

1 Running head: Do grassland plants profit from N partitioning?

2 **Do grassland plant communities profit from N**
3 **partitioning by soil depth?**

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23 Abstract

24 Recent biodiversity–ecosystem functioning experiments in temperate grasslands have shown
25 that productivity positively correlates with plant species richness. Resource partitioning—in
26 particular nitrogen (N) partitioning—has been proposed as one possible mechanism to
27 explain this pattern. There is evidence for interspecific differences in chemical form, soil
28 depth and timing of N uptake. However, it has rarely been tested whether such differences
29 result in increased N exploitation at the plant community-level. Using ^{15}N -labeled litter
30 which was mixed into different soil layers, we tested whether eight common grasses and forbs
31 grown in communities of one, two or four species differ with respect to the proportions of N
32 taken up from different soil depths (N-niche), and how this affects the total N uptake of plant
33 communities. We calculated proportional similarity between species (niche overlap) with
34 regard to N uptake from the labeled soil layers; we further calculated an *a priori* measure of
35 community N uptake based on species N uptake in monoculture (community niche).
36 Interestingly, however, plant community N uptake was not affected by species richness,
37 possibly because community-level N uptake was determined by (diversity-independent) soil
38 N mineralization rates. We nevertheless observed a positive effect of species richness on
39 productivity due to increased aboveground biomass:N ratios. This may indicate increased
40 competition for light resulting in increased amounts of comparably N-poor stem tissue.
41 However, community N content and biomass were positively correlated with the community
42 niche, a measure which is strongly linked to species composition. Thus, our results suggest
43 that the studied species are generalists rather than specialists regarding N uptake depth, and
44 that species composition was more important than species richness in determining
45 community N uptake. Overall, N partitioning may be a less important driver of positive
46 biodiversity–productivity effects in temperate grasslands than previously assumed.

47 **Keywords** ^{15}N , biodiversity–ecosystem functioning, niche, nitrogen, proportional
48 similarity, resource partitioning, rooting depth, species richness, temperate grassland

49 **Introduction**

50 The past two decades have seen a burst of studies addressing the relationship between
51 biodiversity and ecosystem functioning. Experiments in temperate grasslands have
52 repeatedly shown a positive effect of plant species richness on productivity (reviewed e.g., in
53 Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2007, Duffy 2009, Naeem et al.
54 2009, Cardinale et al. 2011). Resource partitioning has often been proposed as a mechanism
55 explaining this relationship. Interspecific differences in resource niches should lead to more
56 complete use of available resources in more diverse plant communities. This has revived an
57 old idea in ecology, going back to Darwin: niches may not only explain species coexistence
58 but also affect ecosystem functioning, through the ecological “division of labour” (Darwin
59 1985, Hector and Hooper 2002).

60 Nitrogen (N) availability limits primary productivity in many ecosystems including
61 temperate grasslands (Vitousek and Howarth 1991), suggesting that N uptake from soil may
62 be an ideal test case for the presence of interspecific resource partitioning by plants. Several
63 studies have investigated partitioning of N with respect to chemical form (such as NH_4^+ ,
64 NO_3^- , and organic N) using ^{15}N -labeling techniques (McKane et al. 2002, Weigelt et al. 2005,
65 Miller et al. 2007, Pornon et al. 2007). Plant species differ in rooting depth (Parrish and
66 Bazzaz 1976, Berendse 1982), depth of root activity (Veresoglou and Fitter 1984, Fitter
67 1986, Mamolos et al. 1995) and depth of water uptake (Gordon and Rice 1992, Nippert and
68 Knapp 2007). However, only a few studies have investigated partitioning of N by soil depth:
69 McKane et al. (2002) observed that arctic tundra plants simultaneously partitioned N by
70 depth, chemical form and time, whereas Kahmen et al. (2006) found no evidence for
71 partitioning of soil N by depth. Moreover, while there is good evidence that plants vary in

72 their capacity to assimilate different chemical forms of N or to exploit different soil depths, it
73 has hardly been tested whether such differences change with species richness and whether
74 they lead to more complete N exploitation in species rich communities.

75 If a mixture is composed of “specialist” species that differ inherently in resource-use patterns,
76 then the mixture should exploit resources more completely than any of the monocultures (in
77 an extreme case the resource capture of complete specialists would be additive). In contrast,
78 if a mixture is composed of “generalist” species that largely overlap in resource use, the
79 mixture would not be expected to exploit more resources than the monocultures. This has
80 been demonstrated elegantly for animals, with specialist and generalist parasitoids (Finke
81 and Snyder 2008), and for bacterial strains (Gravel et al. 2011). Alternatively, species may
82 be able to use a broad range of resources but may behave opportunistically rather than
83 occupying a defined niche. They may behave as generalists under intraspecific competition
84 (in monocultures) but as specialists in the presence of interspecific competition (in species
85 mixtures). Berendse (1982) showed that *P. lanceolata* acquired nutrients from deeper soil
86 layers when grown with the shallow-rooting grass *Anthoxanthum odoratum* than when grown
87 in monocultures. However, a change from generalist to specialist behaviour does not
88 necessarily imply that community-level resource uptake increases with diversity. A more
89 rigorous investigation of the relation between resource partitioning and resource use requires
90 investigating the species’ realised resource niches while manipulating species richness.

