Functional Ecology 2002
16, 454–461

Interspecific and spatial differences in nitrogen uptake in monocultures and two-species mixtures in north European grasslands

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Summary

1. To study the potential for complementarity in nitrogen acquisition from different soil depths, we injected an isotope tracer ($^{15}NH_4Cl$) at 5 and 20 cm depths in plant communities containing *Achillea millefolium* L. and *Festuca ovina* L. or *Phleum pratense* L. and *Trifolium pratense* L. in monocultures and two-species mixtures.
2. In monoculture, *Festuca* and *Phleum* took up tracer at 5 and 20 cm depths. In contrast, *Achillea* and *Trifolium* monocultures acquired the tracer mainly from 5 cm depth. In two-species mixtures, all four species took up tracer at 5 cm depth.
3. *Achillea* N acquisition from 20 cm depth increased in mixture with *Festuca* in comparison to that in monoculture; *Festuca* N acquisition from 20 cm depth decreased, although not significantly. *Trifolium* N acquisition remained unchanged when grown in mixture with *Phleum*. *Phleum* behaved like *Festuca*: its N acquisition from 20 cm depth in mixture was reduced in comparison to monoculture.
4. Our data on *Festuca* and *Achillea* support spatial partitioning in resource acquisition. This was not evident in *Phleum* and *Trifolium* mixture, potentially because *Trifolium* relied on N_2 fixation as N source.
5. These results demonstrate spatial variation among plant species and plant communities in their N acquisition in the field.

Key-words: Nitrogen, nutrient uptake, root length, stable isotope

Functional Ecology (2002) **16**, 454–461

Introduction

Explanatory mechanisms for the coexistence of plants in diverse communities have been sought in niche partitioning. Spatial distribution of roots and temporal separation of nutrient uptake have been proposed as possible below-ground mechanisms of such partitioning in plant communities (Berendse 1981; Berendse 1982; McKane, Grigal & Russelle 1990; Parrish & Bazzaz 1976; Pechácová *et al*. 1999; Veresoglou & Fitter 1984; Yeaton, Travis & Gilinsky 1977). The spatial distribution of roots may be affected by a variety of biotic and abiotic factors. Root growth and proliferation are frequently responsive to resource availability (Arredondo & Johnson 1999; Caldwell, Manwaring & Jackson 1991b; Crick & Grime 1987; Fitter 1994; Robinson 1994; Tibbett 2000). Similarly, environmental stresses such as drought can modify rooting depth (Mamolos, Elisseou & Veresoglou 1995; Reader *et al*. 1992). Finally, interference by adjacent roots, via either competition and resource depletion (Caldwell, Manwaring & Durham 1991a; Caldwell, Manwaring & Durham 1996; D'Antonio & Mahall 1991; Mahall & Callaway 1991) or root-originating allelopathic or inhibitory compounds (Mahall & Callaway 1991; Mahall & Callaway 1992) have also been proposed to be important mechanisms in governing root distribution in soil matrix. Taken together, community composition and resource availability may influence the distribution of roots in plant communities and subsequent nutrient acquisition.

A model presented by Berendse (1979) predicts that a partitioning of vertical rooting depth would result in greater resource acquisition and increase in relative yield totals (according to de Wit & van den Bergh

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1965) in the community. Indeed, when deep-rooting *Plantago lanceolata* was combined in a mixture with a shallow-rooting *Antoxanthium odoratum*, *P. lanceolata* – potentially the inferior competitor in the shallower soil profiles – was forced to utilize deeper soil layers (Berendse 1982). Berendse's model does not directly address spatial or temporal heterogeneity of soil resources. However, it suggests that species may differ in their abilities to exploit soil resources, whether or not they are heterogeneously distributed in time or space. Similarly to Berendse's predictions (Berendse 1979) and experimental work (Berendse 1982), D'Antonio & Mahall (1991) recorded greater rooting depths of the native *Haplopappus* spp. when an adjacent, shallow-rooted invader (*Carpobrotus edulis*) was present in Californian coastal shrublands. Other studies have also shown that species differ in their rooting depths, but have not explicitly addressed whether rooting depths were altered by community composition, or whether or not these differences in rooting depths result in different resource exploitation capabilities (Fitter 1986; Mamolos *et al*. 1995; McKane *et al*. 1990).

