Bell 1989, Dimitrakopoulos and Schmid 2004).

For the ANOVA results shown in Table 1.1 and 1.3, instead of showing only one analysis (e.g., shoot biomass and CE), we present the additional analyses as well for information and to ease interpretation. This can be justified as long as ANOVA is used as an explorative statistical tool (Schmid et al. 2002). In agreement with this philosophy, we did not apply corrections such as Bonferroni methods for multiple testing, because they are notorious for their extreme reductions of statistical power under these circumstances, and can tempt researchers to only present part of their results (Moran 2003).

Results

Shoot and root biomass of communities

Whole-season shoot biomass of communities was approximately doubled by fertilisation accounting for nearly 85% of the total variation (Table 1.1, Fig. 1.1). Shallow soil yielded more shoot biomass at the high nutrient level (Mean±SE shallow: 59.5±1.2 g, deep: 47.1±1.5 g), whereas deep soil yielded more at the low nutrient level (shallow: 21.0±1.0 g, deep: 25.3±0.7 g, nutrients × soil depth interaction). Averaged over all environments, shoot biomass did not differ between monocultures and mixtures. However, on shallow soil with low nutrient level, the mixtures had higher shoot biomass than all the monocultures (transgressive overyielding, Fig. 1.1 and 1.3).

Among the monocultures, the four species showed different responses (significant nutrient × soil depth × monoculture species interaction, Fig. 1.1). At the high nutrient level, A. elatius, H. lanatus, and P. lanceolata did better on shallow soil and L. vulgare did better on deep soil. At the low nutrient level, none of the monocultures did better on shallow soil.

Fertilization generally increased root biomass in absolute terms, but the effect depended on soil depth, species richness, and the identity of monoculture species (see two-way interactions in Table 1.1, Fig. 1.1). In mixtures and in monocultures of A. elatius and Plantago lanceolata, the response to soil depth and nutrients was similar for root and shoot biomass. By comparison, root biomass of H. lanatus and L. vulgare was rather constant, resulting in a clear decrease in the root:shoot ratio at high nutrient level. Under high
nutrient conditions, root biomass was consistently smaller in deep than in shallow soil, whereas under low nutrient conditions there were only slight and inconsistent differences in root biomass between soil depths.

All species extended their roots to the lowest soil layer in monoculture, independent of soil depth (Fig. 1.2). However, root distributions differed significantly among monocultures (depth × monoculture species interaction, % SS = 2, \( P = 0.045 \)). The mean depth of root biomass was 9.0, 9.5, 6.9, and 9.9 cm for *A. elatius*, *H. lanatus*, *L. vulgare*, and *P. lanceolata*, respectively. In agreement with the data used for species selection, *L. vulgare* was the most shallow-rooted, and *P. lanceolata* the most deep-rooted species. In deep soil, the mean depth of root biomass increased for all species (by 57%, 57% 103%, and 123%, same order as above). This indicates that rooting depths are plastic, particularly those of the two herbaceous species. In all four environments, root biomass decreased with soil depth (% SS = 75, \( P \ll 0.001 \)). In mixtures, a larger proportion of root biomass than in monocultures was found in deeper soil layers (depth × species richness interaction, % SS = 1, \( P = 0.033 \), Fig. 1.2).

**Shoot biomass of populations**

The shoot biomass at the population level (mean individual plant biomass per species and pot) was increased by fertilisation (Fig. 1.3, Table 1.2). At the high nutrient level, it was higher in shallow than in deep soil. The four species responded differentially to soil depth both in monoculture (soil depth × monoculture species interaction) and in mixture (soil depth × species in mixture interaction). On shallow, unfertilised soil all species profited from growing in mixture (Figure 1.3). In the other environments, there was at least one species that grew better in monoculture.

**Biodiversity effects**

Composed of a positive complementarity effect (CE) and a negative selection effect (SE), the net effect of biodiversity (NE) on aboveground productivity was only marginally positive overall (Fig. 1.4, Table 1.3). NE was higher on shallow soil than on deep soil, mainly due to higher CE, and marginally higher in unfertilised soil than on fertilised soil (Table 1.3). The negative SE was strongest in deep soil at the high nutrient level (nutrients × soil depth interaction), because in this environment one species, *L. vulgare*, had much less
biomass in mixtures than expected from its monoculture yields in this environment. The tripartite partitioning method of Fox (2005) revealed that SE almost exclusively reflected the dominance effect (DE), whereas trait-dependent complementarity (TDCD) was negligible. Therefore, we only show SE in Fig. 1.4. Values of the relative yield total (RYT, Fig. 1.4, rightmost panel) were significantly larger than 1 overall and like NE higher on shallow than deep soil and at low than high nutrient level. Only on deep soil at high nutrient level, RYT was lower than 1 (0.997).

The net effect of biodiversity (NE) on belowground productivity was positive overall (% SS = 64, \( P < 0.001 \)) and was strongest in fertilised soil, amounting to 12.2, 15.6, 6.1, and 0.3 g for fertilised deep, fertilised shallow, unfertilised deep and unfertilised shallow soils, respectively. Overyielding of mixtures was transgressive (most productive mixture with more roots than most productive monoculture) in all but the unfertilised shallow environment.

The between-species variance component for plant height in mixtures at the first (second) harvest increased from 44.9 (34.5) cm\(^2\) in deep soil at low nutrient level to 58.5 (37.1) cm\(^2\) in deep soil at high nutrient level to 119.8 (59.3) cm\(^2\) in shallow soil at low nutrient level and 128.2 (108) cm\(^2\) in shallow soil at high nutrient level. This indicates stronger differentiation of plant heights between species in mixtures on shallow than on deep soil and also at high compared with low nutrient level.

Discussion

Biodiversity effects and soil depth

The results of this experiment do not support our first hypothesis that deep soil should enhance niche complementarity among species with respect to rooting depth and should thus increase complementarity effects in mixtures. If soil depth had been important for the partitioning of rooting depth among species, we should have found higher values of CE and RYT in deep soil than in shallow soil, particularly under low nutrient conditions, where belowground resource partitioning is presumably more important (e.g. Harpole and Tilman 2007). Contrary to our expectation, CE and RYT were highest in mixtures grown on shallow, unfertilised soil.

Our results contrast with those of Berendse (1982) and Dimitrakopoulos and Schmid
(2004), where RYT or biodiversity effects increased with soil depth, respectively. However, in both of these experiments, soil depth was confounded with soil volume and nutrients within that volume. In Berendse (1982), competition with Anthoxanthum probably caused the deep rooting plant Plantago to access nutrients in lower soil layers that were not accessed in monoculture. Our results suggest that increasing soil depth without increasing the soil volume is not sufficient to increase CE or RYT. In addition to larger CE and RYT, we also found larger between-species variance components for plant height on shallow soil compared with deep soil. This suggests increased complementarity and partitioning of light aboveground, possibly reflecting increased resource partitioning belowground.

**Biodiversity effects and nutrients**

The higher RYT and CE in unfertilised than fertilised soil agree with our second hypothesis and the findings of Berendse (1982). Limiting nutrient conditions may have increased belowground partitioning of resources between species. This is in line with Harpole and Tilman (2007) and the theory of greater niche dimensionality at low nutrients, while fertilisation may decrease the number of limiting resources. This is a potential mechanism to explain the coexistence of higher species numbers in nutrient-poor as opposed to nutrient-rich grasslands, although this is still debated (Craine 2005). Positive effects of fertilization on RYT (Reich et al. 2001, He et al. 2002, Fridley 2003) may be explained by enhanced light partitioning (Fridley 2003). However, we found overyielding in root biomass of mixtures which was most pronounced under fertilised conditions. While root:shoot ratios in most of the monocultures were reduced by fertilisation, they remained nearly constant in mixtures, suggesting considerable inter-specific competition below ground. Thus, possibly supported by the mowing treatment, light partitioning was probably less important than belowground partitioning in our experiment. Even so, belowground partitioning seems not due to different rooting depths of species.

**Explanations for complementarity on shallow soil**

Despite good evidence for niche differences in rooting depth from other studies (Parrish and Bazzaz 1976, Fitter 1986, Berendse 1982, McKane et al. 1990, Mamolos et al. 1995, McKane et al. 2002, Fargione and Tilman 2005, e.g.), our results suggest that other mechanisms may also affect complementarity and overyielding of mixtures. Indeed, we found
a more constant vertical distribution of roots in mixtures compared with monocultures (Fig. 1.2), but it was similar in deep and shallow soil. So, how can the positive effect of shallow soil on CE and RYT be explained?

We speculate that root foraging was less efficient in deep soils than in shallow soils. The larger the soil volume that roots can access, the more nutrients are available. Although soil volume was kept constant in our experiment, and all species were able to extend their roots to the bottom of all soils (in monoculture), it was probably more costly for plants to exploit the space and resources at the bottom of deep soils. This suggests that in deep soil the disadvantage of shallow-rooters was not compensated for by the corresponding advantage of deep-rooters. Whereas in shallow pots plants can exploit rather spherical volumes, plants in deep pots are forced to exploit volumes of oblong shape with a less optimal root length to rooting space ratio. Horizontal constriction in deep pots may have intensified competitive interactions between plants, particularly at the early stage of root growth when all species had roots in the top soil only. In our experiment, some species in deep soils had larger individual aboveground biomass in monocultures than in mixtures (H. lanatus and L. vulgare when fertilised, H. lanatus and A. elatius when unfertilised). This suggests that interspecific competition was stronger than intraspecific competition.

There is evidence that rooting space per se can be regarded as a soil resource (McConnaughay and Bazzaz 1991). Together with resource-independent space requirements due to root morphology and development, plants may primarily compete for space rather than nutrients (McConnaughay and Bazzaz 1992). In their review Schenk et al. (1999) depict various examples for the defense of space through horizontal as well as vertical root segregation between individuals of the same or different species. Species that use resources efficiently and conservatively may profit from active root segregation, as more prodigal species are unable to access the resources within the defended area. To explain the high CE in mixtures on shallow soil, root segregation would have to be stronger between species than within species, and require the recognition of alien roots. Finally, if such territoriality matters, it is plausible that a rather spherical volume of soil is easier to defend than more derived shapes, or in other words that horizontal segregation of roots is less costly than vertical segregation.
Potential caveats of our experiment

Some caveats regarding the employed design and duration of the experiment should be mentioned. First, although deep soil (>17.5 cm) did not enhance partitioning of resources here, the importance of vertical partitioning within shallow soil (<17.5 cm) is unknown. In addition to volume, this could explain the disagreement with Dimitrakopoulos and Schmid (2004) where soil depth ranged from 5 to 15 cm. Second, a number of studies have shown that pot geometry, and in particular the surface area/depth (S/D) ratio of pots affects plant growth (Campbell et al. 1985, Hanson et al. 1987). In line with Dominguez-Lerena et al. (2006), these studies suggest that intermediate values of S/D are optimal, probably through a trade-off between limited aeration and leaching out of nutrients from the rooting zone (mineral deficiencies) in deep pots and increased evapotranspiration in shallow pots. We cannot exclude that part of the positive effect of shallow pots we saw in our study, relate to this trade-off. However, as the additional surface area of shallow pots was covered, differences in evapotranspiration and aeration of the soil should be very small. And, although with an S/D of 6.3 and 39.0 for deep and shallow pots, respectively, our deep pots might be closer to the optimum than the shallow pots, the latter supported higher CE and RYT. Third, we do not have data on root length or absorptive surface area of roots, which would be more directly related to nutrient acquisition than root biomass which was measured here. And last, our experiment was of relatively short duration. Running the experiment over a longer period of time might have changed the outcome of below- and aboveground competition in mixtures vs. monocultures. However, belowground competition (mainly addressed here) is likely to occur earlier than aboveground competition (Fitter 1986). Also, roots were well distributed across the soil profile in all soils, and prolongation of the pot experiment might have distorted the results due to pot-bound foraging behaviour of roots.

Conclusions

From our results we conclude that increased soil depth without simultaneously increased soil volume does not result in enhanced belowground complementarity of species. The positive effects of increased soil depth on complementarity and RYT found in other studies, e.g. by Berendse (1982) and Dimitrakopoulos and Schmid (2004) might have been due to the combination of increased soil depth and volume. Finding the largest biodiversity
effects in shallow pots at low nutrients suggests (1) that belowground partitioning of resources was important, and (2) that horizontal rather than vertical root segregation between roots of different species might have occurred. In line with our results, we would expect a larger effect of species richness on productivity in natural grasslands where the same soil volume is distributed over a shallow, stonefree rather than a deep, rocky profile.

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Chapter 1

Chapter references


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Table 1.1: Analyses of variance for whole-season above-(shoot) and belowground (root) biomass per pot. To account for differences in total root biomass from soil cores due to different soil depths, total root biomass per pot was estimated (rule of proportion), and used as response.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Shoot biomass (% SS)(^a)</th>
<th>Root biomass (% SS)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>1</td>
<td>0.27 *</td>
<td></td>
</tr>
<tr>
<td>Nutrients</td>
<td>1</td>
<td>84.89 ***</td>
<td>25.57 ***</td>
</tr>
<tr>
<td>Soil depth</td>
<td>1</td>
<td>1.57 ***</td>
<td>5.19 ***</td>
</tr>
<tr>
<td>Species richness (SR)</td>
<td>1</td>
<td>0.10</td>
<td>16.57 ***</td>
</tr>
<tr>
<td>Monoculture species (MS)</td>
<td>3</td>
<td>2.11 ***</td>
<td>23.60 ***</td>
</tr>
<tr>
<td>Nutrients × soil depth</td>
<td>1</td>
<td>6.49 ***</td>
<td>11.36 ***</td>
</tr>
<tr>
<td>Nutrients × SR</td>
<td>1</td>
<td>0.11</td>
<td>6.48 ***</td>
</tr>
<tr>
<td>Soil depth × SR</td>
<td>1</td>
<td>0.16</td>
<td>0.075</td>
</tr>
<tr>
<td>Nutrients × MS</td>
<td>3</td>
<td>0.65 **</td>
<td>4.88 *</td>
</tr>
<tr>
<td>Soil depth × MS</td>
<td>3</td>
<td>0.94 ***</td>
<td>1.28</td>
</tr>
<tr>
<td>Nutrients × soil depth × SR</td>
<td>1</td>
<td>0.00</td>
<td>1.21 .</td>
</tr>
<tr>
<td>Nutrients × soil depth × MS</td>
<td>3</td>
<td>0.77 *</td>
<td>0.49</td>
</tr>
<tr>
<td>Residuals</td>
<td>43/12(^b)</td>
<td>1.94</td>
<td>3.30</td>
</tr>
</tbody>
</table>

\(^a\) % sums of squares (SS) indicate increases in multiple \(R^2\) (explained variance) due to the addition of this term to the model. Marginally significant terms are indicated by dots (. \(P<0.1\)), significant terms by asterisks (* \(P<0.05\); ** \(P<0.01\); *** \(P<0.001\)). Note that the full model explains more than 95% of the total variance for both variables (% SS residual < 5).

\(^b\) The residual degrees of freedom for above- and belowground biomass were 43 and 12, respectively, as belowground biomass was analysed for one block only.
Table 1.2: Analysis of variance for whole-season aboveground (shoot) biomass of populations, based on biomass of individual plants.

<table>
<thead>
<tr>
<th>Line</th>
<th>Source of variation</th>
<th>d.f.</th>
<th>Error(^a) (% SS)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Block</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>Nutrients</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>Soil depth</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>Species richness (SR)</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>Monoculture species (MS)</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>Species in mixture</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>7</td>
<td>Nutrients × soil depth</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>8</td>
<td>Nutrients × SR</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>9</td>
<td>Soil depth × SR</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>10</td>
<td>Nutrients × MS</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>11</td>
<td>Soil depth × MS</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>12</td>
<td>Nutrients × species in mixture</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>13</td>
<td>Soil depth × species in mixture</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>14</td>
<td>Nutrients × soil depth × SR</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>15</td>
<td>Nutrients × soil depth × SR × species in mixture</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>16</td>
<td>Pot residuals</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Species residuals</td>
<td>84</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) The line number of the error term used for the calculation of F-values.

\(^b\) % sums of squares (SS) indicate increases in multiple $R^2$ (explained variance) due to the addition of this term to the model. Significant terms are indicated by asterisks (*$P<0.05$; **$P<0.01$; ***$P<0.001$). Note that the full model explains more than 85 % of the total variance (% SS residual < 15).