91 Neighbor removal experiments have investigated partitioning between chemical forms of N in
92 the presence and absence of interspecific competition (Miller and Bowman 2002, Ashton
93 et al. 2008). In an earlier experiment in the field, we assessed plant uptake of three chemical
94 forms of N from two soil depths using ^{15}N -tracer solutions (von Felten et al. 2009). We found
95 a decrease in niche overlap among species across three levels of species richness. However,
96 these studies could not relate interspecific niche differentiation between species to community
97 N use, either because plant species richness was confounded with plant density (Miller and

98 Bowman 2002, Ashton et al. 2008), or because community-level N acquisition was not
99 measured (von Felten et al. 2009).

100 We conducted a ^{15}N -labeling experiment to test for interspecific differences in N uptake from
101 deep and shallow soil among temperate grass and non-legume forb species. Unlike other
102 studies on N partitioning, using liquid tracers, we mixed ^{15}N -labeled litter into the soil,
103 allowing a more precise and homogeneous distribution and microbial mobilisation of the
104 label. We further tested whether N uptake niches (quantified as the fraction of N acquired
105 from separate soil layers) and their overlap (quantified as proportional similarity, Colwell and
106 Futuyma 1971, Feinsinger et al. 1981) depended on species richness. Finally, we tested
107 whether plant community N uptake in mixtures increased with species richness and whether
108 it can be predicted from the “community niche” (Salles et al. 2009), an *a priori* index based
109 on N uptake of the component species in monoculture.

110 **Methods**

111 **Experimental Design**

112 We set up a factorial mesocosm experiment combining a plant species richness and a soil
113 labeling treatment. The species richness treatment consisted of growing all possible
114 communities of one, two and four species that could be assembled from two separate pools of
115 four species (22 communities; Table 1). Each pool contained two common grasses and forbs.
116 Working with two non-overlapping species pools allows more generalizable results that are
117 not restricted to a particular species pool. The soil labeling treatment consisted of mixing
118 ^{15}N -labeled litter with the top soil layer (0-20 cm, “shallow” soil layer treatment) or the
119 bottom soil layer (20-40 cm, “deep” soil layer treatment). Additional mesocosms without soil
120 ^{15}N labeling were established outside the experiment to determine natural background ^{15}N
121 abundances in plant biomass.

122 The experimental communities were set up in boxes of 40 cm × 60 cm area and 40 cm depth.
123 These boxes were subdivided into eight (2 × 4) rectangular compartments. The
124 experimental treatments were applied in a “split-plot” design: one plant community of a
125 given composition was grown in each box (all eight compartments) and soil labeling
126 treatments were applied to half a box (four adjacent compartments). The four compartments
127 of half a box were used for destructive harvests.

128 **Set-up of Mesocosms**

129 Our experiment was set up in the experimental garden of the Institute of Environmental
130 Sciences, at the University of Zurich (Switzerland). Mesocosms were built from
131 polypropylene boxes that were subdivided using waterproof polyphenol-resin-coated boards.
132 Individual compartments (18 cm × 13.5 cm) were sealed using silicone and polyurethane lute
133 to avoid any transfer of ¹⁵N to neighboring compartments. Each compartment had four
134 10 mm drainage holes at the bottom and was fitted with a drainage mat (Enkadrain,
135 Colbond, Arnhem, The Netherlands). The boxes were insulated using 2 cm thick styrofoam
136 boards to minimize warming by sunlight.

137 Each compartment was filled with sieved, natural field soil (0.35±0.01 % N and 3.6±0.04 %
138 C, pH = 7.6) that was compressed to prevent subsequent settling of the soil. 1.4 g ¹⁵N-labeled
139 *Festuca rubra* material containing 2.5 mg ¹⁵N (1.4 g *F. rubra* × 1.18 % N × 15 atom% ¹⁵N)
140 was mixed either into the deep (lower 20 cm), the shallow (upper 20 cm), or no soil layer.
141 The same amount of non-labeled *F. rubra* material was mixed into all non-labeled layers.
142 The *F. rubra* material was obtained by growing plants for 11 weeks on quartz sand supplied
143 with nutrient solution. The nutrient solution contained ¹⁵N-labeled KNO₃ (15 atom% ¹⁵N) or
144 KNO₃ at natural abundance levels to produce ¹⁵N-labeled and non-labeled litter. The plants
145 were cut to the ground, dried and shredded into pieces of ca. 2 cm.

146 From June 7 to 10, 2005, twelve five-week old seedlings were transplanted into each box
147 compartment. The seedlings were organised in three rows with four individuals each; the

148 middle row was offset by half the distance between individuals, i.e. plantlets arranged on a
149 hexagonal grid with constant spacing between individuals. Each row contained the full
150 species set present in the respective community, with species positions randomized within
151 rows. Plant communities were weeded regularly and were watered daily with an automated
152 irrigation system except for rainy days. Daily average temperatures at the site (502 m asl)
153 ranged from -9.1°C (January 29, 2005) to 26.1°C (July 28, 2005).

154 **Harvests and sample preparation**

155 Box compartments were destructively harvested after nine weeks (8–21 August 2005), after
156 15 weeks (19 September–7 October 2005) and after 11 months (15–29 May 2006). In
157 addition, plant biomass in all remaining compartments was cut to 2 cm at 15 weeks (end of
158 the growing season 2005). For each destructive harvest, two compartments per box were
159 randomly selected (one from each ^{15}N -treatment). At the same time, one compartment to
160 which no ^{15}N -label had been added was harvested to assess background ^{15}N abundances to
161 calculate ^{15}N enrichment.