Recently, the connection between community composition or species richness and ecosystem function has received considerable attention. Although a larger proportion of the positive effect of the species richness on productivity may be attributable to a probability of including certain species in the communities – the 'sampling effect' (Aarssen 1997; Hector 1998; Tilman 1999) – complementary resource use may have an additional effect (Hector 1998; Hector *et al*. 1999; Spehn *et al*. 2000; Tilman 1999). We aimed to test if it is possible to detect differential capabilities for nutrient acquisition among species growing in monocultures and two-species mixtures. We examined if naturally occurring species in northern Sweden would acquire nitrogen at different depths when grown in monoculture than when grown in mixture. Differing abilities in N acquisition from different soil depths suggest potential among species to utilize resources in different soil depths, whether they are heterogeneously or homogeneously distributed.

Materials and methods

SITE DESCRIPTION AND EXPERIMENTAL DESIGN

The field site was located at the Swedish University of Agricultural Sciences in Umeå, Sweden (63°45′ N, 20°17′ E, 12 m a.s.l.). The soil type was a fine silty sand with little clay (4·1% clay, 57·9% silt, 38·0% fine sand), pH 6·0. In the 3 years prior to the experiment, the field had been used for barley, potato and barley cultivation. It had been fertilized every year, the last being 1995, with an application of 400 kg ha^{-1} N-P-K (11-5-18). During the summer of 1995, 7·5 g ha^{-1} herbicide (Expresspreparat, DuPont Agro, Malmö, Sweden) was applied to reduce weed growth. The last barley crop was harvested in the fall of 1995, and the site was ploughed without removal of straw. In spring 1996 the site was repeatedly harrowed. No fertilizer was added from 1996 onwards. As a result of this agricultural history, the field was considered adequately homogeneous to allow comparisons among the different, adjacent, human-made communities established for this and other experiments.

Six plant communities, four monocultures and two mixtures, containing perennial, native vascular plants (*Festuca ovina* L. and *Achillea millefolium* L. or *Phleum pratense* L. and *Trifolium pratense* L.) were established in June, 2 years before conducting the tracer injection experiment. We selected these two pairs of species because within each pair, individuals reach approximately the same above-ground height, but the species have different rooting patterns with respect to root length. Furthermore, species within each pair share similar habitat requirements: *Festuca* and *Achillea* occur typically on dry sites, whereas *Phleum* and *Trifolium* are commonly cultivated as forage species and occur naturally on roadsides and meadows. The two herbs form a dominant tap root, whereas the two grasses have fibrous root systems.

The six plant communities were sown on six separate rectangles, each measuring 5·0 × 2·2 m. All communities received a total density of 2000 seeds m^{-2}, i.e. monocultures received 2000 seeds m^{-2} of one single species, while two-species mixtures received 1000 seeds m^{-2} of each of the two species.

ROOT LENGTH

Within each plant community, at the peak of the growing season in early August 1998, two random soil samples 20 cm deep were collected with a 4 cm diameter corer. The samples were divided into two depths: 0–10 and 10–20 cm. Each sample was cleaned free of soil on a screen under running tap water, spread evenly on a transparent screen, and digitized using a flatbed scanner. We were unable to separate roots in the mixtures. Therefore the total root lengths for both monocultures and mixtures are presented in the results. The root lengths were estimated from the digitized images with the DELTA-T SCAN image analysis system (DELTA-T Devices Ltd, Cambridge, UK).