\(^c\) Observations from monocultures and mixtures are given weight 1 and 0.25, respectively.
Table 1.3: Analyses of variance for the net effect of biodiversity (NE), the effects of complementarity (CE) and selection (SE), as well as for the relative yield total (RYT).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>NE(^a)</th>
<th>CE(^a)</th>
<th>SE(^a)</th>
<th>RYT(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.f.</td>
<td>(% SS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1</td>
<td>7.75</td>
<td>14.57</td>
<td>23.63 ***</td>
</tr>
<tr>
<td>Block</td>
<td>1</td>
<td>13.04 *</td>
<td>14.48 *</td>
<td>0.80</td>
</tr>
<tr>
<td>Nutrients</td>
<td>1</td>
<td>7.97</td>
<td>3.72</td>
<td>18.11 **</td>
</tr>
<tr>
<td>Soil depth</td>
<td>1</td>
<td>11.98 *</td>
<td>8.91</td>
<td>5.19</td>
</tr>
<tr>
<td>Soil depth × Nutrients</td>
<td>1</td>
<td>0.09</td>
<td>0.15</td>
<td>10.65 *</td>
</tr>
<tr>
<td>Residuals</td>
<td>27</td>
<td>59.16</td>
<td>58.18</td>
<td>41.63</td>
</tr>
</tbody>
</table>

\(^a\) Data analysed were whole-season aboveground biomass values from \(n=32\) four-species mixtures (8 per environment) and corresponding sets of \(n=32\) monocultures (2 per species in each environment).

\(^b\) % sums of squares (SS) indicate increases in multiple \(R^2\) (explained variance) due to the addition of this term to the model. Marginally significant terms are indicated by dots (\(P<0.1\)), significant terms by asterisks (\(*P<0.05; **P<0.01; ***P<0.001\)). Note that the full model explains 41, 42, 58, and 51% of the total variance for NE, CE, SE, and RYT, respectively.
Figure 1.1: Mean biomass per pot (in g) for the four-species mixture and the monocultures of *Arrhenatherum elatius*, *Holcus lanatus*, *Leucanthemum vulgare*, and *Plantago lanceolata* when grown in pots of different soil depth and nutrient level. The error bars (right panel, top and bottom) show 2 standard errors of the difference between means (SED) and apply to all top and bottom panels, respectively.
Figure 1.2: Mean proportion of root biomass in layers of 5 cm thickness in monocultures (n=4, one per species) and four-species mixtures (n=4) grown in pots of different soil depth at low (a) and high (b) nutrient level. The error bar (lower right panel) shows 2 standard errors of the difference between means (SED) and applies to all panels.
Figure 1.3: Mean biomass per individual (in g) for *Arrhenatherum elatius*, *Holcus lanatus*, *Leucanthemum vulgare*, and *Plantago lanceolata* when grown in monoculture and in the four-species mixture in pots of different soil depth and nutrient level. The error bar (right panel) shows 2 standard errors of the difference between means (SED) and applies to all panels.
Figure 1.4: Mean biodiversity effects on aboveground biomass and relative yield total (RYT) for the mixtures in different environments. The net effect (NE) is the sum of the complementarity effect (CE) and the selection effect (SE). Error bars show 2 standard errors of the difference between means (2 SEDs).
Chapter 2

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

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. 2.1).

**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 2.1). 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. 2.4). The treatments were three chemical forms of $^{15}$N-labeled N ($\text{NO}_3^-$, $\text{NH}_4^+$, 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\textsuperscript{N} tracer application

Each subplot received 6.95 mg 15\textsuperscript{N} (27.8 mg 15\textsuperscript{N} m\textsuperscript{-2}) homogeneously spread over 52 injection points receiving 2 ml tracer solution (4.4 mmol l\textsuperscript{-1} 15\textsuperscript{N}) each. Injection points were spaced by 7.5 cm in a hexagonal grid. Tracer solutions for the three chemical forms of N were K15\textsubscript{NO}_3, 15\textsubscript{NH}_4\textsubscript{Cl} (98 % 15\textsuperscript{N}), and U-13\textsuperscript{C}_2-15\textsuperscript{N}-glycine (98 % 13\textsuperscript{C}, 98 % 15\textsuperscript{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\textsuperscript{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\textsuperscript{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\textdegree 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 δ\textsuperscript{15}N and δ\textsuperscript{13}C (glycine treatments) were analyzed with an isotope ratio mass spectrometer (Delta\textsuperscript{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\textsuperscript{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 $\delta^{15}$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 2.2 and Appendix Table 2.4). For each labeled plant sample, [$^{15}$N$_{ex}$] was calculated from the $^{15}$N concentration in excess atom percent $^{15}$N:

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

(2.1)

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):

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

(2.2)

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):

\[ PS = 1 - 0.5 \sum_{i=1}^{6} |p_{1i} - p_{2i}| \]  

(2.3)

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. 2.2 and 2.3, 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 2.1), 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 2.2). 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 2.4).

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 2.1) 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 2.3). 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 $\delta^{15}$N, relative to natural abundance values. Across the whole tracer experiment, $\delta^{15}$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 $\mu$mol 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 2.2). 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. 2.2). 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. 2.1, 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. 2.2).

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 Nasholm 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 2.3; Fig. 2.3, 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 2.3; Fig. 2.3, 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. 2.2). 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 2.4). 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 2.4).

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 2.3; Fig. 2.3). 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 $\text{NO}_3^{-}$ and $\text{NH}_4^{+}$ ($N_{\text{min}}$) concentrations in the soil (see Appendix Fig. 2.5). This effect was stronger in Set 2 (set×legume presence interaction, $P<0.05$) and in shallow soil (depth×legume presence interaction, $P<0.05$). $N_{\text{min}}$ concentrations were generally higher in shallow than in deep soil ($P<0.01$). In Set 1, concentrations of $\text{NO}_3^{-}$ were higher than those of $\text{NH}_4^{+}$ whereas in Set 2, concentrations of $\text{NH}_4^{+}$ 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. 2.1) 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. 2.2, 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. 2.1, 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. $N_{\text{min}}$ 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 $N_{\text{min}}$ concentrations, is in line with Palmborg et al. (2005), Roscher et al. (2008); together with the simultaneous decrease in $[^{15}\text{N}_{\text{ex}}]$ 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 $\text{NO}_3^-$ 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 $\text{N}_2$ 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. 2.1), but without resulting in increased niche breadth of mixtures compared to monocultures (Hypothesis 3, Fig. 2.1). 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.

Acknowledgments

Many thanks to Luca Wacker and Oksana Baudois for their cooperation at the field site in Reckenholz and the generous exchange of data, and to Romain Barnard as well as numerous field assistants for their help during the labeling campaigns. We are grateful to Ansgar Kahmen for useful discussions on our results. Funding was provided through the University of Zurich and the Swiss National Science Foundation (grant no. 31-65224-01 to B.S.), as well as ETH Zurich and a PSC-Syngenta Graduate Research Fellowship from the Zurich-Basel Plant Science Center (to N.B., A.H., and P.N.).
Chapter references


Symposia on Quantitative Biology 22:415–427

Hutchinson GE (1959) Homage to Santa-Rosalia or why are there so many kinds of animals. American Naturalist 93:145–159


Table 2.1: 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 labeled between 26-28 May (Set 1), Pool 2 between 19-21 July 2004 (Set 2). Functional groups are grasses (g), legumes (l), and forbs (f).

<table>
<thead>
<tr>
<th>Community</th>
<th>SR, FG</th>
<th>Rep</th>
<th>Plots</th>
<th>Pop</th>
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<tr>
<td>Pool 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>each species in monoculture</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grasses, Forbs, Legumes</td>
<td>12</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>all six species</td>
<td>12</td>
<td>2</td>
<td>6</td>
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</tr>
<tr>
<td>Pool 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>each species in monoculture</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grasses, Forbs, Legumes</td>
<td>12</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>all six species</td>
<td>12</td>
<td>2</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 2.2: Mixed model analysis of variance of excess $^{15}$N ($^{15}$N$_{ex}$, in $\mu$mol g$_{dw}$$^{-1}$ over natural background) for populations ($n=60$). Data are averaged per species over all $^{15}$N treatments (three chemical N forms $\times$ two soil depths). This analysis shows the general patterns of $^{15}$N uptake. See Appendix Table 2.4 for a more detailed analysis of the 6-species mixtures.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Error</th>
<th>% SS</th>
</tr>
</thead>
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<tr>
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<td>P</td>
<td>39.9  ***</td>
</tr>
<tr>
<td>Legume presence</td>
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<td>P</td>
<td>13.9  ***</td>
</tr>
<tr>
<td>Species richness</td>
<td>1</td>
<td>P</td>
<td>0.5   ns</td>
</tr>
<tr>
<td>Set$\times$Legume presence</td>
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<td>P</td>
<td>9.7   ***</td>
</tr>
<tr>
<td>Set$\times$Species richness</td>
<td>1</td>
<td>P</td>
<td>0.3   ns</td>
</tr>
<tr>
<td>Functional group</td>
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<td>R</td>
<td>4.9   *</td>
</tr>
<tr>
<td>Legume vs. others</td>
<td>1</td>
<td>R</td>
<td>2.9   *</td>
</tr>
<tr>
<td>Grasses vs. Forbs</td>
<td>1</td>
<td>R</td>
<td>2.0   .</td>
</tr>
<tr>
<td>Legume presence$\times$Grasses vs. Forbs</td>
<td>1</td>
<td>R</td>
<td>3.2   *</td>
</tr>
<tr>
<td>Species richness$\times$Functional group</td>
<td>2</td>
<td>R</td>
<td>0.9  ns</td>
</tr>
<tr>
<td>Plot residuals (P)</td>
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<td>10.1</td>
</tr>
<tr>
<td>Residuals (R)</td>
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<td>16.7</td>
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<tr>
<td>MODEL</td>
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<td>73.2</td>
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</tbody>
</table>

$^a$ 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 2.3: Analyses of variance of Levins’ normalized B ($B_n$) and Proportional Similarity (PS) for species and species pairs, respectively.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>B$_n^a$</th>
<th>PS$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.f</td>
<td>Error$^b$</td>
</tr>
<tr>
<td>Set</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>Species richness</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>Legume presence</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>Dominance</td>
<td>1</td>
<td>R</td>
</tr>
<tr>
<td>Species (within Dom.)</td>
<td>9</td>
<td>R</td>
</tr>
<tr>
<td>Species richness×Dominance</td>
<td>1</td>
<td>R</td>
</tr>
<tr>
<td>Species richness×Species (within Dom.)</td>
<td>10</td>
<td>R</td>
</tr>
<tr>
<td>Plot residuals (P)</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Residuals (R)</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>MODEL</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ $B_n$ 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 2.1: 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).
Figure 2.2: 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).
Figure 2.3: 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 ($\frac{1}{6} < \text{Levins’ B} < 1; 0 < \text{PS} < 1$). 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 2.3 for the ANOVA.
# Appendix

Table 2.4: Analysis of variance of excess $^{15}$N ($[^{15}\text{N}]_{ex}$, $\mu$mol $^{15}$N g dry weight$^{-1}$ over natural background) of species from the six different N sources in the 6-species mixtures of Set 1 and 2 ($n=72$ for each set).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>% SS$^a$</th>
<th>% SS$^a$</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Set 1</td>
<td>Set 2</td>
</tr>
<tr>
<td>Species</td>
<td>5</td>
<td>33.1</td>
<td>47.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Functional group</td>
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<td>40.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Species within FG</td>
<td>3</td>
<td>12.8</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>***</td>
<td>*</td>
</tr>
<tr>
<td>Soil depth</td>
<td>1</td>
<td>13.8</td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td>Chemical N form</td>
<td>2</td>
<td>13.7</td>
<td>12.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td>Species×Soil depth</td>
<td>5</td>
<td>10.7</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>Functional group×Soil depth</td>
<td>2</td>
<td>3.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Species within FG×Soil depth</td>
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<td>7.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Species×Chemical N form</td>
<td>10</td>
<td>8.1</td>
<td>4.4</td>
</tr>
<tr>
<td>Functional group×Chemical N form</td>
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<td>3.4</td>
<td>3.7</td>
</tr>
<tr>
<td>Species within FG×Chemical N form</td>
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<td>4.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Soil depth×Chemical N form</td>
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<td>5.2</td>
<td>7.1</td>
</tr>
<tr>
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<td>2.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Residuals</td>
<td>36 ($24$)</td>
<td>12.3</td>
<td>17.0</td>
</tr>
<tr>
<td>MODEL</td>
<td>35($33$)</td>
<td>87.7</td>
<td>83.0</td>
</tr>
</tbody>
</table>

---

$^a$ % 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 (. $P<0.1$).

$^b$ d.f. for Set 2 in brackets, when different from Set 1.
Figure 2.4: Lay-out of an experimental plot containing six subplots (a–f) to which the six $^{15}$N treatment combinations (3 chemical N forms $\times$ 2 soil depths) were randomly applied.
Figure 2.5: Plant available soil $\text{NO}_3^-$ and $\text{NH}_4^+$ ($N_{\text{min}}$) in shallow and deep soil, for plots of Set 1 (top) and 2 (bottom) depending on legume presence. $n=44$ for $\text{NO}_3^-$ and 45 for $\text{NH}_4^+$. The error bar (upper panel) shows 2 standard errors of the difference between means (SED) and applies to both panels.
Chapter 3

Preferences for different nitrogen forms by coexisting plant species and soil microbes: comment

Harrison et al. (2007) reported on an interesting $^{15}$N labeling study in Ecology 88(4). Under field conditions, they assessed whether coexisting plant species of temperate grasslands show preferences for different chemical forms of nitrogen (N), including ammonium nitrate (inorganic N) and three amino acids of varying complexity (organic N). The authors found that all plant species were able to take up the full range of amino acids offered to them, as shown by $^{15}$N and $^{13}$C enrichment in plant tissues. However, plants all preferred inorganic over organic N, indicated by higher $^{15}$N enrichments after ammonium nitrate compared to organic N labeling. We do not object the general interpretation of the results and the authors main conclusions. Yet, we would like to comment on the plant uptake of intact amino acids. When testing for significant relationships between excess $^{13}$C and $^{15}$N of plants to infer direct uptake of amino acids Näsholm et al. (1998), Harrison et al. (2007) should have accounted for the different C:N ratios of the amino acids used. The amino acid tracers were U-$^{13}$C$_2$-$^{15}$N-glycine, U-$^{13}$C$_3$-$^{15}$N-serine, and U-$^{13}$C$_9$-$^{15}$N-phenylalanine (all $^{15}$N 98% and $^{13}$C 98%), and their ratios of C:N atoms are 2:1, 3:1, and 9:1 respectively. While the authors point out that these differences in available C may affect the preferences of plants and microbes, they omitted to consider the methodological consequences. One common problem (among others, see e.g. Jones et al. 2005) when using dual-labeled amino acids to study organic N uptake by plants is detecting the $^{13}$C label in plants. Due to the high C:N ratio of plants and the high abundance of $^{13}$C (ca. 1.08 at. % in C$_3$ plants), the dilution of C is usually 60-150 times higher than that of $^{15}$N Näsholm and Persson (2001). Finding a significant relationship between excess $^{13}$C and $^{15}$N requires separating the shift in $^{13}$C resulting from direct amino acid uptake from natural variation and analytical error. However, this is often not possible, due to rather low concentrations of tracer $^{13}$C. As a solution, Näsholm and Persson (2001) suggested to concentrate the labeled fraction of the plant material studied, by extracting the soluble fraction containing the label. For assessing the uptake of intact amino acids using the dual-labeling approach, the critical step is to assure that there is a theoretical possibility of detecting this uptake. From the measured values of $\delta^{15}$N (after labeling with $^{15}$N) the theoretical shift in $\delta^{13}$C corresponding to 100 % intact uptake can be calculated Näsholm and Persson (2001). Thereby it can be determined whether this shift is distinguishable from “noise”.