162 Aboveground biomass was harvested at the species level. Roots were washed on a 2 mm
163 sieve. We attempted to also retrieve roots at the species level by extracting individuals
164 separately, but the proportion of residual roots that could not be allocated to species was
165 very large. We thus only present root data at the community level. All plant material was
166 dried (80°C , 48 h) and weighed. All samples were ground and N and ^{15}N measured by
167 isotope ratio mass spectrometry (Delta^{plus} XP IRMS, Finnigan MAT, coupled to a Flash
168 EA 1112 NC elemental analyzer, CE Instruments).

169 The consistency and spatial distribution of the ^{15}N -labeling treatments were assessed by
170 measuring $\delta^{15}\text{N}$ in extractable soil N ($n=72$ measurements, 6 compartments \times 2 treatments
171 \times 2 depths at each of three harvests). For each sample, 15 g fresh soil sieved to 2 mm were
172 extracted with 50 ml 0.03 M K_2SO_4 for 30 min. ^{15}N was measured in freeze-dried extracts by
173 isotope ratio mass spectrometry (Delta^{plus} XP IRMS, Finnigan MAT, coupled to a EuroEA

174 3000 elemental analyzer, HEKAtech).

175 Data analysis

176 For each sample we calculated ^{15}N tracer concentration ($[^{15}\text{N}_{ex}]$; atom% excess or mg excess
177 ^{15}N per g N) and ^{15}N tracer content ($^{15}\text{N}_{ex}$; g excess ^{15}N).

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

$$DF = \frac{[^{15}\text{N}_{ex}]_{deep}}{[^{15}\text{N}_{ex}]_{deep} + [^{15}\text{N}_{ex}]_{shallow}} \quad (1)$$

$$SF = 1 - DF \quad (2)$$

180 where $[^{15}\text{N}_{ex}]_{deep}$ and $[^{15}\text{N}_{ex}]_{shallow}$ are aboveground ^{15}N tracer concentrations from a pair of
181 compartments with the respective ^{15}N treatments ($n=2$ pairs per population and harvest).

182 To quantify niche overlap, we calculated the proportional similarity index (Colwell and
183 Futuyma 1971, Feinsinger et al. 1981) based on DF and SF, i.e. $n=2$ N sources, between
184 pairs of species (indicated as species 1 and 2):

$$PS = 1 - 0.5 \sum_{i=1}^n |p_{1i} - p_{2i}| \quad (\text{general form}) \quad (3)$$

$$PS = 1 - 0.5 (|DF_1 - DF_2| + |SF_1 - SF_2|) \quad (n=2) \quad (4)$$

185 Values of proportional similarity (PS) range from zero to complete overlap (=1) between
186 species. For each species pool, proportional similarity was calculated between all species
187 when grown in monoculture (six pairwise combinations). Within mixtures, proportional
188 similarity was calculated between pairs of species (one combination in 2-species mixtures, six
189 combinations in 4-species mixtures).

190 To quantify the niche space occupied by the whole plant community, we calculated the
191 expected “community niche” (CN, Salles et al. 2009). CN *a priori* predicts the N uptake of
192 a mixture by summing the maximum observed monoculture N uptake from deep and shallow

193 soil across species present in the mixture:

$$CN = \sum_{i=1}^n \max(P_{i,1}, \dots, P_{i,m}) \quad (\text{general form}) \quad (5)$$

$$CN_{depth} = \max_{deep}(^{15}N_{ex,1}, \dots, ^{15}N_{ex,m}) + \max_{shallow}(^{15}N_{ex,1}, \dots, ^{15}N_{ex,m}) \quad (n=2) \quad (6)$$

194 where n is the number of resources (here: n=2 soil depths), m is the number of species in the
 195 mixture (here: m=1,2, or 4) and P_{ij} is the performance of species j on soil depth i. Here,
 196 $^{15}N_{ex,j,deep}$ and $^{15}N_{ex,j,shallow}$ is the total (above- and belowground) ^{15}N uptake of species j in
 197 the deep and the shallow ^{15}N treatment in monoculture (mean of two replicates per
 198 monoculture \times ^{15}N treatment \times harvest combination). CN of each plant community
 199 combines the maximum N uptake from shallow and deep soil in monoculture across all
 200 species present in the community. We also included values of CN for monocultures in our
 201 analyses (although these were not calculated *a priori*), to account for effects of CN at all
 202 levels of species richness. This yielded 66 values of CN, 22 species compositions (Table 1) \times
 203 three harvests.

204 Data were analyzed using linear mixed effects model ANOVA since we used a hierarchical
 205 experimental design (function lme in R version 2.11.0, R Development Core Team 2010). For
 206 the analysis of ^{15}N enrichment of aboveground biomass (n=576, 192 populations \times 3
 207 harvests) we fitted harvest, ^{15}N treatment and the interaction term as fixed factors.
 208 Treatment unit and compartment were used as random factors. The deep fraction of tracer
 209 uptake (DF) was analyzed separately for monocultures and mixtures. For the analysis of DF
 210 in monocultures (n=48, 8 species \times 3 harvests \times 2 replicates) we fitted species, harvest and
 211 the species \times harvest interaction as fixed factors. Species pool and the compartment pair
 212 (see calculation of DF) were used as random factors. For the analysis in mixtures (n=240,
 213 [12 compositions \times 2 species \times 2 replicates + 2 compositions \times 4 species \times 4 replicates] \times 3
 214 harvests), we additionally fitted species richness (two vs. four species) and included all
 215 interaction terms (see Table 2). In addition, we fitted a second model with the species term