INJECTION OF ^{15}N AND FOLIAR SAMPLE PROCESSING

Within each of the six communities with different species composition, we selected 10 representative plots measuring 10 × 10 cm. To minimize isotope dilution effects, individual plants of any given species were visually judged to be equal in size among the 10 plots. These 10 plots were then randomly assigned to two different N tracer treatments (see below), five for each injection depth. This design totalled five replicates of

Table 1. Plant communities used in the study. ^{15}N tracer was injected at two depths (5 and 20 cm) in each of the five plots within the six communities with different species composition. Five plots of each community were used for each of the two injection depths

Community	Species
Monoculture	*Achillea millefolium* L.
Monoculture	*Festuca ovina* L.
Mixture	*Achillea millefolium* L. and *Festuca ovina* L.
Monoculture	*Phleum pratense* L.
Monoculture	*Trifolium pratense* L.
Mixture	*Phleum pratense* L and *Trifolium pratense* L.

two different injection depths within six plant communities; 60 plots in total (Table 1). Individuals of all four species were flowering at the time of injection in monocultures and mixtures, indicating close similarities in phenology.

All plant communities were injected with ^{15}N tracer within a 2 h period in mid-July 1998. No precipitation occurred during the injection experiment. A well for ^{15}N injection, 5 or 20 cm deep, was created by pushing an open-ended plastic cylinder (≈7 mm diameter) into the ground in the four mid-points of the lines defining the 10×10 cm plot. A total of 12·5 ml of 2·133 mM $^{15}NH_4Cl$ (98 at % ^{15}N) solution was injected into each of the four wells in each plot. The total N injected per plot equalled ≈0·16 g m^{-2}, a minute quantity compared to the total soil N pool (total N 1·3 mg g^{-1}), but substantial with regard to NO_3^- (0·06 mg $NO_3^- l^{-1}$ soil water and 0·33 mg $NO_3^- l^{-1}$ soil water in plots with *Phleum* and *Phleum* plus *Trifolium*, respectively) and NH_4^+ (0·4 μg $NH_4^+ l^{-1}$ soil water and 0·8 μg $NH_4^+ l^{-1}$ soil water in plots with *Phleum* and *Phleum* plus *Trifolium*, respectively; Cecilia Palmborg, unpublished lysimeter data, August 1998).

Approximately 10 mg (dry weight) of the topmost, green leaves were collected before injection. The individual plants were always adequately large, or the plots contained more than one individual, to allow repeated harvests from the same layer of the canopy within a plot. The sampling was repeated 2, 6 and 24 h after injection. All foliar samples were dried at 60 °C for 24 h and homogenized with a ball mill. The samples were analysed for ^{15}N abundance using a Europa Scientific (Crewe, UK) isotope ratio mass spectrometer interfaced with a Europa Scientific ANCA-NT preparation module (Ohlsson & Wallmark 1999). Results for ^{15}N abundance before injection are expressed in the standard notation ($\delta^{15}N$) in parts per thousand (‰) relative to the international standard (atmospheric N_2 at 0·3663 at %, Junk & Svec 1958; Mariotti 1983):

$$\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where R = mole ratio of $^{15}N/^{14}N$.

Results after injection are expressed as increase in concentration of the heavy isotope in dried foliar tissue (nmol of ^{15}N mg^{-1} dry foliar tissue) resulting from the injection, i.e. the difference between the ^{15}N concentration before injection and at 2, 6 and 24 h after injection. We assumed that the applications of N would not significantly affect the growth of plants, and that creating an N-enriched patch would not affect the spatial distribution of roots during the short 24 h period. Furthermore, we mainly estimated whether or not the uptake occurred, rather than making quantitative comparisons among the target species.

SOIL SAMPLES

After the final foliar sample was collected, 24 h after injection, a soil core 2·5 cm in diameter was removed from a depth of 30 cm in the centre of each plot. Two samples representing the 0–10 and 15–25 cm horizons were separated from each soil core. The soil samples were dried at 80 °C for 48 h and homogenized using a rod mill. The samples were analysed for ^{15}N abundance, as described above.

Results

Although the differences were frequently non-significant (Figs 1 and 2, least squares means pairwise comparisons; SAS 1989), greater root lengths generally occurred at 0–10 than 10–20 cm depth (Tables 2 and 3, ANOVA; SAS 1989). *Achillea* monoculture had few roots deeper in the soil, significantly fewer than *Festuca* monoculture. *Phleum* had significantly greater root length at 0–10 than at 10–20 cm, while *Trifolium* had the same root length at both soil depths. These data agree with our earlier observations and thus supported our choice of species for this study.