Given the high amount of C in phenylalanine, it is not surprising that Harrison et al.
Chapter 3

(2007) found a significant relationship between excess $^{13}\text{C}$ and $^{15}\text{N}$ across all species for this amino acid but not for glycine and serine. In their paper, Fig. 2A shows that shoot $^{15}\text{N}$ enrichment over all plant species was highest for glycine and lowest for phenylalanine (among organic N forms), while shoot $^{13}\text{C}$ enrichment was similar for all amino acids (Fig. 2C). This almost opposite pattern for $^{13}\text{C}$ and $^{15}\text{N}$ enrichment also applies for single species (Fig. 1), roots (Fig. 3), and microbes (Fig. 4). In the latter, $^{13}\text{C}$ enrichment was actually highest when labeled with phenylalanine, and lowest in the case of glycine. We think that these results are due to the different C:N ratios of the three amino acids rather than indicating higher uptake of phenylalanine compared to glycine and serine, what is particularly unlikely given that phenylalanine is the largest and most complex amino acid tested. Though, without significant relationships between excess $^{13}\text{C}$ and $^{15}\text{N}$ in plant tissues, the proportion of amino acids taken up as intact molecule can not be estimated for glycine and serine. Moreover, although no data on amino acid concentration in the soil solution are shown, it is likely that phenylalanine is the least abundant of the three amino acids, and glycine the most abundant. Thus, the dilution of the added $^{15}\text{N}$ tracer (equal for all N forms) by the natural abundance pool was probably least for phenylalanine and strongest for glycine, again leading to an overestimation of phenylalanine uptake when assessed by $^{15}\text{N}$ labeling.

We fully agree with Harrison et al. (2007), that a rigorous test to detect organic N uptake by plants requires compound specific isotope analysis (a combination of gas chromatography with isotope ratio mass spectrometry, see e.g. Persson and Näsholm 2001). But clearly, the results of Harrison et al. (2007) demonstrate that the use of the Näsholm et al. (1998) method to infer direct uptake of amino acids, without resolving the problem of low $^{13}\text{C}$ enrichment is unreliable, if not misleading.

Acknowledgments

We thank one anonymous referee for helpful comments on the manuscript.
Chapter references


Chapter 4

Nitrogen partitioning by soil depth among grassland plants modulated by species richness

Abstract

Resource partitioning due to niche separation among species has often been suggested as a mechanism to explain the positive relationship between plant species richness and productivity in temperate grasslands. Species differences in resource use may result in better resource exploitation of species-rich plant communities. Nitrogen (N) is quantitatively the most important mineral nutrient for plants, and is seen as the major limiting nutrient to primary production in temperate ecosystems. A number of 15N-labeling studies recently investigated chemical partitioning of N. However, hardly any of them addressed partitioning of N by soil depth, although species differences in the vertical distribution of root biomass and activity are well known. In a pot experiment with plant communities of one, two, and four species of forbs and grasses, we tested how species richness affects species’ N uptake from shallow vs. deep soil. An organic 15N label was mixed directly to natural field soil (shallow or deep), allowing it to undergo microbial mineralization. Tracer uptake by plants was determined at three destructive harvests in two consecutive years. We hypothesized that with increasing species richness, (1) dominant species take up more N from shallow soil whereas subordinates switch to deep soil, and (2) that niche overlap with respect to N uptake among species decreases; (3) that smaller niche overlap is correlated with higher complementarity effects, and (4) that total N uptake of communities increases with species richness, due to increased uptake from deep soil. Unexpectedly, in the 2005 (first year), dominant species took up a higher fraction of 15N from deep soil than subordinate species, which were restricted to shallow soil. Niche overlap between species decreased with species richness, albeit not in the way expected: low niche overlap was most frequent in mixtures with high selection effects, i.e., mixtures dominated by species with higher-than-average yield in monoculture. The situation in 2006 was closer to our expectations, indicating that communities were not yet fully established in 2005. However, also no negative correlation between niche overlap and the complementarity effect was found. Nevertheless, total 15N uptake was increased in mixtures vs. monocultures over all harvests. Similarly, niche overlap between species generally decreased with species richness mainly because species in monoculture were more similar with respect to 15N uptake from deep vs. shallow soil than species in mixtures. We conclude that (1) the presence of interspecific competition had a larger effect on species’ N use patterns than species richness as such, and (2) that complementarity in depth of N up-
take is not the main mechanism underlying the widely observed phenomenon of positive biodiversity–productivity relationships.
Introduction

The principle of competitive exclusion (Volterra 1926, Gause 1934) predicts that whenever two competing species coexist in a stable environment, they do so by differing in their niches. Otherwise, one species will exclude the other. While there are many good examples of coexisting animals due to niche specificity or character displacement (Brown and Wilson 1956, Hutchinson 1957 and 1959), the coexistence of plant species on a small scale still poses a puzzle, given that plants all require the same small set of resources (i.e. light, space, carbon, water, mineral nutrients). One possible solution is differential use of common resources (resource partitioning), i.e., coexisting plant species specialize on different resource forms and in where they obtain them in space and time. But, good evidence for resource-based plant niches is rare (Silvertown 2004).

Recent biodiversity – ecosystem functioning research has again raised the issue of plant resource niches. In temperate grasslands, increased productivity of species rich plant communities (as reviewed e.g. in Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2006 and 2007) suggests more complete resource use in mixtures, probably due to species complementarity. Notably, the use of additive partitioning (Loreau and Hector 2001) to quantify the importance of the complementarity effect (resource partitioning or positive interactions that lead to increased total resource use) vs. the selection effect (dominance by species with particular traits) for the better productivity of mixtures, statistically 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). To demonstrate the underlying biological mechanisms, however, remains difficult.

Since N is the key limiting resource to primary productivity in many ecosystems including temperate grasslands (Vitousek and Howarth 1991), complementary use of N is an obvious hypothesis for an underlying mechanism of species complementarity. After the ability of plants to directly take up organic N (such as amino acids) was shown (Chapin et al. 1993), partitioning of N with respect to chemical form (NH$_4^+$, NO$_3^-$, different amino acids) was frequently investigated (McKane et al. 2002, Miller and Bowman 2002, Weigelt et al. 2005, Kahmen et al. 2006, Miller et al. 2007, Pornon et al. 2007, von Felenet et al. in press, see also Chapter 2). Furthermore, there is plenty of evidence for plant species differing in rooting depth (e.g., Parrish and Bazzaz 1976, Berendse 1982), depth of root activity (e.g., Veresoglou and Fitter 1984, Mamolos et al. 1995), and depth of water up-
take (e.g., in water limited grassland types, Gordon and Rice 1992, Nippert and Knapp 2007). However, only a very small number of studies investigated partitioning of N by soil depth. Whereas McKane et al. (2002) found simultaneous partitioning of N by depth, chemical form, and time in arctic tundra, Kahmen et al. (2006) found no clear patterns in temperate grasslands, and no evidence either for partitioning of soil depth or a positive effect of species richness on the absolute exploitation of soil N pools. Spatiotemporal partitioning of N as a mechanism for coexistence was supported by McKane et al. (1990), and Fargione and Tilman (2005) showed that deep rooting grassland species had a better chance of coexisting with a dominant C₄ bunchgrass exploiting NO₃⁻ predominantly in shallow soil.

In the absence of interspecific competition (monoculture), species occupy their fundamental niche, whereas interspecific competition reduces the fundamental to the realized niche, with the shape of the latter depending on competitors (Hutchinson 1957). Most research related to N partitioning by plants focused on differences among species in the uptake of different N forms either in situ (e.g., McKane et al. 2002) or in the laboratory (e.g., Chapin et al. 1993, Kielland 1994). However, despite the role of interspecific competitors on species’ realized niches, N partitioning until now has not been investigated across an experimentally manipulated gradient of species richness. Apart from von Felten et al. (in press, see Chapter 2), we know of only one study that investigated partitioning of N (by chemical form) with plant species grown in different neighborhoods, finding that species varied in the capacity to take up ¹⁵N-labeled NH₄⁺, NO₃⁻, and glycine in intact neighborhoods and in interspecific pairs, and that neighbor identity influenced the capacity of species to take up different forms of N (Miller et al. 2007). Thus, except the study done in the field by von Felten et al. (in press), the study presented here is to our knowledge the first investigating the partitioning of N by soil depth, as modulated by species richness under more controlled conditions (in pots).

Usually, N uptake patterns are analysed by injection of ¹⁵N enriched N forms at one point in time (pulse-labeling). While required for studying chemical partitioning of N, because of rapid transformation of N in the soil (e.g., McKane et al. 1990 and 2002, Kahmen et al. 2006), this approach is not essential to investigate the depth of uptake and even has the disadvantage of assessing only one point in time. In this study, we tested whether temperate grassland plant species partition soil N by depth, using a different approach: we
mixed an organic $^{15}$N label directly into natural field soil (in pots), allowing it to undergo microbial decomposition. In addition, we related the magnitude of partitioning to plant species richness (one, two, or four species) of communities that we established in a common garden experiment. Hence, our experimental design is novel in several aspects: (1) the solid organic $^{15}$N label can be more precisely and homogeneously distributed in the soil compared liquid $^{15}$N tracers, (2) the label simulates belowground litter input more realistically as it is released via soil microbes; using a gradient of experimentally manipulated plant species richness (3) allows the comparison of fundamental niches (monocultures) with realized niches (mixtures) and realized niches in mixtures of different diversity, as well as (4) decoupling effects of species richness from other effects, e.g., nutrient availability, typically confounded with species richness in observational studies. We asked whether single species change their N uptake from deep versus shallow soil depending on species richness to decrease niche overlap with interspecific competitors. We tested whether the N uptake of species relates to dominance patterns in mixtures and whether niche overlap between species can be related to complementarity (complementarity effect CE, sensu Loreau and Hector 2001) in mixtures? We hypothesized that with increasing species richness, (1) dominant species take up more N from shallow soil whereas subordinates switch to deep soil, and (2) that niche overlap with respect to N uptake among species decreases; (3) that smaller niche overlap is correlated with higher complementarity, and (4) that total N uptake of communities increases with species richness, due to increased uptake from deep soil.

Materials and Methods

Experimental Design

Our experiment was set up in the experimental garden of the Institute of Environmental Sciences, at the University of Zurich (Switzerland). We used two pools of four common temperate grassland species (Table 4.1) to avoid results restricted to a particular mixture. For each pool, we grew all four monocultures, all six pairwise mixtures, and the full 4-species mixture. Three replicates of each monoculture and pairwise mixture, and six replicates of each 4-species mixture were grown in boxes ($40 \times 60$ cm wide and 40 cm deep). Boxes were subdivided into eight compartments. In May 2005, we grew seedlings
of each species in small pots (1.3×1.3 cm wide, 3 cm deep) in the experimental the garden for five weeks. Between June 7-10, 12 seedlings were planted per compartment in three alternate rows of four (6912 plants in total). An even number of individuals per species was represented in each row, with positions within rows randomized. Plant communities were weeded regularly and were watered daily with an automated irrigation system except on rainy days.

Before planting of seedlings, three organic $^{15}$N labeling treatments — shallow, deep, and no $^{15}$N — were applied to sets of four compartments (half a box), yielding a shallow/no $^{15}$N, a deep/no $^{15}$N, and a shallow/deep box for each species composition. In a separate box, we grew four full mixtures of both species pools to determine background $\delta^{15}$N values for each species, in case compartments without $^{15}$N within the experiment were contaminated with label, e.g. during set-up or by litter fall. Note that in this paper, we will focus on treatments “shallow” and “deep” only (compared to background $^{15}$N). All boxes were randomly distributed on a flat area of 7 m × 6.25 m. A detailed description of the experimental design is given in Table 4.1.

$^{15}$N labeling of the soil

The organic $^{15}$N label and non-labeled material were produced in the greenhouse. *Festuca rubra* was grown on quartz sand supplied with nutrient solution either containing K$^{15}$NO$_3$ (15 at% $^{15}$N) or isotopically unenriched KNO$_3$. Shoots were harvested after 11 weeks, dried, and shredded into pieces of app. 2 cm.

The inside of the boxes was vertically subdivided with waterproof boards into eight compartments. Subdivisions were sealed with silicone (wood–wood angles) and polyurethane lute (wood–polypropylene angles) to prevent leaking of the $^{15}$N label into neighboring compartments. Four drainage holes (10 mm wide) were drilled into the bottom of each compartment. Compartments were lined with a plastic mesh to enable easy removal of the soil core, and a drainage mat (Enkadrain) was added at each bottom. Then, compartments were filled with sieved, natural field soil to include active microbial populations (soil pH=7.6). The $^{15}$N treatments were applied by mixing $^{15}$N-labeled *F. rubra* material to the lower (deep) or the upper soil layer (shallow) or to neither layer. The same amount of non-labeled *F. rubra* material was mixed to the respective other layer of the soil. The amount of $^{15}$N tracer added per labeled compartment was 2.5 mg $^{15}$N in 1.45 g
plant material. The lower soil layer of each compartment was compressed (20 cm thick) to minimize subsequent settlement before the top layer was added and compressed too. To limit soil warming by direct radiation, boxes were insulated by 2 cm thick styrofoam walls.

The strength and spatial distribution of the $^{15}$N-labeling treatments were assessed by measuring $\delta^{15}$N in $K_2SO_4$-extracts of soil mineral N ($n=72$ measurements, 6 compartments $\times$ 2 treatments $\times$ 2 depths $\times$ 3 harvests). The treatment was very stable across harvests; average soil $\delta^{15}$N for the shallow $^{15}$N treatment were 45.0±1.8 and 10.7±0.3 % in the upper and lower part; for the deep $^{15}$N treatment 8.5±0.2 and 46.4±1.5 %, respectively. These $\delta^{15}$N values indicate that unlabeled soil parts remained unlabeled (background $\delta^{15}$N of soil: 10.9±0.3 %) for the duration of the experiment (11 months), and that the strength of the label was similar for both $^{15}$N treatments.

**Harvests and sample preparation**

Compartments were destructively harvested twice in 2005: after nine weeks (between 8-21 August) and after 15 weeks (19 September to 7 October), and once in 2006: after 11 months (between 15-29 May). Each time, we randomly selected 2 compartments per box (one from each $^{15}$N-treatment), i.e., we harvested two replicates for each combination of species composition and $^{15}$N treatment. At the same time, we harvested a “background”-compartment for each plant pool. To separate roots from soil, the soils were soaked in water. For monocultures, shoots were cut directly above the soil surface, and all roots were washed together. For mixtures, individual plants were pulled out, recovering as many of their roots as possible (with help of water from a sprinkler), were washed and sorted to species before roots and shoots were separated. All residual roots that could not be allocated to species were washed from the soil using 2 mm sieves, and were treated separately. Plant material was dried for 48 h at 80°C and weighed. For each population, i.e., individual species per compartment (see Table 4.1), shoots and roots were ground separately. Large samples were homogenized with a centrifuge mill before a subsample was ground to powder with a ball mill. Plant $\delta^{15}$N was analyzed with an isotope ratio mass spectrometer (IRMS, DeltaPlus XP, Finnigan MAT) coupled to an elemental analyzer (Flash EA 1112 NC, CE Instruments).

To check the vertical distribution of root $\delta^{15}$N we did a layered root harvest (1–10, 10–
20, 20–30, and 30–40 cm soil depth) in one monoculture with shallow and one with deep $^{15}$N treatment for each of the species *A. elatius*, *L. vulgare*, *D. glomerata*, and *T. officinale* in 2006. Root $\delta^{15}$N values at different soil depths clearly reflected $\delta^{15}$N of the soil, i.e., higher root $\delta^{15}$N of deep than shallow roots in the deep $^{15}$N treatment, and reverse in the shallow $^{15}$N treatment (Appendix Fig. 4.6). Since in mixtures it was not possible to separate the whole root system among species (only roots from the top), we did not use species specific root $\delta^{15}$N data and used shoot $\delta^{15}$N for analyses on the population level only. In contrast, both root and shoot data were used for calculations of tracer content and $^{15}$N uptake from deep soil on the community level.

**Calculations and Data Analysis**

For each population shoot and root sample, we calculated tracer concentration $[^{15}\text{N}_{\text{ex}}]$ (at\% excess or mg excess $^{15}$N/g N) and tracer content $^{15}\text{N}_{\text{ex}}$ (g excess $^{15}$N).