216 replaced by functional group (grasses vs. forbs). Species pool, species composition and the
217 compartment pair were used as random factors. Because the errors did not significantly
218 deviate from normality, there was no need to transform DF. In addition, we calculated the
219 Spearman rank correlation coefficient between species ranks in DF and aboveground biomass
220 within mixtures (ranks 1–4 in 4-species mixtures and ranks 1–2 in 2-species mixtures).
221 Proportional similarity was arc sine square root transformed to meet the assumption of
222 normal errors. We fitted the fixed factors species richness (three-level factor and log-linear
223 effect), species pair, harvest and the interactions species richness \times harvest and species pair
224 \times harvest (Table 3). Random factors were species pool, species composition and the specific
225 species pair.

226 To assess the effect of species richness on aboveground biomass and N content on the
227 community level, we used a model with pool and species composition as random factors, and
228 species richness (log-linear effect), harvest and the interaction term as fixed factors.
229 Similarly, we tested the effect of the “community niche” (CN) on aboveground biomass and
230 aboveground N content. CN was fitted as continuous explanatory variable instead of species
231 richness. We used pool and composition \times harvest as random factors, since CN is defined per
232 composition and harvest. Note that mean numbers given in the text are always mean \pm SE.

233 Results

234 ^{15}N labeling of soil

235 The ^{15}N -labeling treatments were stable across harvests; average soil $\delta^{15}\text{N}$ was 45.0 ± 1.8 ‰
236 in the upper layer and 10.7 ± 0.3 ‰ in the lower layer for the shallow ^{15}N treatment, and
237 8.5 ± 0.2 ‰ and 46.4 ± 1.5 ‰ for the deep ^{15}N treatment, respectively. These $\delta^{15}\text{N}$ values
238 indicate that unlabeled layers remained unlabeled (background $\delta^{15}\text{N}$ of soil: 10.9 ± 0.3 ‰) for
239 the duration of the experiment (11 months), and that the ^{15}N enrichment was similar for

240 both treatments.

241 Addition of ^{15}N labeled plant material led to considerable enrichment of plant aboveground
242 ^{15}N , which was higher in compartments with the shallow than with the deep ^{15}N treatment
243 across all populations (Appendix Tables 1 and 2). As a consequence, the fraction of ^{15}N
244 tracer taken up from deep soil (deep fraction, DF) was on average lower than 0.5
245 (mean= 0.36 ± 0.007). Note that the shallow fraction $\text{SF}=1-\text{DF}$ (Eq. 2).

246 ^{15}N uptake from deep and shallow soil in monocultures

247 When grown in monoculture, species did not differ in the fraction of ^{15}N tracer taken up
248 from deep soil (DF, see Appendix Fig. 1 and Table 3). However, the deep fraction increased
249 across harvests from a mean of 0.31 ± 0.02 in August 2005 to 0.43 ± 0.01 in May 2006
250 ($F_{2,16}=17.8$, $P<0.001$), most likely due to deeper root penetration over time. This might be
251 due to depletion of nutrients in the shallow soil layer, as indicated by decreasing ^{15}N
252 enrichment of aboveground biomass on the shallow treatment but stable ^{15}N enrichment on
253 the deep treatment (harvest \times ^{15}N treatment interaction, Appendix Table 2).

254 Consistent with no difference in DF among species, values of proportional similarity (PS)
255 were large, with an overall mean of 0.94 ± 0.01 . PS was ≥ 0.8 between all but one species pair
256 at harvest 1 (0.74 between *L. perenne* and *T. officinale*, but PS of the second replicate was
257 0.93), and always ≥ 0.85 at harvests 2 and 3 (Fig. 1).

258 ^{15}N uptake from deep and shallow soil in mixtures

259 When grown in mixtures of two or four species, species differed significantly in the fraction of
260 ^{15}N tracer taken up from deep soil, but species differences changed over time (species \times
261 harvest interaction, Table 2 and Appendix Fig. 1). Grasses took up more ^{15}N from deep soil
262 than forbs at harvests 1 and 2, whereas the forbs took up more ^{15}N from deep soil than the
263 grasses at harvest 3 (functional group \times harvest interaction, Appendix Table 4). It seemed
264 that changes in functional group effects over time were more pronounced than changes in
265 species effects. However, a model comparison based on AIC and a likelihood ratio test

266 indicated that the model including species effects fit the data better than the model
267 including functional groups instead of species (Likelihood-ratio=116.9, $P<.001$). As for the
268 monocultures, DF generally increased over time (across harvests) in mixtures. However, the
269 species richness of mixtures did not affect DF.

270 We found a positive correlation between the aboveground biomass of plant populations and
271 the fraction of N taken up from deep soil (DF), which means that plant species with high
272 biomass in mixture took up more N from deep soil than species with low biomass in mixture
273 (Spearman rank correlation coefficient $\rho=0.51$, $P<0.001$, see Appendix Fig. 1 and 2). This
274 may indicate that the roots of smaller plant species did not reach deeper soil layers within 11
275 months.