Tracer injection at the two different soil depths was successful (soil $\delta^{15}N$ before experiment 4·4 ± 1·0‰;

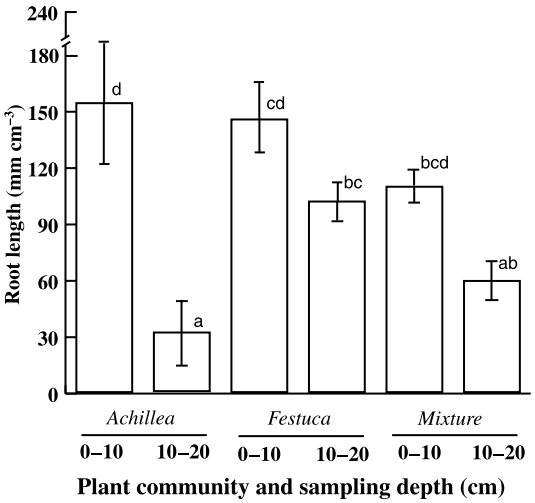

Fig. 1. Root-length density (mm cm^{-3}; mean ± 1 SD) in monocultures and a mixture of *Achillea millefolium* and *Festuca ovina* at two soil depths (0–10 and 10–20 cm). Different letters indicate significant differences at $\alpha = 0{\cdot}05$ based on least squares means pairwise comparisons.

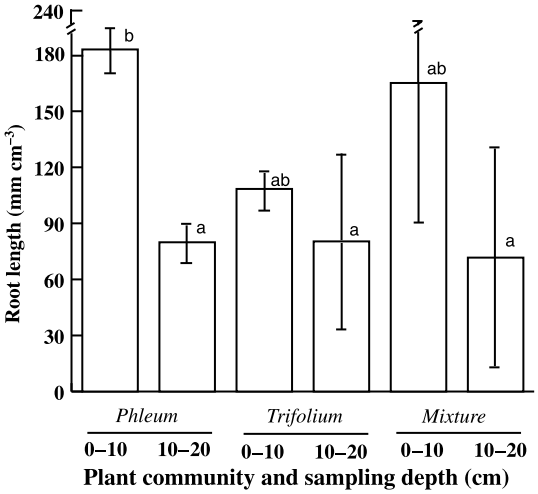

Fig. 2. Root-length density (mm cm^{-3}; mean ± 1 SD) in monocultures and a mixture of *Phleum pratense* and *Trifolium pratense* at two soil depths (0–10 and 10–20 cm). Different letters indicate significant differences at $\alpha = 0{\cdot}05$ based on least squares means pairwise comparisons.

Table 2. ANOVA of root lengths at two sampling depths (0–10 and 10–20 cm) among plant communities comprising *Achillea millefolium* and *Festuca ovina* monocultures and mixture

Source	df	*F*	*P*
Community	2	4·56	0·062
Sampling depth	1	43·4	0·001
Interaction	2	5·83	0·039

Table 3. ANOVA of root lengths at two sampling depths (0–10 and 10–20 cm) among plant communities comprising *Phleum pratense* and *Trifolium pratense* monocultures and mixture

Source	df	*F*	*P*
Community	2	0·95	0·439
Sampling depth	1	10·6	0·018
Interaction	2	1·00	0·421

after injection 11·6 ± 4·7‰; mean ± 1 SD). Although there were no significant differences in ^{15}N isotope composition among the soil samples (ANOVA; SAS 1989), the tracer mainly remained in the soil layer to which it was injected (data not shown). The fact that NH_4^+ is less mobile than NO_3^- is an advantage in this type of experiment, and may have contributed to minimal ^{15}N leakage between the two soil horizons studied.