Then, for each population, the fraction of $^{15}$N tracer taken up from either deep soil (deep fraction, DF) or shallow soil (shallow fraction, SF) was calculated.

$$DF = \frac{[^{15}\text{N}_{\text{ex}}]_{\text{deep}}}{[^{15}\text{N}_{\text{ex}}]_{\text{deep}} + [^{15}\text{N}_{\text{ex}}]_{\text{shallow}}} \quad (4.1)$$

$$SF = 1 - DF \quad (4.2)$$

Hereby, $[^{15}\text{N}_{\text{ex}}]_{\text{deep}}$ and $[^{15}\text{N}_{\text{ex}}]_{\text{shallow}}$ came from a compartment replicate with the respective $^{15}$N treatment ($n=2$ per population at each harvest). Shoot samples were used only, due to incomplete species specific root samples.

Based on DF and SF, we calculated the Proportional Similarity index (Schoener 1970, Colwell and Futuyma 1971) between pairs of species as a measure of niche overlap:

$$PS = 1 - 0.5 \sum_{i=1}^{n} |p_{S1i} - p_{S2i}| \quad \text{(general form)} \quad (4.3)$$

$$PS = 1 - 0.5 (|DF_{S1} - DF_{S2}| + |SF_{S1} - SF_{S2}|) \quad \text{(here: } n=2) \quad (4.4)$$

Values of Proportional Similarity are proportions ranging from 0 to 1, for no to complete overlap between species (S1 and S2 denote two different species). For each species pool, Proportional Similarity was calculated between all species when grown in monoculture, i.e., fundamental niche overlap (six pairwise combinations). Within mixtures, Proportional Similarity was calculated between pairs of species, i.e., realized niche overlap (one combination in 2-species mixtures, six combinations in 4-species mixtures).
The deep fraction of tracer uptake (DF) and Proportional Similarity between pairs of plant species (PS) were analysed with generalized linear models (GLMs) and Analysis of Deviance. Because these are proportion data, we used the logit link function and binomial (quasibinomial) error distribution. Due to a hierarchical experimental design (harvest as a repeated factor), we used Quasi-\(F\)-tests based on the ratio of the deviances divided by their degrees of freedom, just as \(F\)-tests in ordinary analysis of variance (Egli and Schmid 2001). In addition, we calculated the Spearman rank correlation coefficient between species’ within mixture ranks in DF and shoot biomass (ranks 1–4 in 4-species mixtures, and ranks 1–2 in 2-species mixtures).

We applied the additive partitioning method (Loreau and Hector 2001) to the biomass data to partition the net effect (NE) of biodiversity on productivity into a complementarity effect (CE) due to niche separation or facilitative interaction of species, and a selection effect (SE) due to dominance of species with particular traits. This was done for all mixtures by comparing each species’ biomass in the mixture with that in monoculture. Then—for all mixtures—we tested for correlations between the average Proportional Similarity between species pairs in a mixture, and the CE of the mixture. As Proportional Similarity was based on deep fractions calculated from a pair of compartments (one D, one S), NE, CE, and SE were averaged over the respective pairs for correlation analysis.

Further, tracer content \(^{15}\text{N}_{\text{ex}}\) of plant communities was summed up across all populations, and percentage tracer recovery was calculated from the ratio \(^{15}\text{N}_{\text{ex}}/2.48\text{mg}^{15}\text{N}\) (tracer added per compartment). Hereby, we added the above and belowground parts of all plants grown within the same soil compartment, including roots that could not be allocated to species. We used linear mixed model ANOVA to test whether the \(^{15}\text{N}_{\text{ex}}\) of communities increased with species richness. Hereby, we fitted (1) community biomass (to correct for the effect of biomass), (2) species pool, (3) species richness, (4) \(^{15}\text{N}\) treatment, (5) time of harvest, and (6) all interactions between terms 2–5.

**Results**

Our organic \(^{15}\text{N}\) labeling led to significant plant shoot \(^{15}\text{N}\) enrichment, which was higher in compartments with shallow than with deep \(^{15}\text{N}\) treatment for all species. The \(^{15}\text{N}\) enrichment and the difference between \(^{15}\text{N}\) treatments decreased over time: for August
2005, September 2005, and May 2006, mean δ\(^{15}\)N was: 64.7, 60.9, and 49.3‰ for the deep \(^{15}\)N treatment, and 138.7, 115.2, and 64.3‰ for the shallow \(^{15}\)N treatment, respectively.

In August 2005, the \(^{15}\)N treatment explained most of the variation in \([^{15}\text{N}_{ex}]\) (76.4% SS, Appendix Table 4.4). Species differed in \(^{15}\)N uptake from deep and shallow soil (\(^{15}\)N treatment × Species interaction).

The fraction of \(^{15}\)N tracer taken up from deep soil (deep fraction, DF) was mostly lower than 0.5 (median=0.36). DF differed among species and functional groups, as well as across harvests, generally increasing with time from an average of 0.30 in August 2005 to 0.43 in May 2006 (Table 4.2, and Fig. 4.1 and 4.2). The time of harvest changed species and functional group responses, e.g. DF was larger for grasses at the first two harvests in 2005, but larger for forbs at the last harvest 2006 (Functional group × Harvest interaction), but the main effects of Species and Harvest explained more variation than the interaction between both (i.e., 25.5 and 20.8% vs. 8.6% Dev., Table 4.2). DF was constant across levels of species richness, and no clear among species divergence in DF was indicated (SR × species n.s.). Unexpectedly, in the first year, species dominating the mixtures had higher DF than subordinate species, e.g., *H. lanatus* and *L. perenne* in all 2-species mixtures and in the 4-species mixture (Fig. 4.1 and Fig. 4.2), indicated by a significant correlation between species’ within community ranks for abundance and for DF (Spearman’s rank correlation coefficient \(\rho_S\): 0.71 in August 2005, 0.70 in September 2005, both \(P<0.001\)). In 2006, there was no correlation between DF and the abundance of species in mixtures, and often dominant species had lower DF than their suppressed competitors, e.g., *D. glomerata* in all 2-species mixtures and in the 4-species mixture (and similarly *A. elatius*).

Niche overlap (Proportional Similarity) between species differed among levels of species richness (SR fitted as 3-level factor, Table 4.3, Fig. 4.3), and as hypothesized, niche overlap linearly decreased with species richness (sign. linear contrast of SR, Table 4.3). However, while species in mixtures overlapped less than species in monocultures (Mono vs. Mix contrast, Table 4.3), niche overlap between species did not differ between mixtures of two or four species (Mix. SR contrast, Table 4.3). Within a certain level of species richness, Proportional Similarity also depended on the species composition, and on the species pair in 4-species mixtures. However, differences among compositions and species pairs in Proportional Similarity changed with time of harvest (interactions with Harvest in Table
4.3, but see also Fig. 4.1 and Fig. 4.2 for DF).

Mixtures had higher biomass than monocultures, but the linear effect of species richness on community biomass was only marginally significant (Fig. 4.5, see also Chapter 5, Table 5.3). The net effect (NE) of species richness on plant productivity was positive overall (5.2% SS, $F_{1,191}=10.2$, $P<0.01$ for the intercept), but varied considerably among harvests (4.9% SS, $F_{2,168}=5.1$, $P<0.01$, Appendix Fig. 4.7). There was a marginally positive NE for species pool AHLP at harvest 1, but NE was not significantly different from zero at harvest 2 and 3. For species pool DLRT, NE was positive at harvest 2 (n.s. at harvest 1 and 3). The complementarity effect (CE) also varied between species pools and harvests, and was marginally positive only for pool DLRT at harvest 2 (n.s. in all other cases). There were significant positive selection effects (SE) for DLRT (harvest 1 and 2), but negative SE for both species pools at harvest 3, indicating that species with higher- and lower-than-average monoculture yields dominated the mixtures in 2005 and 2006, respectively (Loreau et al. 2001). This was mostly due to changes in forb biomass in May 2006: the forbs *R. acris* and *T. officinale* (DLRT) as well as *L. vulgare* (AHLP) had high monoculture biomass, but were dominated by the grasses in mixture (Fig. 4.2). In contrast, *P. lanceolata* (AHLP), which had relatively low monoculture biomass, was comparatively strong in mixture.

In contrast to our hypothesis, smaller average niche overlap (Proportional Similarity, PS) between species within a mixture was not related to larger complementarity effects (CE) at any harvest. Unexpectedly, there was a positive correlation between PS and CE in August and September 2005, and a negative correlation between PS and the selection effect (SE) in August 2005 (Fig. 4.4).

$^{15}$N tracer recovery in plant community biomass (roots+shoots) was 3.79% on average and varied between 0.8 and 9.6% (see also Fig. 4.5, panel E). Plant community $^{15}$N tracer content was not affected by species richness (as a linear term), but was higher in mixtures than in monocultures (two-level contrast, $F_{1,68}=4.081$, $P<0.05$), thus only partly confirming our hypothesis. Community $^{15}$N tracer content was positively related to community biomass ($F_{1,175}=498.5$, $P<0.001$, Fig. 4.5, panels A and E). In line with data on the deep fraction DF of populations (see Fig. 4.1 and 4.2), tracer content was greater in communities with shallow than with deep $^{15}$N treatment ($F_{1,20}=351.3$, $P<0.001$), and increased in communities with deep $^{15}$N treatment relative to those with shallow $^{15}$N.
treatment over time (Treatment × Harvest interaction, $F_{2,175}=91.4$, $P<0.001$). Overall for both $^{15}$N treatments, $^{15}$N tracer content decreased with time ($F_{2,175}=118.8$, $P<0.001$, Fig. 4.5). The higher $^{15}$N tracer content of mixtures than of monocultures after correcting for biomass (fitted first in the model) results from higher $^{15}$N concentrations that over-compensated for the reduced N concentrations of mixtures (Fig. 4.5, panels B and D). Similar to community $^{15}$N tracer content, the community $^{15}$N-uptake from deep soil (DF, shoots + roots) was unaffected by species richness (linear term), but there was a trend for higher DF in mixtures than in monocultures (0.9 % Dev., $P<0.1$, Fig. 4.5, panel F). Hence, the higher community $^{15}$N content and $^{15}$N concentration of mixtures might be due to increased $^{15}$N-uptake from deep soil.

**Discussion**

Our study is novel in investigating partitioning of N by soil depth using an organic $^{15}$N label and in combining this with experimentally manipulated levels of species richness. Our results do not support the first of our hypotheses and even an opposite relationship was found for the first two harvests in 2005: in mixtures, dominant species took up a higher fraction of $^{15}$N from deep soil (deep fraction, DF) than subordinate species. Supporting our second hypothesis, niche overlap between species decreased with species richness, mainly because species in monoculture were more similar with respect to $^{15}$N uptake from deep vs. shallow soil than species in mixtures. Our third hypothesis, a negative correlation between niche overlap and the complementarity effect could not be confirmed. For both harvests in 2005, even an opposite relationship was found. Our fourth hypothesis was partly supported by higher total $^{15}$N uptake of mixtures than of monocultures, which might partly be due to higher community $^{15}$N uptake from deep soil, in both relative and absolute terms.

Berendse (1982) suggested the term “phenotypic character displacement” to describe the behavior of *Plantago lanceolata*, using more nutrients from deeper soil layers in the presence of the competitively superior grass *Anthoxanthum odoratum*. Similarly, McKane et al. (2002) found that the most productive species used the most abundant nitrogen forms, and less productive species used less abundant forms. N is usually more abundant (higher input and mineralization) and easier to access in shallow than deep soil.
We therefore expected stronger competitors (dominant species) in mixture to take the major part of N from shallow soil, and weaker competitors (subordinate species) to increasingly switch to N uptake from deep soil in the presence and with the number of stronger competitors. As a consequence, niche overlap between species and interspecific competition would be reduced with species richness, resulting in increased complementarity effects and increased community N uptake, due to better exploitation of N from deep soil. However, our results from 2005, with higher DF of dominant than of subordinate species, suggest that root systems of subordinates remained relatively small and thereby even more restricted to shallow soil in mixtures than in monocultures. In 2006, however, this relationship disappeared, in line with frequently reversed dominance patterns. The observed decrease in niche overlap shows that species’ N uptake patterns indeed became less similar in mixture than in monoculture, albeit in 2005 not in the way expected: dominant species increased and subordinates decreased N uptake from deep soil. The pattern in 2006 was closer to our expectations, although there was no negative rank correlation between DF and the biomass of a species. The positive correlation of niche overlap with the complementarity effect (CE) in August and September 2005 was paralleled by negative correlations with the selection effect (but n.s. in September 2005). Thus, lower niche overlap between species was more frequent in mixtures dominated by species with higher-than-average yield in monoculture suppressing the subordinates, than in mixtures where all or most species benefited. Thus, low values of niche overlap reflect relatively low N uptake from deep soil by subordinate species and high uptake from deep soil by dominant species, and not vice versa (as expected). Nevertheless, total $^{15}$N uptake was increased in mixtures vs. monocultures, with a trend for increased relative uptake from deeper soil.

Increased total $^{15}$N uptake of mixtures is in line with Spehn et al. (2005), van Ruijven and Berendse (2005) as well as Roscher et al. (2008), reporting an increase in aboveground plant N with species richness. In all earlier studies, the increase in N was also due to increased biomass. Whereas in Spehn et al. (2005) and Roscher et al. (2008), the increase in aboveground biomass was to some degree driven by legume presence and their additional N input, van Ruijven and Berendse’s experiment was done without legumes, like the one presented here. They also found increased mixture biomass paralleled by a decrease in N concentration for most species, reported as increase in N use efficiency. In fact, it is
reasonable to assume that a decrease in plant N concentration is a consequence of increased growth with limited N supply. Increasing N use efficiency without increasing absolute N uptake by one species would result in an unfavorable advantage to its competitors. Both together, increased total N uptake and increased N use efficiency indicate a positive effect of species richness on community N use.

The increase in community $^{15}$N uptake of mixtures without negative a relationship between PS and CE suggests that mechanisms other than complementarity in depth of N uptake play a role in creating positive biodiversity effects. These may relate to resources other than N, or with respect to N, complementarity in timing of N uptake may play an important role, in line with McKane et al. (1990) and Fargione and Tilman (2005). In fact, time of harvest or interactions with “Harvest” were significant in all analyses, often with a different result for the third harvest in 2006 than for the first two harvests in 2005, suggesting that N partitioning in time could have been relevant in our study. Finally, instead of one or a few niche axes, multiple niche axes simultaneously may create high-dimensional niches (Harpole and Tilman 2007, Clark et al. 2007). Alternatively, species’ N uptake patterns in 2006 are more close to our expectations based on earlier studies (Berendse 1982, McKane et al. 2002, Fargione and Tilman 2005) than the patterns in 2005. It is likely that larger DF of dominant species in 2005 simply reflect faster growth of shoots and roots of these species while communities established, and that plants not yet occupied their N uptake niches. However, the positive effect of mixtures on $^{15}$N uptake did not become stronger in 2006.

In spite of lower niche overlap in mixtures than in monocultures, we found no clear divergence in the $^{15}$N uptake patterns of plant species, i.e. species specific, directed shifts towards higher or lower uptake from deep vs. shallow soil, suggesting that species responses in mixture were neighbor specific. For instance, L. vulgare had higher relative N uptake from deep soil (DF) than A. elatius in May 2006, and had continuously increased DF over time (Fig. 4.1). In contrast, L. vulgare had a lower DF than H. lanatus or P. lanceolata at all harvests (compare also R. acris when grown with D. glomerata vs. L. perenne and T. officinale). In another tracer study with NO$_3^-$, NH$_4^+$, and glycine, Miller et al. (2007) found a similar result: competition within interspecific neighbor pairs often caused reduced uptake of a particular chemical form of N, as well as shifts to uptake of an alternative form, whereby neighbor identity mattered. Generally, we observed
more pronounced differences between monocultures and mixtures compared to differences between mixtures of two and four species. This indicates that the presence or absence of interspecific competition had a larger effect on species’ N use patterns than species richness as such.

Conclusions

We conclude that interspecific competition changed the N uptake patterns of plant species in a neighbor specific way, but that species richness of mixtures did not affect the strength of this “phenotypic character displacement”. Together with the lack of a negative relationship between niche overlap and complementarity effects even in the second year of our experiment, this indicates that the observed decrease in niche overlap rather facilitated coexistence of multiple species than directly lead to better functioning of mixtures, as has often been suggested. It is important to note that varying patterns of N uptake by different plant species do not necessarily imply better functioning of mixtures with respect to N uptake. However, from increased community $^{15}$N uptake in mixtures, without a negative relationship between niche overlap and the complementarity effect, we conclude that mechanisms other than complementarity in depth of N uptake (e.g., complementarity in timing of N uptake or with respect to other resources) underly the widely observed phenomenon of positive biodiversity–productivity relationships.
Chapter 4
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Chapter references


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Table 4.1: **Experimental Design:** Combinations of species compositions and $^{15}$N treatments. Plant communities were grown in compartments (aggregated in boxes of 8), $^{15}$N treatments were applied to sets of four compartments per box (treatment unit). We show the detailed design for one harvest of species pool AHLP, including *Arrhenaterum elatius* (A), *Holcus lanatus* (H), *Leucanthemum vulgare* (L), and *Plantago lanceolata* (P). Totals are given for pool DLRT, including *Dactylis glomerata* (D), *Lolium perenne* (L), *Ranunculus acris* (R), and *Taraxacum officinale* (T) with identical design, and for both pools together. Since in this paper, only the $^{15}$N labeled compartments are considered (D and S), the total numbers including compartments with no $^{15}$N are given in brackets. Three destructive harvests were conducted, at each of which one out of four equally treated compartments was harvested (all harvested $^{15}$N labeled compartments: $n=96 \times 3=288$, as in Table 4.2).

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<th>Species richness</th>
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<td><strong>AHLP Composition</strong></td>
<td>A</td>
<td>H</td>
<td>L</td>
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<td>AH</td>
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<td>AP</td>
<td>HL</td>
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<td>LP</td>
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<td>(2)</td>
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<td>(4)</td>
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<td>4 (6)</td>
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<th>R</th>
<th>T</th>
<th>DL</th>
<th>DR</th>
<th>DT</th>
<th>LR</th>
<th>LT</th>
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<td>4 (6)</td>
<td>4 (6)</td>
<td>4 (6)</td>
<td>4 (6)</td>
<td>4 (6)</td>
<td>4 (6)</td>
<td>4 (6)</td>
<td>4 (6)</td>
<td>8 (12)</td>
<td><strong>48 (72)</strong></td>
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<td>32 (48)</td>
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<td></td>
<td>48 (72)</td>
</tr>
<tr>
<td></td>
<td>16 (24)</td>
</tr>
<tr>
<td></td>
<td><strong>96 (144)</strong></td>
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<td></td>
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<td>96 (144)</td>
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<tr>
<td></td>
<td>64 (96)</td>
</tr>
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<td><strong>192 (288)</strong></td>
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<td></td>
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<td>12 (12)</td>
</tr>
<tr>
<td></td>
<td><strong>72 (72)</strong></td>
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Nomenclature follows Lauber and Wagner (1998)
Table 4.2: Analysis of Deviance for the fraction of $^{15}$N tracer taken up from deep soil (DF) by populations of individual species ($n=288$). Significance levels refer to Quasi-$F$ tests derived from mean deviance ratios (, $P<0.1$, $* P<0.05$, $** P<0.01$, $*** P<0.001$).

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>Deviance (D)</th>
<th>% D</th>
<th>Mean D</th>
<th>Quasi-$F$</th>
<th>$P(&gt;Q.-F)$</th>
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<tbody>
<tr>
<td>Pool</td>
<td>1</td>
<td>28.0</td>
<td>1.6</td>
<td>28.0</td>
<td>9.7</td>
<td>**</td>
</tr>
<tr>
<td>Species richness (SR)</td>
<td>2</td>
<td>15.6</td>
<td>0.9</td>
<td>7.8</td>
<td>2.7</td>
<td>.</td>
</tr>
<tr>
<td>Composition (Com)$^a$</td>
<td>20</td>
<td>72.7</td>
<td>4.1</td>
<td>3.6</td>
<td>1.3</td>
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</tr>
<tr>
<td>Species$^b$</td>
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<td>75.5</td>
<td>26.1</td>
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<td>5.5</td>
<td>98.3</td>
<td>34.0</td>
<td>***</td>
</tr>
<tr>
<td>Within FG$^c$</td>
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<td>20.0</td>
<td>71.0</td>
<td>24.5</td>
<td>***</td>
</tr>
<tr>
<td>SR×Species</td>
<td>6</td>
<td>10.4</td>
<td>0.6</td>
<td>1.7</td>
<td>0.6</td>
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<tr>
<td>Com×Species</td>
<td>12</td>
<td>32.3</td>
<td>1.8</td>
<td>2.7</td>
<td>0.9</td>
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<tr>
<td>Harvest (H)$^d$</td>
<td>2</td>
<td>369.3</td>
<td>20.8</td>
<td>184.7</td>
<td>63.4</td>
<td>***</td>
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<tr>
<td>Pool×H</td>
<td>2</td>
<td>28.1</td>
<td>1.6</td>
<td>14.1</td>
<td>4.8</td>
<td>*</td>
</tr>
<tr>
<td>SR×H</td>
<td>4</td>
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<td>0.9</td>
<td>4.1</td>
<td>1.4</td>
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<tr>
<td>Com×H</td>
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<td>Species×H</td>
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<td>***</td>
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<tr>
<td>FG×H</td>
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<td>82.5</td>
<td>4.6</td>
<td>41.3</td>
<td>14.2</td>
<td>***</td>
</tr>
<tr>
<td>Within FG×H</td>
<td>10</td>
<td>69.7</td>
<td>3.9</td>
<td>7.0</td>
<td>2.4</td>
<td>*</td>
</tr>
<tr>
<td>SR×Species×H</td>
<td>12</td>
<td>25.6</td>
<td>1.4</td>
<td>2.1</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>com×Species×H</td>
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<td>67.3</td>
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<td>1.0</td>
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<tr>
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<td>138.7</td>
<td>7.8</td>
<td>2.9</td>
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<td></td>
</tr>
<tr>
<td>Residual</td>
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<td>15.7</td>
<td>2.9</td>
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<td>76.5</td>
<td>9.5</td>
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<td></td>
</tr>
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</table>

$^a$ Compositions as specified in Table 4.1.

$^b$ Species in mixtures, as fitted after Composition.

$^c$ Contrasts within Species (FG: grasses vs. forbs), fitted when one of them was significant.

$^d$ Harvest and interactions with Harvest were tested against the Residual mean deviance, all other terms against the Population residual mean deviance. Harvests: August 2005, September 2005, and May 2006.
Table 4.3: Analysis of Deviance for Proportional Similarity between pairs of species (n=288). Significance levels refer to Quasi-\(F\) tests derived from mean deviance ratios (\(P<0.1, \ast P<0.05, \ast\ast P<0.01, \ast\ast\ast P<0.001\)).

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>Deviance (D)</th>
<th>% D</th>
<th>Mean D</th>
<th>Quasi-(F)</th>
<th>(P(&gt;Q.-F))</th>
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<td>Pool</td>
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<td>0.8</td>
<td>18.6</td>
<td>2.7</td>
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<tr>
<td>Species richness (SR)</td>
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<td>292.69</td>
<td>13.1</td>
<td>146.3</td>
<td>21.6</td>
<td>**</td>
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<tr>
<td>Mono. vs. Mix.(^a)</td>
<td>1</td>
<td>292.68</td>
<td>13.1</td>
<td>292.7</td>
<td>43.2</td>
<td>**</td>
</tr>
<tr>
<td>Mix. SR(^a)</td>
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<td>0.01</td>
<td>0.0</td>
<td>0.01</td>
<td>0.0</td>
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</tr>
<tr>
<td>Composition (Com)(^b)</td>
<td>14</td>
<td>181.70</td>
<td>8.1</td>
<td>13.0</td>
<td>1.9</td>
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<tr>
<td>Species pair (SP)</td>
<td>10</td>
<td>138.34</td>
<td>6.2</td>
<td>13.8</td>
<td>2.0</td>
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</tr>
<tr>
<td>Harvest (H)(^c)</td>
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<td>24.65</td>
<td>1.1</td>
<td>12.3</td>
<td>2.6</td>
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<tr>
<td>Pool×H</td>
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<td>43.61</td>
<td>1.9</td>
<td>21.8</td>
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<td>*</td>
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<tr>
<td>SR×H</td>
<td>4</td>
<td>49.96</td>
<td>2.2</td>
<td>12.5</td>
<td>2.7</td>
<td>*</td>
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<tr>
<td>Mono vs. Mix.×H</td>
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<td>43.94</td>
<td>2.0</td>
<td>22.0</td>
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<tr>
<td>Mix. SR×H</td>
<td>2</td>
<td>6.02</td>
<td>0.3</td>
<td>3.0</td>
<td>0.6</td>
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</tr>
<tr>
<td>Com×H</td>
<td>28</td>
<td>233.79</td>
<td>10.4</td>
<td>8.3</td>
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<tr>
<td>SP×H</td>
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<td>160.42</td>
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<td>8.0</td>
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<tr>
<td>Specific pair residual(^d)</td>
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<td>Residual</td>
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<td>635.82</td>
<td>28.4</td>
<td>4.7</td>
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\(^a\) Contrasts within SR, fitted only when one of them was significant. Note that the linear effect of SR is significant too (7.3 \% Dev., \(P<0.001\)).

\(^b\) Compositions as specified in Table 4.1.

\(^c\) Harvest and interactions with Harvest were tested against the Residual mean deviance, whereas all other terms where tested against the Specific pair residual mean deviance. Harvests: August 2005, September 2005, and May 2006.

\(^d\) Specific pairs are the pairs of species (either two monocultures or two species within a mixture) between which Proportional Similarity was calculated (\(n=96\)) at each of three harvests (Total \(n=3\times96=288\)).
Figure 4.1: Fraction of $^{15}$N uptake from deep soil (DF) and shoot biomass per species in all 2-species mixtures of pool AHLP (left two columns of panels) and pool DLRT (right two columns of panels). Error bars show standard errors of the mean, $n=2$ for each bar.
Figure 4.2: Fraction of $^{15}$N uptake from deep soil (DF) and shoot biomass per species in all Monocultures (upper panels) and 4-species mixtures (lower panels) of pool AHLP (left two columns of panels) and pool DLRT (right two columns of panels). Error bars show standard errors of the mean, monocultures: $n=2$, 4-species mixtures: $n=4$ for each bar.
Figure 4.3: Proportional Similarity between pairs of species depending on species richness at all three harvests. Values for individual pairs of species are shown (circles and triangles), and means per species pool and species richness level (horizontal lines). Note that at species richness 1, species pairs were pairs of different monocultures, whereas for species richness 2 and 4, species pairs were pairs within mixtures.
Figure 4.4: Correlation of Proportional Similarity between species (averaged across pairs in 4-species mixtures) with the Complementarity effect (CE) and the Selection effect (SE) of mixtures at all three harvests. Open circles: species pool AHLP, filled circles: species pool DLRT. Pearson’s correlation coefficients $r$ and $P$-values are given, respectively ($n=32$ at each harvest).
Figure 4.5: Plant community biomass (A), N concentration (B), N content (C), $^{15}$N tracer concentration (D), $^{15}$N tracer content (E), and community deep fraction DF (F) by harvest and species richness. All panels refer to total community biomass, i.e. shoots+roots (per compartment). Error bars show standard errors of the mean SE, $n=32, 48, \text{ and } 16$ for bars of monocultures, 2-species mixtures, and 4-species mixtures, respectively (exception: DF with $n=16, 24, \text{ and } 8$.)
Appendix

Figure 4.6: $\delta^{15}$N of roots in layers of 10 cm thickness at 0–10, 10–20, 20–30, and 30–40 cm soil depth with deep $^{15}$N treatment (solid lines) and shallow $^{15}$N treatment (dashed lines), in monocultures of *L. vulgare*, *T. officinale*, *A. elatius*, and *D. glomerata*. 
Figure 4.7: Additive partitioning of yield effects in mixtures of species pool AHLP (left) and DLRT (right) by harvest and species richness. The net effect (NE) is the sum of the complementarity effect (CE), and the selection effect (SE). Error bars are means ± SE, significance levels indicate the difference from zero for harvest means across species richness levels (, $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$). NE was not significantly different from zero for pool AHLP overall (all harvests), but positive for pool DLRT ($P<0.001$).
Table 4.4: Analysis of Variance for shoot $^{15}\text{N}$ enrichment $[^{15}\text{N}_{\text{ex}}]$ in August 2005 ($n=192$, $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$).

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Error</th>
<th>% SS$^a$</th>
<th>$F$</th>
<th>$P(&gt;F)$</th>
</tr>
</thead>
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<td>B</td>
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<td>10.93</td>
<td>**</td>
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<tr>
<td>Species richness (SR)</td>
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<td>0.23</td>
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</tr>
<tr>
<td>Pool×SR</td>
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<td>B</td>
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<td>2.73</td>
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<tr>
<td>$^{15}\text{N}$ Treatment (T)</td>
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<td>TU</td>
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<tr>
<td>Pool×T</td>
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<td>0.9</td>
<td>19.83</td>
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<td>SR×T</td>
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<td>TU</td>
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</tr>
<tr>
<td>Pool×SR×T</td>
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</tr>
<tr>
<td>Species</td>
<td>6</td>
<td>R</td>
<td>0.7</td>
<td>2.77</td>
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<tr>
<td>Functional group (FG)$^b$</td>
<td>1</td>
<td>R</td>
<td>0.4</td>
<td>8.87</td>
<td>**</td>
</tr>
<tr>
<td>Within FG$^b$</td>
<td>5</td>
<td>R</td>
<td>0.3</td>
<td>1.54</td>
<td></td>
</tr>
<tr>
<td>SR×Species</td>
<td>12</td>
<td>R</td>
<td>0.9</td>
<td>1.72</td>
<td></td>
</tr>
<tr>
<td>SR×FG</td>
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<td>R</td>
<td>0.0</td>
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<tr>
<td>SR×Within FG</td>
<td>10</td>
<td>R</td>
<td>0.8</td>
<td>1.98</td>
<td>*</td>
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<tr>
<td>T×Species</td>
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<td>R</td>
<td>7.6</td>
<td>30.64</td>
<td>***</td>
</tr>
<tr>
<td>T×FG</td>
<td>1</td>
<td>R</td>
<td>2.6</td>
<td>63.07</td>
<td>***</td>
</tr>
<tr>
<td>T×Within FG</td>
<td>5</td>
<td>R</td>
<td>5.0</td>
<td>24.16</td>
<td>***</td>
</tr>
<tr>
<td>SR×T×Species</td>
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<td>R</td>
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<td>1.41</td>
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<tr>
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<tr>
<td>Treatment unit (TU)$^c$</td>
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<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual (R)</td>
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<td>3.0</td>
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<tr>
<td>MODEL</td>
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<td>89.3</td>
<td></td>
<td></td>
</tr>
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$^a$ % sums of squares (SS) indicate increases in multiple $R^2$ (explained variance) due to the addition of this term to the model. Note that the full model explains 89.3% of the total variance.

$^b$ Contrasts within Species (FG: grasses vs. forbs), fitted when one of them was significant.

$^c$ A set of four compartments with the same $^{15}\text{N}$ treatment (Table 4.1).
Chapter 5

Effects of plant species richness on partitioning of N between plants and microbes

Abstract

We studied the effects of manipulated levels of plant species diversity on N partitioning between plants and microbes. Many experiments have shown a general increase in plant biomass production with species richness in temperate grasslands. Also, plant species richness was shown to affect N cycling, e.g., to decrease soil mineral N concentrations and nitrate leaching, and to increase N stocks in plant biomass, suggesting better N exploitation in plant mixtures. Although many plant–soil interactions including competition for soil N are known, it has not been tested so far whether plant diversity can change partitioning of N between plants and microbes. We measured plant and microbial N in communities of one, two, and four plant species (forbs and grasses) to test whether plant species richness increases the plant share of N. In addition, we mixed a $^{15}$N labeled organic substrate to the soil and tracked its allocation into plants and microbes to test whether plant–microbe partitioning of N changes over time. By adding the label either to the shallow or the deep soil layer, we could also test for differences due to soil depth. The species richness of plant communities did neither affect the partitioning of N and $^{15}$N between plants and microbes, nor microbial N. There was only a trend for increased plant N in mixtures, although plant biomass was significantly increased in mixtures compared to monocultures, due to increased biomass:N. However, partitioning of N and microbial N depended on the plant species composition. Over time, the plant share of N remained constant, whereas the share of $^{15}$N decreased. Moreover, plants competed better for $^{15}$N with microbes in the shallow soil layer at the beginning of the experiment, probably due to low root density in deep soil. We conclude that plant–microbe partitioning of N is resistant to changes in plant species richness, although we cannot exclude that changes in constituent N pools or fluxes occurred but compensated one another. Further, increased plant biomass:N suggests that increased biomass production in species rich plant communities may only partly be explained by better N exploitation.
Introduction

One aspect of anthropogenic global change is the dramatic loss of biological species. To understand the consequences of biodiversity loss is fundamental, since biodiversity provides humans with many “ecosystem services” such as food production, climate regulation, maintenance of soil fertility, or watershed protection (Daily 1997). Intensive research was carried out on plants in temperate grasslands, where a positive relationship between experimentally manipulated levels of plant species richness and productivity was found as a general pattern (Hector et al. 1999, Tilman et al. 2001, van Ruijven and Berendse 2003, Roscher et al. 2005). More efficient plant resource use in mixtures due to complementarity of species, in particular with regard to nitrogen (N) uptake, has often been suggested as an underlying mechanism (Tilman et al. 1997b, Loreau 1998, Loreau et al. 2001).

However, very little attention so far was paid to the plant–soil interface. Until recently, the common view of the terrestrial N cycle was based on the assumption that plants compete poorly with soil microbes, using N that microbes “left over”. But recent research has challenged this classical paradigm: although microbes decompose organic matter, thus, providing many essential plant resources (e.g., N) plants directly compete with microbes for N (Hodge et al. 2000a, Schimel and Bennett 2004). Hence, if plant species richness enhances the productivity of plant communities, this might alter plant–microbe competition and therefore N partitioning between plants and microbes. This could have fundamental consequences, since plants are mostly N limited (Vitousek and Howarth 1991).

Plants and soil microbes compete for soil NO$_3^-$, NH$_4^+$, and probably even for soluble organic N (Fig. 5.1). However, significant plant uptake of organic N is thought to be restricted to extremely N limited conditions found in arctic or alpine regions (Schimel and Bennett 2004). The balance between mineralization and microbial immobilization of N depends on the C:N ratio of the substrate being decomposed and the decomposer’s need for N relative to C (Harte and Kinzig 1993, Hodge et al. 2000a). High substrate C:N ratios tend to favour immobilization of N, whereas low substrate C:N ratios result in net mineralization of N by soil microbes, reducing and augmenting N available for plants, respectively. Because of their major role in the mineralization process, it was assumed that microbes are the superior competitors for soil N (Rosswall 1982). Also, microbes were shown to take up substantially more NH$_4^+$ and NO$_3^-$ than plants in the short-term,
i.e., 24h (Jackson et al. 1989). However, it was suggested that plants effectively compete for N if its distribution in the soil is spatially heterogeneous (patches with low C:N ratio), and in the long run, because of the slower turnover of plant roots than of microbes (Hodge et al. 2000a andb).

Biodiversity experiments have shown several effects of plant species diversity on N cycling. Plant functional group richness or plant species richness was often found to decrease concentrations of soil NO$_3^-$ or soil mineral N (Tilman et al. 1997a, Hooper and Vitousek 1998, Niklaus et al. 2001b, Spehn et al. 2005, Oelmann et al. 2007) and leaching of NO$_3^-$ (Scherer-Lorenzen et al. 2003). Niklaus et al. (2006) observed decreased N$_2$O emissions, mainly driven by effects of plant species richness on soil nitrate and nitrification. Plant species richness was shown to increase N pools in plant mixture biomass (Spehn et al. 2005, van Ruijven and Berendse 2005, Oelmann et al. 2007), but also to increase microbial biomass N (Hooper and Vitousek 1998, Spehn et al. 2000, Zak et al. 2003) and gross N mineralization (Zak et al. 2003). Moreover, the total N use of plants and microbes, based on resource reductions relative to bare ground treatments was enhanced with functional group richness (Hooper and Vitousek 1998). In summary, these results support the idea that species richness enhances N exploitation of a plant community.

Research on elevated CO$_2$ has revealed much on the mechanisms by which plant communities (here as modulated by elevated CO$_2$) can affect soil microbes, such as the quantity of resources entering the soil through the effect on plant productivity, the quality of litter, and changes in the functional composition of plant communities (see e.g., Wardle 2002). As direct effects of CO$_2$ enrichment on soil microbes probably are of little consequence, because of the high levels of CO$_2$ already present in soils (Vanveen et al. 1991), these mechanisms could work in a similar way if plant communities are modulated by plant species richness. The response of gross N mineralization under elevated CO$_2$ is variable, but most often positive as also observed with increased species richness (Zak et al. 2003), potentially increasing mineral N availability to plants (Zak et al. 2000). However, gross mineralization is difficult to interpret without taking into account microbial immobilization. In fact, it was shown that increased release of substrate into the rhizosphere under elevated CO$_2$ can enhance mineral nutrient sequestration by the expanded microbial community and cause a negative feedback on plant growth, even under fertile conditions (Diaz et al. 1993, de Graaff et al. 2006).
More complex species-specific interactions between plants and soil microbes might also be important in this context. Plant species identity can alter the community composition of belowground primary consumers (Wardle et al. 2003). Conversely, species richness of arbuscular mycorrhizal fungi can enhance biodiversity, productivity, and nutrient capture of calcareous grassland plant communities (van der Heijden et al. 1998). Such species-specific associations between plants and microbes suggest that microbes can mediate complementary resource use by plants (Reynolds et al. 2003).

In consequence, diversity effects on plant performance might well alter N partitioning between plants and microbes. In this study, we test how plant species richness affects partitioning of N between plants and microbes by measuring plant and microbial N pools at three consecutive, destructive harvests in experimental plant communities of one, two, and four species. We expect that three main mechanisms could affect plant–microbe partitioning of N. First, plant species richness could increase N exploitation by plants through complementary N uptake by different species, resulting in a larger plant share of N. Second, increased plant biomass can increase carbon inputs to the soil and thus enhance microbial activity and N mineralization. This would lead to an increase in plant available N, probably resulting in a constant plant share of N. Third, increased carbon inputs could lead to a negative feedback on plant N uptake by enhanced microbial immobilization of N, resulting in increased microbial and decreased plant N. In addition to analysing plant and microbial N pools, we used a standard $^{15}$N labeled organic substrate (plant material) mixed to either the shallow or the deep layer of the soil assess plant–microbe competition in different depths of the soil. Further, we measured plant and microbial $^{15}$N across three harvests to test whether the partitioning of N from that substrate changes over time. We expected plant species richness to increase the plant share of $^{15}$N due to complementary N uptake of plant species (Hypothesis 1), and to increase total biotic $^{15}$N recovery (Hypothesis 2). Further, we expected microbes to be better competitors in the short-term but plants to win in the long-term (Hypothesis 3).
Materials and Methods

Experimental Design

Our experiment was set up in an experimental garden near Zurich, Switzerland. Plant communities of one, two, or four common temperate grassland species were grown on natural field soil, in boxes (40 × 60 cm wide and 40 cm deep). We used two pools of four plant species to avoid results restricted to a particular species pool (Table 5.1). For each pool, we grew all four monocultures, all six pairwise mixtures, and the full 4-species mixture. Three replicate boxes of each monoculture and pairwise mixture were set up, and six replicates for each 4-species mixture. Boxes were subdivided into eight compartments.

In May 2005, seedlings were grown in the garden for five weeks after germination in the greenhouse. Between June 7-10, 12 seedlings were planted per compartment in three alternate rows of four (6912 plants in total). An even number of individuals per species was represented in each row, i.e. two or one per species in 2-and 4-species mixtures, with positions within rows randomized. Plant communities were weeded regularly and were watered daily with an automated irrigation system except on rainy days.

Before planting of seedlings, three organic $^{15}$N labeling treatments — shallow, deep, and no $^{15}$N — were applied to sets of four compartments (half a box), yielding a shallow/no $^{15}$N, a deep/no $^{15}$N, and a shallow/deep box for each species composition. Note that in this paper, we will focus on treatments “shallow” and “deep” only (compared to background $^{15}$N). All boxes were placed on a flat area of 7 m × 6.25 m. A detailed description of the experimental design is given in Table 5.1.

$^{15}$N labeling of the soil

The organic $^{15}$N label and non-labeled material were produced in the greenhouse. *Festuca rubra* was grown on quartz sand supplied with nutrient solution either containing K$^{15}$NO$_3$ (15 at% $^{15}$N) or isotopically unenriched KNO$_3$. Shoots were harvested after 11 weeks, dried, and shredded into pieces of app. 2 cm.

The inside of the boxes was vertically subdivided into eight compartments. Four drainage holes (10 mm wide) were drilled into the bottom of each compartment. Compartments were fitted with a drainage mat (Enkadrain). Then, compartments were filled with natural, sieved field soil to include active microbial populations (soil pH=7.6). The


$^{15}$N treatments were applied by mixing $^{15}$N-labeled $F. ~rubra$ material to the lower (deep) or the upper soil layer (shallow) or to neither layer. The same amount of non-labeled $F. ~rubra$ material was mixed to the respective other layer of the soil. The amount of $^{15}$N tracer added per labeled compartment was 2.5 mg $^{15}$N in 1.45 g plant material. The lower soil layer of each compartment was added first (approx. 20 cm high). The soil was compressed to minimize subsequent settlement before the top layer was added and compressed too. To limit soil warming by direct radiation, boxes were insulated by 2 cm thick styrofoam walls.

**Soil sampling and sample preparation**

Between August 8 and 21, 2005, two compartments per box (one from each $^{15}$N-treatment) were destructively harvested below and above ground (two replicates for each combination of species composition and $^{15}$N). Between September 19 and October 7, 2005, and between May 15 and 29, 2006, a subset of compartments was harvested to test for changes over time (Table 5.1).

Three horizontal cores (27 mm diameter, 13 cm long) were taken from the shallow and deep soil layer of each harvested compartment. Cores were pooled for each layer and kept at $4{^\circ}C$ until the end of a harvest.

Microbial biomass C, N, and $^{15}$N were extracted using the chloroform-fumigation-extraction method (Vance et al. 1987). To determine microbial biomass C and N, 15 g fresh soil sieved to 2 mm were extracted with 50 ml 0.5 M K$_2$SO$_4$ for 30 min. To determine $^{15}$N, 0.03 M K$_2$SO$_4$ was used in order to have higher N to K$_2$SO$_4$ ratios to allow subsequent measurement with our EA-IRMS system (see below). One subsample was extracted directly, one was extracted after chloroform fumigation for 24 hours. Hence, from each soil sample, four subsamples were extracted. Extracts were filtered and kept frozen at $-18{^\circ}C$ until analysis.

Total organic C and N were measured in 0.5 M K$_2$SO$_4$ extracts with an automated TON/TOC-analyser (DIMA TOC-100, Dimatec, Essen, Germany). Microbial biomass N ($N_{mic}$) was calculated as

$$N_{mic} \, (\mu g \cdot g^{-1} \, soil) = \frac{N_f - N_{nf}}{k_{EN}}$$

where $k_{EN}=0.54$ is the extraction efficiency for microbial N (after Brookes et al. 1985).
$N_f$ and $N_{nf}$ are the organic N contents ($\mu g \cdot g^{-1} soil$) of the extracts of fumigated and non-fumigated samples, respectively. Likewise, microbial biomass C ($C_{mic}$) was calculated using $k_{EC}=0.45$.

$\delta^{15}N$ of freeze-dried 0.03 M K$_2$SO$_4$ extracts was analysed with an isotope ratio mass spectrometer (IRMS, Delta$^+$ XP, Finnigan MAT, Germany) coupled to an elemental analyzer (EuroEA 3000 Serie, HEKAtech, Germany). Microbial biomass $\delta^{15}N$ was calculated using an isotope mixing model:

$$\delta^{15}N_{mic} (\permil) = \frac{\delta^{15}N_f \cdot N_f - \delta^{15}N_{nf} \cdot N_{nf}}{N_f - N_{nf}}$$

where $\delta^{15}N_f$ and $\delta^{15}N_{nf}$ are the $\delta^{15}N$ of the extracts of fumigated and non-fumigated subsamples, respectively. Due to the non-linear $\delta^{15}N$ values, this model is an approximation. However, comparison with a more complicated but exact method (Niklaus et al. 2001a) yielded equivalent results, due to moderate $^{15}N$ enrichment of samples in this study (12.0 to 138.2 $\permil$).

**Plant sampling and sample preparation**

Shoots and roots were collected and pooled from all species per harvested compartment (total plant biomass). Roots were washed using 2 mm sieves. Plant material was dried for 48 h at 80°C and ground with a ball mill. Plant $\delta^{15}N$ was analyzed with an isotope ratio mass spectrometer (IRMS, Delta$^+$XP, Finnigan MAT, Germany) coupled to an elemental analyzer (Flash EA 1112 NC, CE Instruments, Italy). $^{15}N$ of unlabeled plants and of soil microbes was determined from a separate box containing four full mixtures of both species pools, sampled concomitantly with the regular harvests.

**Data analysis**

For the analysis of plant community biomass, N, biomass:N, and $\delta^{15}N$ in August 2005 (first harvest, $n=96$ compartments), we used linear mixed model ANOVA (see Table 5.2). We fitted (1) species pool, (2) species richness, (3) species pool×species richness, and (4) species composition in this order. Species richness (three levels) was split into a contrast for monocultures vs. mixtures which always explained most of the variation due to species richness, and a contrast for 2-species vs. 4-species mixtures. For $\delta^{15}N$ we additionally fitted (5) $^{15}N$ treatment (shallow or deep) and its interactions with terms 1–3. Factors
1–4, which varied at the level of boxes were tested against the box residuals, $^{15}\text{N}$ treatment (and its interactions), which varied at the treatment unit level (four compartments per box receiving the same $^{15}\text{N}$ treatment) were tested against the residuals. Similarly, we analysed soil microbial C, N, C:N, and $\delta^{15}\text{N}$ in August 2005 (see Table 5.3), but microbes were measured from both soil layers separately ($n=96$ compartments$\times2$ soil layers $=192$).

In addition to terms 1–4, we fitted (5) $^{15}\text{N}$ label (layer with $^{15}\text{N}$ label or without) and for the analysis of $\delta^{15}\text{N}$ we also fitted (6) soil layer (deep or shallow) and interactions between soil layer and terms 1–3 (and 5 for $\delta^{15}\text{N}$). Here, $^{15}\text{N}$ label was tested against the treatment unit residuals, soil layer and its interactions against the residuals.

$^{15}\text{N}$ tracer content $^{15}\text{N}_{\text{ex}}$ was calculated for each plant species in the community as $g$ excess $^{15}\text{N}$ (excess at% $^{15}\text{N}$-$\%$ species biomass [g]) and added from all species. For microbes, $^{15}\text{N}_{\text{ex}}$ was calculated for each soil layer (excess at% $^{15}\text{N}$-$\%$ mic [g$\cdot$g$^{-1}$ soil]$\cdot$ weight of soil layer [g]), and added from both soil layers. Total biotic N and $^{15}\text{N}$ was calculated by adding plant and microbial N and $^{15}\text{N}_{\text{ex}}$, and was analysed with the same model as used for plant biomass. Further, we calculated the fraction of total biotic N and $^{15}\text{N}_{\text{ex}}$ found in plant biomass ($N_{\text{Plant}}$ and $^{15}\text{N}_{\text{Plant}}$) for each community. For the analysis of $N_{\text{Plant}}$ and $^{15}\text{N}_{\text{ex}}$ in August 2005, which are fractions between 0 and 1, we used a generalized linear model (binomial error distribution and logit link function) and analysis of deviance (ANDEV, Table 5.4). Factors fitted and error terms were the same as for plant biomass (see above). Due to the hierarchical experimental design, we used Quasi-$F$-tests based on mean deviance ratios, just as $F$-tests in ordinary analysis of variance (Egli and Schmid 2001).