276 Proportional similarity with regard to N uptake from shallow and deep soil significantly
277 decreased with species richness (as a three-level factor and as a log-linear effect). However, it
278 was the presence of interspecific competition rather than the number of interspecific
279 competitors that mattered, since PS was similar in mixtures of two and four species (Fig. 1,
280 Table 3). Moreover, species in mixtures were still relatively similar (mean = 0.86 ± 0.01). PS
281 was ≥ 0.65 at harvest 1, and ≥ 0.6 at harvest 2 and between all but one species pair at harvest
282 3 (PS=0.47 between *L. vulgare* and *P. lanceolata*, but 0.92 for the other replicate). The
283 effect of both species richness and specific species pairs changed over time (across harvests).

284 **Effects of species richness and community niche on community biomass and N** 285 **content**

286 Total community biomass increased with species richness (log-linear effect, $F_{1,19}= 6.52$,
287 $P<0.05$), with average biomass in 2-species and 4-species mixtures amounting to 112 % and
288 116 % of the biomass in monocultures. However, the N content of the plant communities did
289 not increase with species richness, since the increase in biomass was paralleled by a decrease
290 in N concentration (at harvest 1 for instance, from an average of 1.04 % in monocultures to
291 0.96 and 0.94 % in 2-species and 4-species mixtures, respectively).

292 Community biomass also increased with the community niche (CN), predicted *a priori* from
293 species N uptake in monocultures (Appendix Fig. 3, $F_{1,59} = 28.1$, $P < 0.001$), independent of
294 harvest time. Moreover, there was a significant positive relationship between CN and the N
295 content of plant communities (Fig. 2, $F_{1,59} = 27.0$, $P < 0.001$). This relationship between CN
296 and community N content was stronger at harvests 2 and 3 than at harvest 1, indicated by a
297 significant CN \times harvest interaction. Although CN generally increased with species richness,
298 CN of the mixtures never exceeded CN of the “best monoculture”, indicating that CN is
299 largely determined by species composition rather than species richness. However, at each
300 harvest, a different species performed best at taking up N from both depths in monoculture
301 (*L. perenne* at harvest 1, *H. lanatus* at harvest 2 and *L. vulgare* at harvest 3).

302 Discussion

303 Limited partitioning of soil N acquisition by depth

304 In our mesocosm experiment, we found limited evidence for differences in N resource niches
305 and, in particular, vertical N partitioning as major drivers of positive
306 biodiversity–productivity relationships in temperate grassland plant communities, although
307 this has often been proposed (HilleRisLambers et al. 2004, Spehn et al. 2005). While we
308 showed interspecific differences in the proportion of N derived from deep and shallow soil
309 layers when species were grown in mixture, we found no effect of species richness on the total
310 N uptake of plant communities.

311 Whereas linking N uptake from deep and shallow soil of individual species to N uptake of
312 communities of varying species richness is a novel aspect of our study, we also found limited
313 evidence for vertical N partitioning in our earlier studies. In a field experiment with plant
314 communities comprising one, three or six species, we also showed higher N uptake from
315 shallow soil than from deep soil, despite the different choice of soil depths for the shallow and
316 deep layer (0–3 cm and 7–12 cm, von Felten et al. 2009). In that experiment, root

317 distributions of plant communities were unaffected by species richness (Wacker 2007). In a
318 pot experiment with pots of different depths but constant volume, we found stronger net
319 biodiversity and complementarity effects in shallow pots, indicating that horizontal root
320 segregation might have been more important than the partitioning of rooting depths (von
321 Felten and Schmid 2008). Moreover, when Mommer et al. (2010) used a DNA-based
322 technique to compare species-specific root distributions, they found that although
323 four-species mixtures of two common grasses and forbs produced significantly more roots
324 than the monocultures of the same species, this overyielding of root biomass was not due to
325 vertical niche differentiation. Instead, recent evidence suggests that pathogen-mediated root
326 overproduction in species mixtures determines the patterns of community productivity and
327 overyielding (de Kroon et al. 2012).

328 In general, water can be accessed earlier in the top soil after precipitation events, and as long
329 as there is enough moisture, more N is available in the top soil where litter and atmospheric
330 N inputs arrive and N turnover mostly occurs. This should favour shallow roots, at least
331 under moist conditions, while deep roots are certainly beneficial under dry conditions.
332 However, allocating a high proportion of resources to deep roots may be unfavorable,
333 whenever competitors take more effective advantage of resources available in shallow soil
334 layers and exclude deep-rooted species (Schenk 2008). Hence, although the trade-off between
335 deep and shallow roots may prevent plants from exclusively exploiting the shallow soil
336 horizon, there may be a general advantage of shallow vs. deep rooting.

337 **Generalists rather than specialists**

338 No differences in species N uptake from shallow and deep soil in monoculture combined with
339 only slight differences in mixtures and high niche overlap (proportional similarity) suggest
340 that the species used here are generalists rather than specialists with regard to N uptake
341 from different soil depths. The decrease in proportional similarity (PS) of species pairs with
342 regard to N uptake from shallow and deep soil with increasing species richness confirms our

343 findings in an earlier experiment (von Felten et al. 2009). Although decreasing PS could
344 indicate a change from generalist behavior of species in monoculture to specialist behaviour
345 in mixture, it did not result in higher N uptake of communities with increasing species
346 richness. Had there been a “division of labour” or had subordinate species taken refuge in
347 deep soil, we should have seen higher N uptake with increasing species richness. Instead, it
348 seems that the decrease in PS was due to subordinate species being constrained to using N
349 from shallow soil, precluded from growing deep roots by dominant species. Our results
350 contrast with Berendse (1982), where the deep-rooting forb *P. lanceolata* derived more
351 nutrients from deeper soil layers when grown with the competitively dominant grass *A.*
352 *odoratum*.