The $\delta^{15}N$ data for leaves collected before injection were left untransformed and analysed by ANOVA (SAS 1989). *Festuca* ($\delta^{15}N$ 4·52 ± 0·60‰) and *Achillea* ($\delta^{15}N$ 5·41 ± 0·47‰) monoculture $\delta^{15}N$ did not differ before injection (Table 4). However, plants in mixed communities were more ^{15}N-enriched than in monocultures (*Festuca* $\delta^{15}N$ 6·17 ± 0·95, *Achillea* $\delta^{15}N$ 6·80 ± 2·34). In contrast, monocultures of *Phleum* ($\delta^{15}N$ 7·58 ± 1·17‰) and *Trifolium* ($\delta^{15}N$ 0·31 ± 0·16) clearly differed at the

Table 4. ANOVA of initial $\delta^{15}N$ values in *Achillea millefolium* and *Festuca ovina* monocultures and mixture

Source	DF	*F*	*P*
Species	1	1·99	0·174
Mixture	1	7·98	0·011
Interaction	1	0·06	0·803

Table 5. ANOVA of initial $\delta^{15}N$ values in *Phleum pratense* and *Trifolium pratense* monocultures and mixture

Source	df	*F*	*P*
Species	1	269·71	0·001
Mixture	1	14·44	0·001
Interaction	1	7·53	0·013

time of injection (Table 5): *Phleum* was more enriched than *Trifolium* in ^{15}N. When in mixture, *Phleum* ($\delta^{15}N$ 5·09 ± 0·71) was less enriched in ^{15}N than in monoculture, while *Trifolium* in mixture (−0·32 ± 0·58) did not differ from *Trifolium* in monoculture.

The foliar ^{15}N excess data were analysed separately by ANOVA (SAS 1989) for different sampling times after tracer injection, because of increasing variance with time since injection. The data were $\log_{10}$-transformed because of large differences in variance among the different treatments within a given sampling time. All species took up the injected ^{15}N, and this was usually evident from sampling of the topmost leaves within 2 h after injection. The ^{15}N excess increased with time since injection. For brevity and clarity, the results are presented separately for each species and each point in time (Tables 6–9), but focus on the final sampling time, 24 h after injection.

Achillea when in monoculture took up tracer exclusively from 5 cm depth (Table 6). In contrast, *Festuca* when in monoculture acquired tracer equally from both 5 and 20 cm depths (Table 7). When in mixture, both *Festuca* and *Achillea* took up tracer at 5 cm depth. However, *Festuca* N acquisition from 20 cm depth in mixture was reduced in comparison to that in monoculture, although not significantly. *Achillea* N acquisition in mixture, in contrast, was greater from 20 cm depth in comparison to that in monoculture. *Achillea* N acquisition from 20 cm depth in monoculture barely increased over the experimental period.

Phleum had a significant uptake of tracer both from 5 and 20 cm depth when in monoculture (Table 8). Although the differences in the acquired quantities were large, on average four times greater at 5 than at 20 cm, they were not significant. *Trifolium* acquired soil ^{15}N nearly exclusively from 5 cm depth (Table 9). When in mixture, both *Phleum* and *Trifolium* acquired tracer from 5 cm depth. *Phleum* N acquisition from 20 cm depth in mixture remained small throughout the experiment, and was very small in comparison to monoculture. *Trifolium* acquired soil N nearly

Table 6. *Achillea millefolium* ^{15}N excess (mean ± 1 SD) after tracer injection. Different letters in the ^{15}N excess column indicate significant differences at $\alpha = 0{\cdot}05$ based on least squares means pairwise comparisons of $\log_{10}$-transformed values within that sampling time

				ANOVA			
Time (h)	Community	Injection (cm)	^{15}N excess (nmol mg^{-1})	Source	df	*F*	*P*
2	Monoculture	5	22·6 ± 24·6a				
	Monoculture	20	2·51 ± 4·16a	Mixture	1	0·05	0·829
	Mixture	5	1·00 ± 0·30a	Depth	1	2·26	0·157
	Mixture	20	0·41 ± 2·29a	Interaction	1	1	0·336
6	Monoculture	5	32·7 ± 24·4a				
	Monoculture	20	0·99 ± 0·83a	Mixture	1	0·46	0·507
	Mixture	5	5·74 ± 7·87a	Depth	1	0	0·959
	Mixture	20	0·51 ± 0·39a	Interaction	1	2·29	0·153
24	Monoculture	5	185 ± 256c				
	Monoculture	20	3·97 ± 3·73a	Mixture	1	3·92	0·069
	Mixture	5	86·0 ± 30·9bc	Depth	1	27·0	0·001
	Mixture	20	26·7 ± 18·9b	Interaction	1	5·55	0·035