To additionally test for changes over time, a subset of compartments from one pool was used for each harvest (pool AHLP, see Table 5.1, $n=12$ compartments$\times3$ harvests($\times2$ soil layers)$=36(72)$). For plant biomass, N, and biomass:N, we fitted (1) species richness, (2) species composition, (3) harvest, and interactions of terms 1 and 2 with harvest, using ANOVA. Hereby, harvest was split into a linear contrast and the remainder. Additional terms and their interactions with harvest were fitted for plant $\delta^{15}\text{N}$ ($^{15}\text{N}$ treatment), for microbial N and $\delta^{15}\text{N}$ (soil layer), and for microbial $\delta^{15}\text{N}$ ($^{15}\text{N}$ label). For $N_{\text{Plant}}$ and $^{15}\text{N}_{\text{Plant}}$, we used again a generalized linear model and ANDEV, but the same factors as for plant N and $\delta^{15}\text{N}$, respectively (including $^{15}\text{N}$ treatment for $^{15}\text{N}_{\text{Plant}}$).

Further, we tested Pearson correlations between plant and microbial N, $^{15}\text{N}$, and be-
tween the plant biomass:N and microbial C:N.

Note that mean numbers given in the text are always mean±SE.

Results

Nitrogen in August 2005

In August 2005, there was no linear change of plant N with species richness, but a trend for higher plant N in mixtures than in monocultures (Table 5.2). Plant community biomass responded more strongly with a trend for a linear increase with species richness, and significantly higher biomass in mixtures than in monocultures (Table 5.2). In line with plant N and biomass, biomass:N decreased with species richness, mainly due to higher biomass:N in mixtures than in monocultures (Table 5.2). Plant N and community biomass were larger in pool AHLP than in pool DLRT. Plant species composition within levels of species richness, did not significantly change plant N or community biomass, although composition explained a large amount of variation. However, composition had a highly significant effect on plant biomass:N.

In August 2005, there was no effect of plant species richness on soil microbial N, C, and C:N. Species composition had a marginally significant effect on microbial N. Microbial N was significantly higher in the shallower than in the deep soil layer (deep: 85.7±1.4, shallow: 96.3±1.5µg·g⁻¹ soil), as was microbial C (Table 5.3). Microbial C:N remained constant across all treatment combinations.

On average, the plant fraction of biotic N (N_{Plant}) accounted for 23.5 % (±<0.1 %) in August 2005 (see also 5.5). N_{Plant} was unaffected by species richness (Table 5.4, Fig. 5.3, left panels), but differed significantly among communities. Total biotic N was unaffected by species richness too (Table 5.5).

There was no correlation between microbial N and plant N (Fig. 5.4, panel A). Instead, we found a positive correlation between microbial C:N and plant biomass:N (Fig. 5.4, panel C).
Nitrogen across harvests

Plant N linearly increased across harvests (harvest linear: $P_{1,14}<0.05$, 7.3% SS, Fig. 5.4, panel B), whereas plant community biomass and biomass:N were largest at the second harvest in September 2005 (harvest: $P_{2,14}<0.05$, 14.6% SS for biomass, $P_{2,14}<0.05$, 16.6% SS for biomass:N, Fig. 5.4, panel D).

Microbial N increased across harvests (August 05: 90.6±3.8, September 05: 99.1±2.5, May 06: 114.5±2.0 µg·g$^{-1}$ soil, harvest linear: $P_{1,47}<0.001$, 33.0%, Fig. 5.4, panel B). Microbial N remained larger in the shallow than in the deep soil layer. A highly significant species composition×harvest interaction ($P<0.001_{8,47}$, 23.3% SS, 11.4% by linear contrast), indicated that changes in microbial N across harvests depended on the plant species composition. Like plant biomass:N, microbial C:N was highest in September 2005 ($P_{1,47}<0.001$, 16.0% SS, Fig. 5.4, panel D). Moreover, there was an interaction between species richness and harvest ($P_{2,47}<0.01$, 6.4% SS), because microbial C:N was higher in the full mixture of pool AHLP than in the monocultures in August 2005 and May 2006, but not in September 2005.

The fraction of N in plants ($N_{Plant}$) was not affected by plant species richness. There was no general decrease or increase of $N_{Plant}$ across harvests, but similar to microbial N, there was a trend for changes in $N_{Plant}$ that depended on the plant species composition (composition×harvest: $P<0.1_{8,14}$, 33.7% SS, 19.2% by linear contrast).

$^{15}$N in August 2005

Mean plant community $\delta^{15}$N was 103.4±2.8‰ compared to unlabeled plant communities with a $\delta^{15}$N of 5.2±0.3‰. Plant community $\delta^{15}$N was neither affected by species richness, nor by species composition. However, plant community $\delta^{15}$N was strongly affected by the $^{15}$N treatment (Table 5.2), with higher $\delta^{15}$N of communities growing on shallow than on deep labeled soil (means: 136.6±1.9‰ and 80.1±2.0‰, respectively).

Application of the organic $^{15}$N label led to significant, spatially confined $^{15}$N enrichment of microbial N in labeled layers in August 2005 (mean: 98.9±1.9‰, Table 5.3), whereas microbial N in unlabeled layers was only slightly enriched (mean: 17.2±0.3‰) compared to background $\delta^{15}$N of unlabeled microbes (mean: 14.4±0.6‰). There was no effect of plant species richness on soil microbial $\delta^{15}$N. $^{15}$N enrichment of microbes was stronger in labeled shallow than labeled deep soil layers (Fig. 5.2). In contrast, microbes in respective
unlabeled layers were slightly more enriched in deep than shallow layers ($^{15}$N label×soil layer interaction, Table 5.3), indicating some leaching of $^{15}$N down the soil column.

In August 2005, the recovery of $^{15}$N in biotic plants and microbes (biotic $^{15}$N) ranged from 4.4% to 16.8% (mean: 9.7±0.3%). Total $^{15}$N was unaffected by species richness (Table 5.5). The plant fraction ($^{15}$N$_{Plant}$) accounted for 40.2% (±<0.1%), and was as well unaffected by species richness (Table 5.4). Plants competed better for $^{15}$N in the shallow soil layer ($^{15}$N treatment, Table 5.4, Fig. 5.3). $^{15}$N$_{Plant}$ is larger than N$_{Plant}$, probably because the $^{15}$N label almost exclusively remained in the labeled soil layer (and microbes therein). The relationship between microbial $^{15}$N$_{ex}$ and plant $^{15}$N$_{ex}$ was significantly positive.

$^{15}$N across harvests

Plant δ$^{15}$N decreased across harvests (linear effect of harvest: $P_{1.11}<0.001$, 44.6% SS, Fig. 5.4, panel F), and was generally higher in communities growing on shallow than deep labeled soil ($P_{1.11}<0.001$, 29.8% SS). Moreover, there was a significant treatment×harvest interaction, indicating that δ$^{15}$N decreased more strongly for communities growing on shallow labeled soil ($P_{1.11}<0.001$, 10.4% SS).

The strength and spatial distribution of microbial $^{15}$N enrichment was constant across harvests (Fig. 5.4, panel F). The difference between labeled and unlabeled layers remained highly significant, indicating that our $^{15}$N treatments remained spatially confined ($^{15}$N Label $P_{1.42}<0.001$, %SS=94.1, Fig. 5.2).

The fraction of plant $^{15}$N ($^{15}$N$_{Plant}$) decreased across harvests (harvest: $p_{2.10}<0.01$, 25.0% deviance, linear contrast: $P_{1.10}<0.001$, 24.0% deviance, Fig. 5.3). Plants still competed better for $^{15}$N in the shallow soil layer ($P_{1.10}<0.01$, 14.8% deviance). As for plant δ$^{15}$N, there was a significant treatment×harvest interaction, indicating that $^{15}$N$_{Plant}$ decreased more strongly for communities growing on shallow labeled soil ($P_{1.10}<0.05$, 5.8% deviance).
Discussion

Plant–microbe partitioning of N

Our results clearly do not support our hypothesis (1) that plant species richness increases the competitive ability of plant communities vs. soil microbes for N, due to complementary N uptake by different plant species. Plant species richness neither affected the partitioning of N between plants and microbes, nor plant or microbial N individually, although there was a trend for increased plant N and significantly increased plant biomass in mixtures compared to monocultures. Hence, a small positive effect on plant N uptake cannot be excluded. However, the effect of species richness on plant biomass was relatively weak here compared to other experiments, and it is possible that results would look different with a stronger increase in plant biomass. Thus, the other two mechanisms considered, which would both operate via plant community biomass, i.e. (2) increased plant and microbial N through increased microbial activity or (3) increased immobilization of N by microbes, could also not be supported across levels of plant species richness.

Interestingly, plant species richness significantly increased plant biomass:N. This is in line with the study of van Ruijven and Berendse (2005), which like ours did not include legumes. Even in the presence of legumes, a negative effect of species richness on N concentration was found (Temperton et al. 2007), although the presence of legumes in mixtures can drive increased N content (Spehn et al. 2005). Increased plant biomass paralleled by increased biomass:N indicates that more plant growth does not necessarily involve higher N uptake by plants. Rather, N might be used more efficiently by plants when in competition with interspecific neighbors, or could indicate that growth is N limited. In contrast to plant biomass:N, microbial C:N was very stable across all treatment combinations.

The plant share of N was significantly affected by the plant species composition, as was plant biomass:N and as a trend also microbial N. The greater importance of plant species composition rather than plant species richness is consistent with many other studies (Hooper and Vitousek 1998, Scherer-Lorenzen et al. 2003, Spehn et al. 2005, Oelmann et al. 2007), and suggests that the presence of certain species affects partitioning of N more strongly than the number of species.

For August 2005, we found no significant correlation between plant N and microbial
N on the level of species compositions (as shown in Fig. 5.4, panel A). Thus, our results do neither support a simultaneous improvement, nor a negative interdependency of plant N and microbial N. However, we cannot exclude some negative feedback of plant biomass on plant N via increased microbial immobilization, as this might be suggested by the inverse relationship between plant biomass and biomass:N. However, the positive relationship between microbial C:N and plant biomass:N suggests that microbes cannot fully compensate higher C inputs by increased N immobilization.

Interestingly, a positive correlation between microbial and plant N was found in September 2005, but no longer in May 2006. This could indicate that feedbacks between plants and microbes change over time.

**Plant–microbe partitioning of $^{15}$N**

The plant share of $^{15}$N ($^{15}$N$_{Plant}$) was unaffected by species richness like the plant share of N. Also, our hypothesis (2) that species richness would increase total biotic $^{15}$N recovery could not be supported. Since across harvests $^{15}$N$_{Plant}$ even decreased, our hypothesis (3) that microbes would be better competitors in the short-term but that plants would win in the long-term (Hodge et al. 2000a andb), could not be confirmed as well. Indeed, plant and microbial N both increased, but plant $\delta^{15}$N decreased whereas microbial $\delta^{15}$N was constant. One caveat when testing this hypothesis may be that shoots in the remaining compartments were cut and discarded in autumn 2005 after the September harvest. Shoot removal might have negatively affected N$_{Plant}$, because plants could not draw back the shoot $^{15}$N before shoot senescence. However, also no increase in $^{15}$N$_{Plant}$ was indicated between August and September 2005 (before the cut).

In August 2005, plants competed better for $^{15}$N in the shallow soil layer, although there was also more microbial biomass than in the deep layer. Since this difference lessened across harvests and virtually disappeared by 2006 (see Fig. 5.3), it may be explained by the root growth of plants: roots more fully explored the whole soil profile, i.e. the deep layer in 2006 than in 2005 (see also Chapter 4, Fig. 4.5, panel F), and thus were relatively more abundant compared to microbes in the shallow layer.

Other studies that reported significant effects of plant species richness on the N cycle, mostly investigated particular pools or fluxes (e.g., Spehn et al. 2000 and 2005, Niklaus et al. 2001b, Zak et al. 2003). In contrast, our study integrates the overall outcome of
competition for N between plant and microbes. However, finding no effect of plant species richness on plant–microbe partitioning of N, does not mutually exclude changes in single, constituent parts of the N cycle, as these might compensate each other.

Conclusions

We conclude that competition for N between plants and microbes is resistant to changes in plant species richness, although we cannot exclude that changes in constituent N pools or fluxes occurred but compensated one another. Further, increased plant biomass:N suggests that increased biomass production in species rich plant communities may only partly be explained by better N exploitation.
Chapter references


Table 5.1: Experimental Design: Combinations of plant species compositions and 15N treatments. Plant communities were grown in compartments (aggregated in boxes of 8), 15N treatments were applied to sets of four compartments per box (treatment unit). The species pool AHLP included *Arrhenaterum elatius* (A), *Holcus lanatus* (H), *Leucanthemum vulgare* (L), and *Plantago lanceolata* (P), DLR T included *Dactylis glomerata* (D), *Lolium perenne* (L), *Ranunculus acris* (R), and *Taraxacum officinale* (T). At each harvest, one compartment per treatment unit was destructively harvested. Microbes were separately analysed for the shallow and the deep soil layers, for the full design in August 2005, but only for a subset of compartments from September 2005 and May 2006, including monocultures and 4-species mixtures of pool AHLP.

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</tr>
</tbody>
</table>
Table 5.2: ANOVA for plant community biomass (g), N (g), biomass:N and δ¹⁵N (‰) in August 2005 (P<0.1, * P<0.05, ** P<0.01, *** P<0.001). n=96 compartments.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Error</th>
<th>% SS</th>
<th>P(F)</th>
<th>% SS</th>
<th>P(F)</th>
<th>% SS</th>
<th>P(F)</th>
<th>% SS</th>
<th>P(F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species pool</td>
<td>1</td>
<td>B</td>
<td>3.7</td>
<td>*</td>
<td>3.8</td>
<td>*</td>
<td>1.8</td>
<td></td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Species richness (SR)</td>
<td>2</td>
<td>B</td>
<td>4.7</td>
<td></td>
<td>2.8</td>
<td></td>
<td>5.2</td>
<td>*</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Mono. vs. Mix.</td>
<td>1</td>
<td>B</td>
<td>4.7</td>
<td>*</td>
<td>2.8</td>
<td></td>
<td>5.0</td>
<td>*</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>2- vs. 4-species</td>
<td>1</td>
<td>B</td>
<td>0.0</td>
<td></td>
<td>0.0</td>
<td></td>
<td>0.2</td>
<td></td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Species pool x SR</td>
<td>2</td>
<td>B</td>
<td>0.8</td>
<td></td>
<td>0.0</td>
<td></td>
<td>1.7</td>
<td></td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Composition</td>
<td>18</td>
<td>B</td>
<td>19.8</td>
<td></td>
<td>14.4</td>
<td></td>
<td>42.9</td>
<td>***</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>¹⁵N Treatment (T)</td>
<td>1</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>82.1</td>
<td>***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species pool x T</td>
<td>1</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SR x T</td>
<td>2</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species pool x SR x T</td>
<td>2</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Box residuals (B)</td>
<td>48</td>
<td></td>
<td>43.8</td>
<td></td>
<td>44.7</td>
<td></td>
<td>34.3</td>
<td></td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td>Residuals (R)</td>
<td>18 (24)</td>
<td></td>
<td>27.2</td>
<td></td>
<td>34.2</td>
<td></td>
<td>14.2</td>
<td></td>
<td>5.1</td>
<td></td>
</tr>
</tbody>
</table>

a Within SR contrasts are presented only for the main effect of SR, since none of the interactions was significant. Linear effect of SR on biomass: 2.7 % SS (.), on biomass:N: 3.7 % SS (*).

b Compositions as specified in Table 5.1.

c d.f. for δ¹⁵N are given in parentheses if different from d.f. for biomass, N, and biomass:N.
Table 5.3: ANOVA for soil microbial N (g) and $\delta^{15}N$ (%/permil) in August 2005.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Error df</th>
<th>% SS</th>
<th>% SS</th>
<th>% SS</th>
<th>% SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species pool</td>
<td>1</td>
<td>11.4(6)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Species richness (SR)</td>
<td>2</td>
<td>1.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Composition</td>
<td>1</td>
<td>28.3</td>
<td>26.5</td>
<td>27.1</td>
<td>1.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Soil layer (SL)</td>
<td>1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Box residuals (B)</td>
<td>1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Treatment unit res. (T)</td>
<td>1</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

No within SR contrasts since SR and contrasts n.s.

No within treatment unit contrasts since treatment unit n.s.

Three $\delta^{15}N$ values were missing ($n=189$).

$F$ and $P$ values are given in parentheses. Bold face indicates $F$ values are different from $F$ for C, N and C:N. There are 0 $\delta^{15}N$ values were

$n=192$ (96 compartments $\times$ 2 soil layers).