353 **Effects of composition vs. species richness**

354 We found no effect of species richness on the N content of the plant communities, because
355 the observed increase in biomass was paralleled by a decrease in N concentration. Lower N
356 concentration in species-rich compared to species-poor communities was also shown in two
357 large biodiversity experiments manipulating the species richness of temperate grassland
358 plants from one to eight and nine species (van Ruijven and Berendse 2005, Roscher et al.
359 2008). van Ruijven and Berendse (2005) suggested that mixtures have a higher nitrogen use
360 efficiency. This could be due to higher biomass of those species with larger biomass:N ratio,
361 consistent with resource-competition theory (Tilman 1982 and 1990) predicting that
362 mixtures are dominated by those species able to most efficiently acquire limiting resources.
363 Alternatively, increasing species richness may have lead to shifts in biomass allocation, i.e., a
364 larger fraction of stems (low N concentration) as opposed to leaves (high N concentration),
365 indicating increased competition for light.

366 Moreover, increasing species richness was shown to increase shoot to root ratios due to
367 constant root but increasing aboveground biomass (Bessler et al. 2009). This may indicate,
368 that complementarity aboveground was more important than complementarity belowground.

369 While more diverse plant communities may be able to capture more light by building a more
370 complex canopy, total N acquisition may be largely set by soil mineralisation rates, which
371 may not change much with diversity. Nevertheless, a combination of above- and belowground
372 niche complementarity may be needed to create strong effects of diversity on productivity.

373 Whereas we found no effect of species richness on the N content of plant communities, the
374 latter could be predicted by the community niche, which heavily depended on species
375 composition. This result is in line with other studies. For instance, just as at each harvest a
376 single (but each time different) species acquired most N from both soil depths, dominant
377 bunchgrasses acquired most of the N from all soil N pools in a study on invasion resistance,
378 and there was no evidence suggesting that functional groups partitioned different soil N pools
379 when biomass was incorporated (James et al. 2008). Similarly, in a study conducted in three
380 temperate grasslands, Kahmen et al. (2006) found that total N uptake at the ecosystem level
381 was determined by species or functional group identity, and thus by community composition
382 rather than species richness. Indeed, Ashton et al. (2008) found enhanced plant N uptake in
383 the presence of an interspecific neighbor, but since they used neighbor removal treatments,
384 the effects of neighboring plants and plant density could not be separated.

385 Our results indicate that species composition was more important in determining biomass
386 production and N content than was species richness. Also, the community niche of a mixture
387 was never larger than that of the best monoculture. However, it is important to note that at
388 each harvest, a different species performed best at taking up N from both depths. This raises
389 the possibility of a temporal N niche (McKane et al. 1990) and implies an advantage over
390 time for communities containing all three species instead of only one, consistent with the
391 insurance hypothesis (McNaughton 1977, Yachi and Loreau 1999).

392 **Potential caveats**

393 There are some potential caveats of this study that should be noted. First, there was a
394 comparatively small effect of species richness on productivity, and we do not know if, under

395 these conditions, species would have coexisted in the long run. We cannot exclude that we
396 might have found stronger differentiation of species in N uptake in an experiment showing a
397 larger biodiversity effect on biomass. Vice versa, since differentiation in N uptake depends on
398 the combination of species, more differentiated species might have produced a larger
399 biodiversity–productivity effect. Second, our experimental plant communities were certainly
400 not fully established yet, especially in the first year of the experiment (harvests 1 and 2).
401 Cardinale et al. (2007) found that biodiversity effects on productivity increased over time,
402 because the magnitude of complementarity increased as experiments were run longer. In
403 fact, some changes between the first and the second year, such as increased N uptake from
404 deep soil by forbs, might have continued and could have led to different N uptake patterns in
405 fully established plant communities. Moreover, the effect of species diversity on plant
406 community N content increased over time in the Jena-Experiment (Oelmann et al. 2011),
407 and we cannot exclude that the absence of such an effect in our experiment is due to its
408 relatively short duration. Third, by measuring N partitioning by soil depth, we investigated
409 only one specific type of resource partitioning. Although our results fit well with those of a
410 field experiment where we used different depth ranges for the shallow and the deep soil layer
411 (von Felten et al. 2009), a different choice of depths for the layers in the mesocosms may
412 have led to different results. Also, other types of resource partitioning (e.g., temporal
413 partitioning and partitioning of different chemical forms) are known and a combination of
414 several may be necessary to explain positive biodiversity–productivity effects.

415 **Conclusions**

416 We conclude that our eight experimental species are generalists rather than specialists
417 regarding N uptake from deep and shallow soil. Also, our results do not support the role of
418 vertical N partitioning as an important driver of positive biodiversity effects on the N
419 capture of mixtures in temperate grasslands. With regard to preferred N uptake from the

420 shallow soil layer and the decrease in proportional similarity of species at increasing levels of
421 species richness, our results match those of an earlier experiment (von Felten et al. 2009).
422 This is notable, since quite different experimental approaches were used (different species
423 pools, mesocosms vs. field, organic ^{15}N label vs. tracer solutions, different choice of soil
424 depths for the shallow and deep layer). Although we observed a decrease in proportional
425 similarity of species (here with regard to N uptake from different soil depths and earlier with
426 regard to N uptake from different soil depths and N forms) both experiments revealed only
427 limited evidence for N partitioning.