Table 7. *Festuca ovina* ^{15}N excess (mean ± 1 SD) after tracer injection. Different letters in the ^{15}N excess column indicate significant differences at $\alpha = 0{\cdot}05$ based on least squares means pairwise comparisons of $\log_{10}$-transformed values within that sampling time

				ANOVA			
Time (h)	Community	Injection (cm)	^{15}N excess (nmol mg^{-1})	Source	df	*F*	*P*
2	Monoculture	5	4·68 ± 2·58a				
	Monoculture	20	1·75 ± 1·23a	Mixture	1	0·28	0·604
	Mixture	5	6·01 ± 5·89a	Depth	1	2·43	0·141
	Mixture	20	1·48 ± 0·45a	Interaction	1	0·23	0·642
6	Monoculture	5	20·2 ± 25·9a				
	Monoculture	20	1·92 ± 0·48a	Mixture	1	1·93	0·186
	Mixture	5	8·98 ± 5·98a	Depth	1	5·51	0·034
	Mixture	20	0·72 ± 0·77a	Interaction	1	1·78	0·204
24	Monoculture	5	87·2 ± 83·0a				
	Monoculture	20	88·3 ± 85·2a	Mixture	1	0·37	0·554
	Mixture	5	73·5 ± 25·8a	Depth	1	0·91	0·356
	Mixture	20	21·6 ± 23·7a	Interaction	1	2·94	0·109

Table 8. *Phleum pratense* ^{15}N excess (mean ± 1 SD) after tracer injection. Different letters in the ^{15}N excess column indicate significant differences at $\alpha = 0{\cdot}05$ based on least squares means pairwise comparisons of $\log_{10}$-transformed values within that sampling time

				ANOVA			
Time (h)	Community	Injection (cm)	^{15}N excess (nmol mg^{-1})	Source	df	*F*	*P*
2	Monoculture	5	12·3 ± 11·7a				
	Monoculture	20	1·07 ± 0·52a	Mixture	1	0·84	0·373
	Mixture	5	2·82 ± 0·82a	Depth	1	2·81	0·113
	Mixture	20	1·83 ± 1·50a	Interaction	1	0·02	0·892
6	Monoculture	5	162 ± 261b				
	Monoculture	20	21·1 ± 13·4b	Mixture	1	4·21	0·059
	Mixture	5	45·8 ± 23·0b	Depth	1	15·8	0·001
	Mixture	20	5·20 ± 2·20a	Interaction	1	1·22	0·286
24	Monoculture	5	336 ± 275b				
	Monoculture	20	82·8 ± 23·5b	Mixture	1	3·43	0·091
	Mixture	5	86·7 ± 59·7b	Depth	1	3·30	0·097
	Mixture	20	5·89 ± 3·98a	Interaction	1	0·28	0·604

Table 9. *Trifolium pratense* ^{15}N excess (mean ± 1 SD) after tracer injection. Different letters in the ^{15}N excess column indicate significant differences at $\alpha = 0{\cdot}05$ based on least squares means pairwise comparisons of $\log_{10}$-transformed values within that sampling time

Time (h)	Community	Injection (cm)	^{15}N excess (nmol mg^{-1})	ANOVA Source	df	*F*	*P*
2	Monoculture	5	1·71 ± 1·00a				
	Monoculture	20	−0·24 ± 1·18a	Mixture	1	4·23	0·058
	Mixture	5	1·96 ± 0·98a	Depth	1	0·83	0·378
	Mixture	20	1·15 ± 0·42a	Interaction	1	1·10	0·311
6	Monoculture	5	29·8 ± 24·4a				
	Monoculture	20	0·82 ± 0·29a	Mixture	1	0	0·981
	Mixture	5	15·0 ± 10·1a	Depth	1	0·21	0·651
	Mixture	20	0·67 ± 0·55a	Interaction	1	0	0·975
24	Monoculture	5	29·0 ± 13·6b				
	Monoculture	20	1·42 ± 1·42a	Mixture	1	0·82	0·379
	Mixture	5	26·9 ± 12·6b	Depth	1	53·3	0·001
	Mixture	20	1·56 ± 0·55 a	Interaction	1	0·96	0·343

exclusively from 5 cm depth even when in mixture: its N acquisition remained unchanged when grown in mixture with *Phleum*.

Discussion

The goals of the present study were to observe N uptake from two different soil horizons among four native grassland species when a pulse of $^{15}NH_4^+$ was injected directly into soil. We created uniquely ^{15}N-enriched patches which were placed either at 5 or 20 cm soil depth. The added $^{15}NH_4^+$ solution had an N concentration five orders of magnitude greater than the soil solution. Although isotopic exchange with endogenous N pools may confound the interpretation of injection studies similar to ours, the $^{15}NH_4^+$ patches in our study were so concentrated in N that minor differences in endogenous available N between soil depths or between monocultures or species mixtures should not affect our interpretations. Additionally, our emphasis lies on the depth of soil N acquisition in one plant species when it occurred with another species, as compared to when it grew alone. Finally, to minimize the potential isotope dilution effects, we specifically selected plant individuals of comparable size.

All species acquired soil N from the upper horizon, whereas the deeper horizon was utilized to a lesser extent. However, our data suggest that the species differed in their ability to utilize N from deeper soil, and the N acquisition was different between individuals grown in a monoculture and in a mixture.

After 24 h, *Achillea* had acquired only minimal quantities of N tracer from the 20 cm depth in monoculture. When grown with *Festuca*, *Achillea* N acquisition from that horizon increased. In contrast, *Festuca* N acquisition from that horizon decreased, although not significantly. These data support niche differentiation among plant species in mixtures: N acquisition depends on whether or not neighbours are present. It remains unclear from our study whether or not the observed differences in N uptake represent changes in rooting depth or root activity. Earlier theory (Berendse 1979), as well as greenhouse and field experiments (Berendse 1981; Berendse 1982; D'Antonio & Mahall 1991) suggest that rooting depths can be altered depending on the presence of neighbours.

Changes in the spatial distribution of roots have frequently been reported in response to uneven or patchy distribution of nutrients (Arredondo & Johnson 1999; Caldwell *et al*. 1991b; Hodge *et al*. 2000; Jackson & Caldwell 1991; Robinson 1994; Tibbett 2000; van Vuuren, Robinson & Griffiths 1996) or other abiotic factors such as drought (Reader *et al*. 1992; Rhizopoulou & Davies 1991). We collected foliar tissues within 24 h after tracer injection. Although exceptionally fast proliferation and nutrient uptake in response to addition of liquid fertilizer has been reported (Jackson & Caldwell 1989; Jackson, Manwaring & Caldwell 1990), plant response to fertile patches in soil usually requires more time – days or weeks rather than hours (Tibbett 2000; van Vuuren *et al*. 1996). Plant proliferation responses to nutrient availability also require relatively large nutrient patches (Hodge *et al*. 2000; Jackson & Caldwell 1991; van Vuuren *et al*. 1996). Therefore it is unlikely that the plants responded to increased supply of N or other abiotic changes resulting from tracer injection. Resource competition or inhibitory mechanisms in mixed communities may control the spatial distribution of roots (Caldwell *et al*. 1991a; Caldwell *et al*. 1996; Mahall & Callaway 1991; Mahall & Callaway 1992). Such mechanisms could result in the observed changes in N acquisition when the community complexity increases.

Our data suggest that *Achillea* and *Festuca* in monocultures and mixtures differ in the depth of N acquisition. As indicated by our root length data, both species occupied both soil depths. However, when in monoculture *Achillea* had a greater root length in the

upper soil horizon. Plants frequently concentrate roots in the uppermost soil layers (Cook & Ratcliff 1984; Fitter 1986; Mamolos *et al*. 1995). Nevertheless, species differ in their ability to obtain nutrients from different soil layers (Berendse 1982; Fitter 1986; Mamolos *et al*. 1995; McKane *et al*. 1990; Veresoglou & Fitter 1984).

Several factors may explain our observations. First, we do not know to what extent the plants took up ^{15}N as ammonium or nitrate. We have seen previously (Näsholm, Huss-Danell & Högberg 2000) that *Phleum* and *Trifolium* can take up ^{15}N from enriched nitrate at this field site. Although we added ^{15}N as ammonium, it is possible that some of it was nitrified during the 24 h period. Nitrification rates at the two soil depths are not known. Second, plant species may differ in their ability to compete for ammonium as well as for nitrate. Third, different plant species may also differ in their rhizosphere flora (Grayston & Campbell 1996; Westover, Kennedy & Kelley 1997) and in the extent to which nitrate can be immobilized in the rhizosphere. Nitrogen acquisition by plants involves more than root length and spatial root distribution.

Fitter (1986) questioned if spatial separation of roots or their activity could be observed in more diverse alluvial grassland communities. Based on the results from natural, species-rich grassland communities, it was concluded that temporal segregation of resource acquisition was essential, whereas there was little support for differentiation in the depth of root activity (Fitter 1986; Veresoglou & Fitter 1984). Our data on *Festuca* and *Achillea* support spatial partitioning in N acquisition. It is unclear whether N acquisition would differ within the growing season; the current study provides only a snapshot in time. Our data from the *Trifolium* and *Phleum* mixture, however, provide little support for vertical resource partitioning: *Trifolium* did not acquire N from the deeper horizon in monoculture or in mixture with *Phleum*. It is possible that *Trifolium* depends more heavily on its symbiotic N_2 fixation in monoculture and in mixed communities, and that its use of soil N is confined to the uppermost soil layer. In support of this, we also observed differences among species in their isotopic composition prior to tracer injection. The initial $\delta^{15}N$ data indicated two different patterns: (i) *Festuca* and *Achillea* were more enriched in ^{15}N when grown together than they were in monoculture; (ii) when in mixture with *Trifolium*, *Phleum* was less enriched in ^{15}N than in monoculture. Furthermore, *Phleum* had nearly double the foliar N concentration [26·4 *vs* 14·3 mg g^{-1} (DW)] in the mixed community with *Trifolium* when compared to the monoculture. This indicates that N_2 fixation associated with *Trifolium* probably contributed to the N economy of the mixed plant communities.

The results from our study utilizing two sets of monocultures and mixtures suggest that spatial partitioning in N uptake may be important in some plant communities, but not in others. The *Achillea* and *Festuca* mixture seemed to support vertical separation in N acquisition, whereas the *Phleum* and *Trifolium* mixture showed no such separation, but *Trifolium* probably relied on its symbiotic N_2 fixation, thus avoiding competition for available soil N. In conclusion, complementary resource use may help to maintain species diversity and minimize competition for resources in some plant communities, but plays a lesser role in others.

Acknowledgements

This project is part of the 'Biodiversity and Ecosystem Function in Terrestrial Herbaceous Plant Communities' (BIODEPTH) project funded by the European Commission (Framework IV, Environment and Climate Program, ENV-CT95-0008). Additional funding was generously provided by the Kempe Foundation (Sweden) and the National Science Foundation EPS-CoR Grant no. 9874732, with matching support from the State of Kansas. Elisabeth Bärlund, Sanna Forsberg and Sebastian Marklund assisted in various stages of experiment maintenance and sample processing. Håkan Wallmark performed all isotope composition analyses. The authors are grateful for permission to use Dr Cecilia Palmborg's unpublished soil nutrient data.

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Received 24 January 2002; accepted 25 January 2002