As specified in Table 5.1.

$\delta^{15}N$ values were missing ($n=189$).
Table 5.4: ANDEV for the fraction of N and $^{15}$N in plant biomass ($N_{Plant}$ and $^{15}N_{Plant}$) in August 2005. Significance levels refer to Quasi-$F$ tests derived from mean deviance ratios ( . $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$). $n=96$ compartments.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Error</th>
<th>% Dev</th>
<th>Quasi-$F$</th>
<th>$P(&gt;Q.-F)$</th>
<th>% Dev</th>
<th>Quasi-$F$</th>
<th>$P(&gt;Q.-F)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species pool</td>
<td>1</td>
<td>B</td>
<td>2.9</td>
<td>2.53</td>
<td>3.3</td>
<td>2.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species richness (SR)</td>
<td>2</td>
<td>B</td>
<td>1.6</td>
<td>0.71</td>
<td>1.8</td>
<td>0.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species pool x SR</td>
<td>2</td>
<td>B</td>
<td>0.0</td>
<td>0.02</td>
<td>0.2</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composition$^a$</td>
<td>18</td>
<td>B</td>
<td>19.8</td>
<td>1.84</td>
<td>*</td>
<td>17.1</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>$^{15}$N Treatment (T)</td>
<td>1</td>
<td>R</td>
<td></td>
<td></td>
<td>15.9</td>
<td>51.04</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>Species pool x T</td>
<td>1</td>
<td>R</td>
<td></td>
<td></td>
<td>0.1</td>
<td>0.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SR x T</td>
<td>2</td>
<td>R</td>
<td></td>
<td></td>
<td>1.0</td>
<td>1.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species pool x SR x T</td>
<td>2</td>
<td>R</td>
<td></td>
<td></td>
<td>2.3</td>
<td>3.74</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Box residuals (B)</td>
<td>48 (46)$^b$</td>
<td>48.2</td>
<td></td>
<td></td>
<td>52.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residuals (R)</td>
<td>24 (17)$^b$</td>
<td>27.5</td>
<td></td>
<td></td>
<td>5.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Compositions as specified in Table 5.1.

$^b$ d.f. for $^{15}N_{Plant}$ are given in parentheses if different from d.f. for $N_{Plant}$. Three $^{15}N_{Plant}$ values were missing ($n=93$).
Table 5.5: Summary table of microbial C, N, and C:N, total biotic (plants + microbes) N, \(^{15}N_{\text{ex}}\), and \(^{15}N_{\text{recov.}}\), as well as plant biomass (BM), N, biomass:N, \(^{15}N_{\text{plant}}\) in August 2005, at different levels of species richness (SR). Significance levels are shown for the effect of species richness (monocultures vs. mixtures in brackets, *P* < 0.1, **P** < 0.05).

<table>
<thead>
<tr>
<th>SR</th>
<th>C</th>
<th>N</th>
<th>C:N</th>
<th>N(^{15}N_{\text{ex}})</th>
<th>(^{15}N_{\text{recov.}})</th>
<th>BM</th>
<th>N</th>
<th>biomass:N</th>
<th>(^{15}N_{\text{plant}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>204.3</td>
<td>35.7</td>
<td>5.7</td>
<td>46.1</td>
<td>0.010</td>
<td>9.4</td>
<td>1052.4</td>
<td>10.5</td>
<td>100.7</td>
</tr>
<tr>
<td>2</td>
<td>218.3</td>
<td>36.4</td>
<td>6.0</td>
<td>47.9</td>
<td>0.010</td>
<td>9.8</td>
<td>1248.8</td>
<td>11.6</td>
<td>108.0</td>
</tr>
<tr>
<td>4</td>
<td>227.2</td>
<td>37.9</td>
<td>6.0</td>
<td>49.3</td>
<td>0.010</td>
<td>9.6</td>
<td>1267.2</td>
<td>11.6</td>
<td>109.6</td>
</tr>
</tbody>
</table>

Note: \(^{15}N_{\text{recov.}}\) from Table 5.2, \(^{15}N_{\text{plant}}\) from Table 5.3, *P* < 0.05, **P** < 0.01.

\(d\) richness (monocultures vs. mixtures in brackets).
Figure 5.1: Conceptual Model of soil nitrogen transformation (see also Hodge et al. 2000a, Schimel and Bennett 2004). Depolymerization (1) of N-containing organic matter compounds by extracellular enzymes is now seen as the critical point in the N cycle. Microbial immobilization (2) competes with plant uptake (3). The balance between microbial immobilization and mineralization (4) is influenced by C:N of both substrate and decomposer. NH$_4^+$ is transformed into NO$_3^-$ by nitrification (5). N can leave the system through denitrification (6) or leaching (7). Soil organic matter accumulates from litter input and root exudates (8) and microbial turnover (9). Until now, effects of plant diversity have been studied mainly with regard to the processes underlayed with gray (see refs in text), whereas our study of N partitioning integrates the soil microbial part.
Figure 5.2: Soil microbial $\delta^{15}$N in the shallow (S) or deep (D) soil layer in both $^{15}$N treatments (shallow $^{15}$N and deep $^{15}$N), across all three harvests. Dashed lines show background $\delta^{15}$N of unlabeled soil microbes. Note that $n=24$ per harvest ($n=6$ per bar), using the same subset across all harvests. Error bars show standard errors of the mean.
Figure 5.3: The fraction of $^{15}$N in plant biomass ($^{15}$N$_{plant}$) from the total of $^{15}$N taken up by plants and microbes (biotic $^{15}$N) by $^{15}$N treatment, at different levels of species richness. Top: species pool AHLP in August 2005 ($n$=48 communities), September 2005, and May 2006 (subset of $n$=12, see Table 5.1). Bottom: species pool DLRT in August 2005 only ($n$=48). Error bars show standard errors of the mean.
Figure 5.4: Scatterplots of microbial N against plant N (A, B), microbial C:N against plant biomass:N (C, D), and microbial $^{15}$N$_{ex}$ against plant $^{15}$N$_{ex}$ (E, F). Points show means for each plant species composition, either for all compositions harvested in August 2005 (left panels A, C, E) or the compositions harvested at all three harvests (right panels B, D, F).
General Discussion
A significant role of resource partitioning through niche differentiation among plant species was often suggested after large scale manipulative biodiversity experiments had consistently shown a positive effect of plant diversity on plant productivity (e.g., Tilman et al. 1996, Hector et al. 1999). However, experimental evidence for resource partitioning is scarce (but see McKane et al. 2002), and validity of the niche concept in plant communities is under debate, particularly since it was challenged by new “neutral models” (Bell 2001, Hubbell 2001). These models assume that plant communities result from a process of random drift of species (similar to random drift of genes) that are competitively equivalent (identical per capita demographic rates).

The aim of this thesis was to experimentally test for nitrogen (N) partitioning across gradients of experimentally manipulated species richness in temperate grassland plant communities. We used a variety of approaches to investigate the problem, including pot and field experiments, manipulating the spatial availability of soil resources, \(^{15}\)N labeling with liquid and solid tracers, and including soil microbes.

We generally found no strong evidence for N partitioning as an important mechanism behind positive biodiversity—productivity effects, or species coexistence. Species in mixtures differed less than expected in their N uptake from different N sources, i.e., from different soil depths (Chapter 2 and 4) and from different chemical forms (Chapter 2). The latter is in line with other studies done in temperate grasslands, also finding limited evidence for chemical partitioning of N, and where inorganic N (Harrison et al. 2007), or nitrate (Kahmen et al. 2006) were the preferred N forms. In contrast, plant species in a N-limited, arctic tundra community were differentiated in timing, depth and chemical form of N uptake (McKane et al. 2002). This could mean, that chemical partitioning of N, in particular with respect to inorganic vs. organic N is more important in arctic or alpine regions, where inorganic N is less available (see Schimel and Bennett 2004). However, demonstrating plant uptake of organic N is technically very difficult (see Chapter 3, von Felten et al. 2008), and its relevance for plant nutrition controversial (Jones et al. 2005). Our results which provide limited evidence for N partitioning by soil depth are in line with Kahmen et al. (2006), but contrast with McKane et al. (1990), who suggested that spatial and temporal partitioning of N is a major determinant of plant community organization. However, plant species in their study where to a greater extent temporally than spatially differentiated, and we only studied differential N uptake in space and chemical form.
Given a constant soil volume, deeper soil did neither increase the net effect of species richness on productivity, nor complementarity effects in Chapter 1 (von Felten and Schmid 2008). However, positive effects of soil depth on mixture performance were observed in experiments where deeper soil was correlated with larger soil volume (Berendse 1982, Dimitrakopoulos and Schmid 2004). It could be argued that our experiment was unrealistic, since soil volume can be exploited to any depth in the field. However, on the same field site as used in Chapter 2, root distributions of plant communities were unaffected by species richness (Wacker 2007). Moreover, a recent comment by Schenk (2008), suggests a general advantage of shallow rooting vs. deep rooting. To allocate a high proportion of resources to deep roots may be disadvantageous, if competitors take more effective advantage of resources available in shallow soil layers and exclude the deep-rooted plants. Only in special environments deep-rootedness may be an advantage. A specialized root system, which allows plants to locate cracks in the underlying rock of very shallow soil and to grow deep roots therein, was shown for shallow-soil endemics (Poot and Lambers 2008). Possibly, the general potential for resource partitioning among plant species by soil depth has been overestimated so far.

On the other hand, we found that species richness consistently decreased niche overlap between plant species with regard to N uptake (Chapters 2 and 4). That is, plant species differed more in their N uptake patterns when grown in mixtures than in individual monocultures. Thus, different plant species interacted and interspecific competition caused shifts in N uptake patterns. While results of Chapter 2 suggest that spatio-chemical partitioning might allow subordinate species to persist in a community by reducing niche overlap with dominant species and among themselves, we could not confirm this result in Chapter 4. Here, subordinate species did not shift to deeper soil, as expected to avoid competition with dominant species, but instead were restricted to N uptake from shallow soil where competition with dominant species should be most intense. These contrasting results may be explained by the short duration of the pot experiment (Chapter 4), where different results in the second year indicated that plant communities were not fully established in the first year. In contrast, the experimental communities used in the field (Chapter 2) where already in the second vegetation season. Thus, the more controlled conditions which are an advantage of the pot experiment, may be partly outbalanced by the short duration. Another caveat of this study which should be noted is the compara-
tively small effect of species richness on productivity in both the field experiment (see also Wacker et al. 2008) and the pot experiments. We cannot exclude, that in an experiment with large biodiversity effects, we could have found stronger differentiation of species in N uptake. Or the other way round, since differentiation in N uptake depends on the combination of species, more differentiated species might have produced larger biodiversity effects.

Mixtures tended to take up more N than monocultures, yet they showed also a greater dilution of it, i.e., had larger plant biomass:N (Chapters 4 and 5). This is in line with (van Ruijven and Berendse 2005), who suggest that species richness increases the “N-use efficiency” of plant communities. Further, plant species richness had no effect on partitioning of N between plants and microbes (Chapter 5). Clearly, it can be said that diverse communities are able to produce more biomass with the same amount of N. However, it is unclear whether this now indicates higher “N-use efficiency”, as plants could actually produce more biomass with the same amount of N, or N limitation, because plants could not increase the amount of N proportional to the amount of C assimilated.

The title of this thesis, “Neutral vs. niche-structured communities: testing for resource partitioning by plants”, asks for an answer to the significance of resource partitioning and niche complementarity in plant communities. The main answer is that partitioning of N in space and chemical form are relatively weak and thus cannot be the major mechanism underlying the observed positive diversity–productivity relationships or explaining plant coexistence in the temperate grasslands studied. However, we can not conclude that resource partitioning as such is not important. On the one hand, plants might differ in the uptake of other resources (water, light, phosphorous), or in the timing of resource uptake. On the other hand, it may be complementarity in higher-dimensional niches rather than along single axes that structures plant communities (Harpole and Tilman 2007, Clark et al. 2007). Such high dimensional niches may include N partitioning in depth and chemical form in combination with other niche axes.

We showed that N uptake patterns of species were affected by the presence and identity of interspecific competitors. This clearly contradicts fitness equivalence (and identical effects of species on one another), the main premise of neutral theory. With fitness equivalence, stochastic events would drive all but one species extinct in the long term. But diversity can be maintained as long as extinction rates are slow enough to be balanced
by speciation. In contrast, without fitness equivalence, the strongest competitor would outcompete all others in the short term. Hence, what mechanisms stabilize a community and keep it diverse? Niches undoubtedly are a stabilizing mechanism. However, the term “niche” is understood in different ways. In its narrow sense and as used in this thesis, it means a resource niche. In that sense, niches provide one out of a few alternatives to neutrality, and others may include competition colonization trade-offs (see Levine and Rees 2002), habitat heterogeneity (MacArthur and Levins 1967), species-specific interactions with soil organisms (Bever 2003), or Janzen-Connell effects (Janzen 1970, Connell 1971, Petermann et al. 2008). Thus, lack of evidence for resource partitioning does not automatically mean evidence for neutrality. In its broader sense, the term “niche” may cover all stabilizing mechanisms. Moreover, niche and neutral processes are not mutually exclusive (Cadotte 2007, Adler et al. 2007), and neither are different stabilizing mechanisms. Rather, the magnitude of niche-based differences or stabilizing mechanisms necessary to stabilize long-term coexistence may depend on how similar species are in average fitness (Chesson 2000), with the purely neutral case being the extreme.

This study was to our knowledge the first that investigated resource partitioning among plant species across a gradient of plant species richness. In conclusion, we could clearly demonstrate that species respond to interspecific competition by changes in N uptake patterns. For example, we could show that niche overlap between species decreased with species richness. However, N uptake patterns of species in mixtures were not as distinct as expected, and our results provide limited evidence for complementary N use as being a main mechanism to explain positive effects of species richness on productivity or species coexistence. Furthermore, the potential for resource partitioning among plant species by soil depth may have been overestimated so far, since we observed larger complementarity effects in shallow soil than in deep soil. Also, although a small effect of plant species richness on total plant N uptake seems possible, higher biomass production in diverse plant communities does not necessarily require higher N uptake, but can be mediated by higher biomass:N ratios. Finally, although our results provide limited evidence for the existence of niches in plant communities, they neither support neutrality as a mechanism. We regard it likely that plant coexistence is mediated by a combination of mechanisms instead of a single most important one.
Chapter references

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mechanisms. Proceedings of the National Academy of Sciences of the United States of America 102:695–700
and soil microbes: Comment. Ecology 89:878–879
Ecology 1:33–41
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Thank you Nina for the good structure you brought into my work, for example with yearly appraisal talks to evaluate work progress and to set future goals, and for your outright support in career questions. It was great to gain you as an enthusiastic advisor of the FrauSchafftWissen peer mentoring group, and naturally, to have a strong female role model with you as doctor mother.

Thank you Andy for teaching me a lot about statistics and the software package R, and also for involving me in teaching your own stats courses which was very instructive. I also greatly enjoyed to be at the pulse of biodiversity research in your group, and to profit from your scientific network. I always enjoyed very much for instance, when the group was allowed to take scientific guests out for dinner.

Thank you Pascal for excellent and frequent practical advice, like building a multi-dibble to get seedlings planted in half the time, or sophisticated R scripts to do very convenient routines, but also giving insight into the depths of programming. This all revealed that while being an advanced scientist, you are still enthusiastic to do lab and field research yourself. Thanks a lot also for many good conversations over lunch or a cup of coffee.
Thank you Michael for your strong support in the Reckenholz $^{15}$N labelling project. Not only you helped with applying the $^{15}$N label in the field, but later you also worked together with me doing KCl extractions in the lab, jointly from Reckenholz and Alp Weissenstein. Thanks also for sharing a lot of ideas on our work, biodiversity research, $^{15}$N labeling techniques, but many other things as well.

Thank you Bernhard for the very generous opportunity to start my PhD on your SNF grant (Reckenholz project), which finally resulted in continuing my PhD on the PSC grant. I greatly enjoyed our stimulating discussions on science and stats, often supposed to be short but ending up long. I admire your enthusiastic way of doing science, and how you lead the IfU. It is more than just an ordinary institute; this became obvious in both cheerful and sad moments.

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