428 A novel aspect of our mesocosm experiment is the specific test whether and how interspecific
429 differences in N uptake from shallow and deep soil lead to enhanced community N uptake at
430 higher levels of species richness. Based on the positive relationship between plant species
431 richness and productivity repeatedly shown in grassland experiments, and differences in
432 species' capacity to assimilate different chemical forms of N or to exploit different soil depths
433 (see references in introduction), N partitioning has been proposed as an important driver.
434 However, our results do not support this hypothesis, as we observed no effect of species
435 richness on community N uptake. On the one hand, the increase in biomass with species
436 richness was paralleled by a decrease in N concentration. On the other hand, community
437 composition was more important than species richness in determining community N uptake.
438 Future studies on resource partitioning should incorporate multidimensional differentiation
439 of resource niches among coexisting plant species, including spatial and temporal
440 differentiation, and different nutrients. Moreover, theoretical models are needed to further
441 assess the importance of resource partitioning as opposed to other mechanisms that can
442 drive positive diversity–productivity relationships, such as reduced relative fitness differences
443 (so called stabilizing effects, see Chesson 2000, Carroll et al. 2011).

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586 Appendices

587 **Appendix A** Table: Mean ^{15}N enrichment of aboveground biomass by harvest and
588 treatment and natural background ^{15}N .

589 **Appendix B** Table: Analysis of Variance for ^{15}N enrichment of aboveground biomass.

590 **Appendix C** Table: Analysis of Variance for ^{15}N uptake from deep soil (DF) in
591 monocultures

592 **Appendix D** Table: Analysis of Variance for the fraction of ^{15}N tracer taken up from deep
593 soil (DF) by populations of individual species grown in mixture.

594 **Appendix E** Figure: Fraction of ^{15}N uptake from deep soil (DF) and aboveground biomass
595 per species in all monocultures and mixtures.

596 **Appendix F** Figure: Relationship between the rank of each species in aboveground
597 biomass and its rank in the deep fraction DF (fraction of ^{15}N uptake from deep soil)
598 within mixture.

599 **Appendix G** Figure: Plant community biomass (above- and belowground) as a function of
600 the calculated community niche for each harvest.

Table 1: **Experimental Design:** Combinations of plant species composition and ^{15}N treatments. Plant communities were randomly assigned to boxes, that were subdivided in eight (2×4) compartments. ^{15}N treatments were randomly applied to sets of four adjacent compartments per box (treatment unit). We show the numbers of harvested compartments 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 species pool DLRT, including *Dactylis glomerata* (D), *Lolium perenne* (L), *Ranunculus acris* (R) and *Taraxacum officinale* (T), and for both pools together (overall). Since in this paper, only the ^{15}N labeled compartments are considered (deep and shallow), the numbers including compartments without ^{15}N are given in brackets. Three destructive harvests were conducted, at each of which one out of four compartments was harvested per treatment unit (all harvested ^{15}N labeled compartments: $n=96 \times 3=288$). Note that we refer to all plants in one compartment as community, and to individual species within a compartment as populations. Nomenclature follows Lauber and Wagner (1998).

| Pool AHLP ^a | Species composition | | | | | | | | | | | all |
|------------------------|---------------------|-------|-------|-------|-------|-------|-----------|-------|-------|-----------|--------|-----------------|
| | A | H | L | P | AH | AL | AP | HL | HP | LP | AHLP | |
| Deep | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 4 | 24 |
| Shallow | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 4 | 24 |
| No ^{15}N | (2) | (2) | (2) | (2) | (2) | (2) | (2) | (2) | (2) | (2) | (4) | (24) |
| Total | 4 (6) | 4 (6) | 4 (6) | 4 (6) | 4 (6) | 4 (6) | 4 (6) | 4 (6) | 4 (6) | 4 (6) | 8 (12) | 48 (72) |
| Pool DLRT ^b | D | L | R | T | DL | DR | DT | LR | LT | RT | DLRT | all |
| Total | 4 (6) | 4 (6) | 4 (6) | 4 (6) | 4 (6) | 4 (6) | 4 (6) | 4 (6) | 4 (6) | 4 (6) | 8 (12) | 48 (72) |
| Overall | Monocultures | | | | | | 2 Species | | | 4 Species | | all |
| Compartments | 32 (48) | | | | | | 48 (72) | | | 16 (24) | | 96 (144) |
| Populations | 32 (48) | | | | | | 96 (144) | | | 64 (96) | | 192 (288) |
| Boxes | 24 (24) | | | | | | 36 (36) | | | 12 (12) | | 72 (72) |

^a *A. elatius* and *H. lanatus* are grasses, *L. vulgare* and *P. lanceolata* are forbs.

^b *D. glomerata* and *L. perenne* are grasses, *R. acris* and *T. officinale* are forbs.

Table 2: Analysis of Variance for the fraction of ^{15}N tracer taken up from deep soil (DF) by populations of individual species grown in mixture ($n=240$). A linear mixed effects model with the random factors species pool, species composition (see Table 1) and compartment pair was used. Significance levels: . $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$. See Appendix Table 4 for the same analysis but with species replaced by functional group (grasses vs. forbs).

| Deep Fraction DF | | | | | |
|--------------------------------|---------|---------|-------|---------|-----|
| Source | Num. df | Den. df | F | $P(>F)$ | |
| Species richness (SR) | 1 | 11 | 0.26 | 0.62 | |
| Species | 7 | 52 | 21.01 | <.001 | *** |
| SR \times Species | 7 | 52 | 0.64 | 0.72 | |
| Harvest (H) ^a | 2 | 128 | 66.02 | <.001 | *** |
| SR \times H | 2 | 128 | 2.67 | 0.07 . | |
| Species \times H | 14 | 128 | 4.69 | <.001 | *** |
| SR \times Species \times H | 14 | 128 | 1.23 | 0.26 | |

^a Harvests: August 2005, September 2005 and May 2006.

Table 3: Analysis of Variance for proportional similarity with regard to N uptake from shallow and deep soil between pairs of species ($n=288$). A linear mixed effects model with the random factors species pool, species composition (see Table 1) and specific pair was used. Specific pairs are the pairs of species (either two monocultures or two species within a mixture community) between which proportional similarity was calculated ($n=96$) at each of three harvests. Significance levels: . $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$).

| Source | Proportional similarity | | | | |
|------------------------------------|-------------------------|---------|--------|---------|-----|
| | Num. df | Den. df | F | $P(>F)$ | |
| Species richness (SR) ^a | 2 | 12 | 19.422 | 0.0002 | *** |
| Species pair (SP) | 11 | 69 | 2.404 | 0.0137 | * |
| Harvest (H) ^b | 2 | 164 | 1.643 | 0.1967 | |
| SR \times Harvest | 4 | 164 | 2.863 | 0.0251 | * |
| SP \times Harvest | 22 | 164 | 2.246 | 0.0021 | ** |

^a Note that the difference between monocultures and mixtures would be highly significant if fitted instead of SR ($F_{1,13}=39.21$, $P<0.001$) and that the log-linear effect of SR is significant too ($F_{1,13}=10.03$, $P=0.007$).

^b Harvests: August 2005, September 2005 and May 2006.

601 **Figure Legends**

- 602 1 Proportional similarity with regard to N uptake from shallow and deep soil
 603 between pairs of species, at different levels of species richness and at differ-
 604 ent harvests, as estimated from a mixed-effects model. Error bars show 95 %
 605 confidence intervals. The model is as shown in Table 3, except for the effect
 606 of species pair that was not included here. Note that the ordinate covers the
 607 whole potential range of proportional similarity values. 30
- 608 2 Plant community N content (in above- and belowground biomass) as a function
 609 of the calculated community niche for each harvest. The positive relationship
 610 is indicated by regression lines, including a 95 % confidence interval. Note that
 611 the community niche for the mixtures was determined *a priori* from ¹⁵N uptake
 612 by individual plant species from deep and shallow soil (Eq. 6), whereas for the
 613 monocultures, it equals ¹⁵N uptake from deep and shallow soil by one species
 614 only. 31

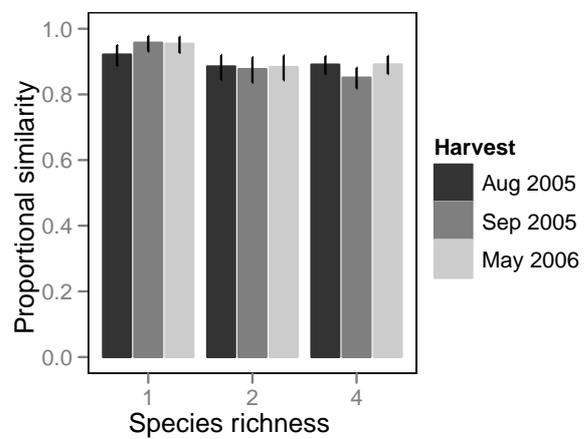


Fig. 1:

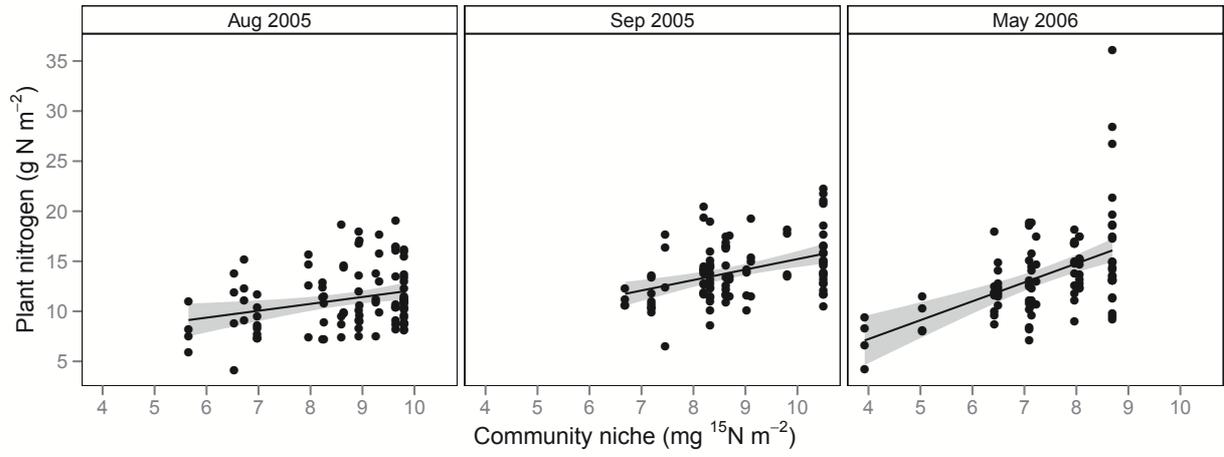


Fig. 2: