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Foliar δ^{15}N values characterize soil N cycling and reflect nitrate or ammonium preference of plants along a temperate grassland gradient

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Abstract The natural abundance of stable ^{15}N isotopes in soils and plants is potentially a simple tool to assess ecosystem N dynamics. Several open questions remain, however, in particular regarding the mechanisms driving the variability of foliar δ^{15}N values of non-N$_2$ fixing plants within and across ecosystems. The goal of the work presented here was therefore to: (1) characterize the relationship between soil net mineralization and variability of foliar $\Delta\delta^{15}$N (δ^{15}Nleaf $-$ δ^{15}Nsoil) values from 20 different plant species within and across 18 grassland sites; (2) to determine in situ if a plant's preference for NO$_3^-$ or NH$_4^+$ uptake explains variability in foliar $\Delta\delta^{15}$N among different plant species within an ecosystem; and (3) test if variability in foliar $\Delta\delta^{15}$N among species or functional group is consistent across 18 grassland sites. $\Delta\delta^{15}$N values of the 20 different plant species were positively related to soil net mineralization rates across the 18 sites. We found that within a site, foliar $\Delta\delta^{15}$N values increased with the species' NO$_3^-$ to NH$_4^+$ uptake ratios. Interestingly, the slope of this relationship differed in direction from previously published studies. Finally, the variability in foliar $\Delta\delta^{15}$N values among species was not consistent across 18 grassland sites but was significantly influenced by N mineralization rates and the abundance of a particular species in a site. Our findings improve the mechanistic understanding of the commonly observed variability in foliar $\Delta\delta^{15}$N among different plant species. In particular we were able to show that within a site, foliar δ^{15}N values nicely reflect a plant's N source but that the direction of the relationship between NO$_3^-$ to NH$_4^+$ uptake and foliar $\Delta\delta^{15}$N values is not universal. Using a large set of data, our study highlights that foliar $\Delta\delta^{15}$N values are valuable tools to assess plant N uptake patterns and to characterize the soil N cycle across different ecosystems.

Keywords Stable isotopes · Mineral nutrition · Competition · Nitrogen · Mineralization · Biodiversity

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Introduction

N is a critical element limiting plant growth in temperate ecosystems (Vitousek and Howarth 1991). Consequently, N dynamics such as N mineralization or plant N uptake determine much of the structure and function of these ecosystems. The assessment of N dynamics is therefore critical for monitoring, understanding and predicting essential functions (biogeochemical cycles and productivity) or structural components (species composition and diversity) of temperate ecosystems. In this respect, the natural abundance of stable ^{15}N isotopes (δ^{15}N) in soils and

in particular in plants has been suggested as an efficient and simple non-invasive tool to assess and monitor ecosystem N dynamics (Handley and Raven 1992; Nadelhoffer and Fry 1994; Högberg 1997; Handley et al. 1998; Evans 2001; Robinson 2001; Amundson et al. 2003; Pardo et al. 2006; Houlton et al. 2007).

Variability in plant and soil δ^{15}N values derives primarily from physiological and biogeochemical processes in the N cycle. Soil processes such as N mineralization, nitrification, denitrification or NH_3 volatilization discriminate against ^{15}N and lead to soil N pools with different δ^{15}N signatures (Mariotti et al. 1981; Handley and Raven 1992; Nadelhoffer and Fry 1994; Piccolo et al. 1994; Robinson 2001). The δ^{15}N signatures of different soils N pools are further imprinted in the δ^{15}N values of plants that utilize these soil N pools for their N nutrition.

Based on the link between foliar and soil δ^{15}N signals, foliar δ^{15}N values can be used as simple but valuable tools to study ecosystem N dynamics, both as tracers or as integrative signals. For example, foliar δ^{15}N values that reflect the δ^{15}N signatures of the plant's specific N sources (e.g. NO_3^- and NH_4^+) could reveal information on the plant's N uptake patterns (Houlton et al. 2007). Alternatively, foliar δ^{15}N values have the potential to characterize N turnover in the soil as integrative proxies. High soil N mineralization rates are often correlated with denitrification or leaching of inorganic N forms. Both processes lead to losses of ^{15}N-depleted N_2O and N_2 or NO_3^-, respectively and leave the remaining inorganic N pool enriched in ^{15}N. Foliar δ^{15}N signals that reflect the ^{15}N enrichment of the inorganic soil pool may therefore indicate the magnitude of N fluxes and N losses in ecosystems (Högberg 1990; Garten and van Miegroet 1994; Austin and Vitousek 1998; Emmett et al. 1998; Korontzi et al. 2000; Pardo et al. 2002; Amundson et al. 2003; Pardo et al. 2006; Templer et al. 2007).

Despite the potential to study ecosystem N dynamics, foliar δ^{15}N signatures can be obscured by a variety of factors such as discrimination during N uptake, mycorrhizal status and type, nodulation, and intra-plant isotope partitioning (Handley et al. 1998). As a result, the variability of foliar δ^{15}N values among different non-N_2 fixing plant species within an ecosystem remains unclear and complicates the use of plant tissue δ^{15}N to investigate plant N uptake patterns or soil N cycling. For example, experimental evidence that relates variability in foliar δ^{15}N values of different species to differences in the plants' N sources comes from only two laboratory studies (Miller and Bowman 2002; Falkengren-Grerup et al. 2004). While both studies give credible evidence for a positive relationship between NH_4^+ uptake and foliar δ^{15}N values, it remains unclear if the direction of this relationship is a universal

pattern that can be used in the interpretation of foliar δ^{15}N values in different natural ecosystems.

Given the uncertainties regarding foliar δ^{15}N values, the goal of the study presented here was to mechanistically address the variability of foliar δ^{15}N values among a large number of different plant species within an ecosystem and to test if differences in foliar δ^{15}N values of different plant species are persistent across a range of 18 different grassland ecosystems. Specifically, our study had three objectives: first, we characterized the relationship between soil net mineralization rates and variability of foliar δ^{15}N values from different plant species within and across 18 different grassland sites; second, we determined if a plant's preference for NO_3^+ or NH_4^- uptake explains the variability in foliar δ^{15}N values among different plant species within grassland sites; and third, we tested edaphic and biotic parameters as drivers of species- or functional group-specific variability in foliar δ^{15}N across 18 grassland sites with different N dynamics.

Materials and methods

Study sites

The study was conducted in the Thüringer Schiefergebirge, Germany (11°00′–11°37′E and 50°21′–50°34′N), using 18 montane grasslands that differed in plant species composition and plant diversity (Table 1). Present-day soils in the area have developed from a carbonate-free, nutrient-poor schist and greywacke bedrock material. All sites were located within an area of 20 by 40 km and were similar in elevation above sea level and exposition. For a detailed description of the sites see Kahmen et al. (2005b). The 18 sites were selected from 78 intensively surveyed grasslands (Kahmen et al. 2005a) based on the following selection criteria: sites had to be ungrazed, cut twice a year (late June and August/September) and must not have received any fertilizer applications for the last 10 years prior to sampling.

Edaphic variables

Soil variables were extensively surveyed 6 times throughout 2002. For each sample, four soil cores (4.3 cm diameter, 10 cm length) were collected at each site, pooled to a single sample and sieved to 2 mm. To determine NO_3^- and NH_4^+ pools in the soils, one part of each soil sample (~ 10 g) was extracted with 50 ml of 1M KCl for 60 min on the same day of sampling. KCl extracts were filtered and then frozen at -20°C and later analyzed using a continuous flow analyzer (SAN Plus; Skalar, Erkelenz, Germany) for NO_3^- and NH_4^+ concentrations. The remaining soil was dried at 35°C

Table 1 Means and SDs of edaphic site variables[a] for the 18 investigated temperate grasslands. *NA* Not available

Site	$\delta^{15}N$ (soil ‰)	NO_3 prod. ($\mu g\ N\ g^{-1}\ d^{-1}$)	NH_4 prod. ($\mu g\ N\ g^{-1}\ d^{-1}$)	N_{min} prod. ($\mu g\ N\ g^{-1}\ d^{-1}$)	NO_3 conc. ($\mu g\ N\ g^{-1}$)	NH_4 conc. ($\mu g\ N\ g^{-1}$)	N_{min} conc. ($\mu g\ N\ g^{-1}$)	C:N	pH	K conc. ($\mu g\ K\ g^{-1}$)	P conc. ($\mu g\ P\ g^{-1}$)
1	5.61 ± 0.31	1.17 ± 0.29	0.05 ± 0.07	1.12 ± 0.22	3.00 ± 1.63	3.98 ± 0.88	6.97 ± 0.81	12.41 ± 0.14	5.97 ± 0.26	286.43 ± 75.51	35.18 ± 6.64
2	4.86 ± 0.19	1.14 ± 0.65	0.12 ± 0.07	1.02 ± 0.58	1.11 ± 1.02	4.70 ± 0.94	5.81 ± 1.11	12.32 ± 0.27	6.26 ± 0.14	129.44 ± 58.49	70.49 ± 11.34
3	5.25 ± 0.40	1.53 ± 0.70	0.12 ± 0.02	1.41 ± 0.67	1.32 ± 1.23	6.99 ± 2.41	8.32 ± 2.84	12.08 ± 0.13	5.91 ± 0.17	44.93 ± 8.99	29.39 ± 2.54
4	6.43 ± 0.21	1.05 ± 0.13	0.10 ± 0.11	0.95 ± 0.02	0.58 ± 0.56	4.07 ± 0.48	4.65 ± 0.45	12.30 ± 0.13	5.68 ± 0.24	45.92 ± 16.25	14.64 ± 2.75
5	4.09 ± 0.21	1.51 ± 0.71	0.23 ± 0.24	1.28 ± 0.47	0.76 ± 1.02	4.77 ± 1.86	5.54 ± 2.79	12.60 ± 0.18	5.79 ± 0.21	49.97 NA	13.92 ± 4.90
6	5.13 ± 0.16	1.40 ± 0.40	0.31 ± 0.37	1.08 ± 0.04	1.08 ± 1.07	5.82 ± 1.31	6.91 ± 2.01	12.08 ± 0.16	5.26 ± 0.22	48.20 ± 11.07	11.44 ± 1.90
7	5.36 ± 0.17	1.22 ± 0.18	0.13 ± 0.23	1.09 ± 0.05	1.09 ± 0.93	5.19 ± 1.09	6.28 ± 1.40	11.04 ± 0.14	5.78 ± 0.22	49.00 ± 22.46	23.16 ± 7.94
8	6.12 ± 0.53	1.35 ± 0.20	0.11 ± 0.15	1.24 ± 0.05	2.91 ± 1.37	4.83 ± 0.93	7.74 ± 1.25	11.99 ± 0.12	6.01 ± 0.15	56.92 ± 20.15	62.45 ± 25.53
9	3.90 ± 0.31	0.82 ± 0.36	0.17 ± 0.01	0.65 ± 0.36	<0.01 NA	5.64 ± 1.87	5.64 ± 1.87	13.50 ± 0.11	5.98 ± 0.28	42.5 ± 93.09	12.02 NA
10	3.39 ± 0.26	0.87 ± 0.46	0.13 ± 0.14	0.74 ± 0.32	0.21 ± 0.33	3.09 ± 1.26	3.30 ± 1.38	12.58 ± 0.26	5.04 ± 0.28	59.91 ± 19.22	20.42 ± 6.51
11	3.67 ± 0.13	0.08 ± 0.11	0.22 ± 0.17	0.30 ± 0.07	0.11 ± 0.28	3.89 ± 1.43	4.00 ± 1.29	13.20 ± 0.21	5.58 ± 0.23	50.62 ± 12.50	12.76 ± 1.81
12	4.49 ± 0.20	0.75 ± 0.56	0.26 ± 0.01	1.02 ± 0.55	0.09 ± 0.21	6.21 ± 0.96	6.30 ± 1.06	12.45 ± 0.08	4.74 ± 0.31	52.63 ± 16.37	26.13 ± 4.42
13	4.54 ± 0.27	0.75 ± 0.19	0.04 ± 0.06	0.79 ± 0.25	0.12 ± 0.30	3.64 ± 1.08	3.76 ± 0.99	12.20 ± 0.11	5.01 ± 0.17	135.16 ± 45.74	15.98 ± 3.10
14	3.88 ± 0.50	0.46 ± 0.31	0.07 ± 0.04	0.38 ± 0.35	<0.01 NA	4.69 ± 0.89	4.69 ± 0.89	12.74 ± 0.34	5.53 ± 0.22	101.47 ± 12.26	11.13 ± 2.66
15	5.17 ± 0.26	0.73 ± 0.05	0.03 ± 0.18	0.69 ± 0.23	0.56 ± 0.69	3.94 ± 0.85	4.51 ± 1.36	12.03 ± 0.07	5.05 ± 0.23	56.12 ± 12.89	39.21 ± 4.38
16	5.16 ± 0.36	0.81 ± 0.14	0.13 ± 0.06	0.68 ± 0.20	0.49 ± 0.55	3.40 ± 0.85	3.98 ± 0.69	13.13 ± 0.16	5.33 ± 0.34	370.45 ± 80.52	55.49 ± 11.93
17	4.02 ± 0.72	0.21 ± 0.19	0.07 ± 0.25	0.28 ± 0.44	0.30 ± 0.49	4.31 ± 1.58	4.61 ± 1.79	14.20 ± 0.55	5.46 ± 0.25	41.34 ± 5.48	19.75 ± 2.30
18	4.05 ± 0.12	0.18 ± 0.02	0.46 ± 0.24	0.64 ± 0.27	<0.01 NA	3.40 ± 1.31	3.40 ± 1.31	15.39 ± 0.16	4.97 ± 0.20	51.16 NA	16.62 ± 2.16

[a] Soil N fluxes [NO_3^- production (*prod.*), NH_4^+ prod., N_{min} prod.] were sampled twice in 2003, while bulk soil $\delta^{15}N$, soil N concentrations (*conc.*) (NO_3^- conc., NH_4^+ conc., N_{min} conc.), C/N, pH, K and P conc. were sampled 6 times in 2002 ($n = 6$)

and extracted for 1 h using a 1M calcium acetate-lactate (CAL) solution. CAL extracts were analyzed with ICP-AES (Optima 3300 DV; Perkin-Elmer, Norwalk, USA) for P and K concentrations. Soil pH was measured in water suspensions. For the determination of soil C:N ratios and total soil N (N_{tot}) and C (C_{tot}) concentrations, dry soil was ground and analyzed with an elemental analyzer (Vario EL II; Elementar, Hanau, Germany). The same dried and ground material was then used for $\delta^{15}N$ analyses of bulk soil with an isotope ratio mass spectrometer (IRMS) (Delta C Finnigan MAT; Bremen, Germany). For statistical analyses, soil variables sampled throughout 2002 were averaged for each plot to a single mean value.

Daily net mineralization rates at each site, i.e., the daily production of plant available NO_3^- and NH_4^+, were determined twice in 2003 (mid May to mid June and mid July to mid August) by in situ incubation (Hart et al. 1994). Four subsamples were collected at each site, pooled, and NO_3^- and NH_4^+ concentrations determined as described above. The remaining pooled soil sample of each site was split into four equal parts, sealed in polyethylene bags and incubated in the soil for 24 or 16 days during the spring and summer campaigns, respectively. After incubation, bags were collected, and soil extracted with 1M KCl and analyzed for NO_3^- and NH_4^+ concentrations as described above. To calculate daily net mineralization rates, NO_3^- and NH_4^+ concentrations at the beginning of the incubation were subtracted from the concentrations determined at the end of the incubation, and the difference divided by the number of incubation days. Daily net mineralization rates from both incubation periods were averaged for the statistical analyses as mean daily net mineralization. Mineralization rates and concentrations were calculated on a soil dry weight basis.

Foliar $\delta^{15}N$

At each site, we collected leaf material from different non-N_2 fixing plant species for $\delta^{15}N$ analyses in mid June 2003. The number of plant species sampled at each site was proportional to the species richness in each plot and covered two thirds of the species at a site. Plant material was dried, ground and analyzed for $\delta^{15}N$ with an IRMS (Delta C Finnigan MAT). In total, 20 different herbaceous plant species were sampled in the 18 sites. For a more detailed analysis of plant trait effects on foliar $\delta^{15}N$ signatures we separated the investigated plant species into the functional groups "grasses" and "forbs". The functional group grasses contained all species belonging to the family Poaceae, while the functional group forbs contained all other species. Species names and functional group identity are listed in Fig. 4.

N uptake

To test the effect of N nutrition on leaf $\delta^{15}N$, NO_3^- and NH_4^+ uptake was determined for 15 plant species (seven forbs and eight grasses) in three of the 18 sites (site 2, site 4 and site 7) using ^{15}N labeled N tracers (see Kahmen et al. 2006). At each site, ^{15}N-labeled NO_3^- and NH_4^+ was injected at two different soil depths (3 and 8 cm) twice a year (spring, 22–24 May 2003; summer, 4–6 August 2003), each treatment in a separate 1-m^2 plot. During each campaign in spring and summer, one grassland was labeled per day. The ^{15}N-labeled NO_3^- and NH_4^+ compounds were injected (using spine syringes) as separate solutions of 9.66 mM $K^{15}NO_3$ and 9.66 mM $^{15}NH_4Cl$ (>98.9 at% ^{15}N). In order to offer both N compounds in each treatment, we added equal amounts (9.66 mM) ^{14}N of the non-treatment N compound to the ^{15}N solution. Injection points in each treatment were distributed evenly across the 1-m^2 plots using a 6.5 × 7.0 cm grid, resulting in 210 injections per plot. Assuming a 2-cm diffusion radius, we injected 2 ml of ^{15}N labeled solution at each injection point, leading to a total of 55.35 mg added ^{15}N m^{-2}. As a result of the large experimental effort, sampling of soil and plant materials was nested within the labeled plots at the three grassland sites. To avoid contamination, the 1-m^2 plots where tracers were applied, were located in secure distance to the 5 × 5 m sampling areas where plant leaves were collected for $\delta^{15}N$ natural abundance analyses.

Three days after injecting the ^{15}N-labeled N compounds into the soil, the vegetation in each treatment was clipped 2 cm above the ground, sorted to species, dried at 70°C for 48 h and weighed. From the sorted biomass, ten individuals from each species were combined to a single bulk sample. Plant material was ground and analyzed for N concentrations and $\delta^{15}N$ values with an elemental analyzer (Vario EL II, Elementar, Hanau, Germany) coupled to an IRMS (Delta C Finnigan MAT).

The recovered ^{15}N tracer in the leaves of the different plant species was used to calculate mean daily NO_3^- and NH_4^+ uptake of the different species in the individual treatments. In the calculations, background natural abundance ^{15}N and N concentration in the leaves of the respective plants as well as plant available N in the soil were taken into account. For a detailed description of the calculations see Kahmen et al. (2006). For the statistical analyses, NO_3^- and NH_4^+ uptake was summed up for the two soil depths and averaged over the two temporal treatments.

Data analysis and statistics

To correct leaf $\delta^{15}N$ values for site-specific differences in background bulk soil $\delta^{15}N$, $\Delta\delta^{15}N$ was calculated for each

Table 2 ^{15}N enrichment of plants compared to soil background values ($\Delta\delta^{15}N^a$) as a function of plant NO_3^- and NH_4^+ uptake and functional (*Funct.*) group effects as tested in ANOVAs[b] (type I SS). The variables for the models were assessed in three sites (site 2, site 4 and site 7)

	$\Delta\delta^{15}N$ for NO_3^- uptake				$\Delta\delta^{15}N$ for NH_4^+ uptake				$\Delta\delta^{15}N$ for NO_3^- / NH_4^+ uptake			
	df	SS	F	P	df	SS	F	P	df	SS	F	P
Soil N flux	2	8.95	5.88	0.008	2	8.95	7.22	0.003	2	8.95	6.94	0.004
Funct. group	1	0.97	1.28	0.269	1	0.97	1.57	0.222	1	0.97	1.51	0.231
N uptake (μg N g^{-1} day^{-1})	1	0.95	1.25	0.275	1	3.85	6.21	0.020	1	3.78	5.87	0.023
Funct. group × N uptake	1	0.52	0.68	0.418	1	1.01	1.62	0.214	1	0.47	0.72	0.404
Error	24	18.25			24	14.86			24	15.47		

[a] $\Delta\delta^{15}N$ was calculated as $\delta^{15}N$ plant $- \delta^{15}N$ soil. Values predicted for $\Delta\delta^{15}N$ by the model are presented in Fig. 3

[b] To control for different effects of plant-available N in the soil of the three sites, the mean daily flux of the respective N species (i.e., NO_3^- flux, NH_4^+ flux and NO_3^-/NH_4^+ flux in the NO_3^- uptake model, NH_4^+ uptake model and NO_3^-/NH_4^+ uptake model, respectively) was first entered into a model as a block factor. Then the factor funct. group (forb, grass) followed by N uptake as a covariate and the interaction of funct. group and N uptake were subsequently entered in the model

species from all sites as the difference between $\delta^{15}N_{plant}$ and $\delta^{15}N_{soil}$ (Amundson et al. 2003). Foliar $\Delta\delta^{15}N$ values represent the ^{15}N depletion of a plant leaf compared to the soil $\delta^{15}N$ background. We tested the effects of NO_3^- and NH_4^+ uptake on $\Delta\delta^{15}N$ of different species in three sites with ANOVA models (type I SS). The ANOVA models allowed to control for site-specific effects of plant available soil N on foliar $\Delta\delta^{15}N$ across these three sites. Further the ANOVA models allowed us to test if the functional group identity of a species had an effect on the relationship between NO_3^- and NH_4^+ uptake and foliar $\Delta\delta^{15}N$ (i.e., a significant interaction effect between functional group and N uptake on $\Delta\delta^{15}N$). In each model, mean daily soil N fluxes were entered first as the block factor (i.e., NO_3^- flux, NH_4^+ flux and NO_3^-/NH_4^+ flux in the NO_3^- uptake model, NH_4^+ uptake model and NO_3^-/NH_4^+ uptake model, respectively), followed by a fixed hierarchical sequence of functional group as factor, N uptake as covariate, and finally the interaction between functional group and N uptake (Table 2). We also used an ANOVA model (type I SS) to test functional group and species-specific effects as well as effects of the plant's relative abundance on the relationship between net N mineralization and foliar $\Delta\delta^{15}N$ across all 18 sites. As above, this model allowed testing for direct effects of individual variables and also for interacting effects of variables. Factors (functional group and species identity) and covariates (abundance and net N mineralization) were entered into the model in a fixed hierarchical sequence (Table 3). All ANOVA models were calculated using SPSS 11 for MAC OS X 10.3.

Results

Soil $\delta^{15}N$ values in the 18 grassland sites varied between 3.39 and 6.43‰ (Table 1). NO_3^- and NH_4^+ pools in the soil were relatively low, ranging from below detection levels

Table 3 ANOVA[a] (type I SS) for plant ^{15}N enrichment compared to soil background values ($\Delta\delta^{15}N^b$) for 20 herb and grass species from 18 different grassland sites

	df	SS	F	P
N_{min}	1	79.63	107.94	<0.001
Funct. group	1	8.12	11.00	0.001
Species	18	39.83	3.00	<0.001
Abundance	1	3.79	5.14	0.025
Funct. group × N_{min}	1	3.63	4.94	0.028
Species × N_{min}	18	46.17	3.48	<0.001
Funct. group × Abundance	1	1.32	1.78	0.184
Species × Abundance	18	29.11	2.19	0.006
Error	149	109.93		

[a] Daily net mineralization (N_{min}) was entered first into the model as a covariate. Funct. group (forbs, grasses) and species identity were entered as factors and abundance (% cover) as covariate
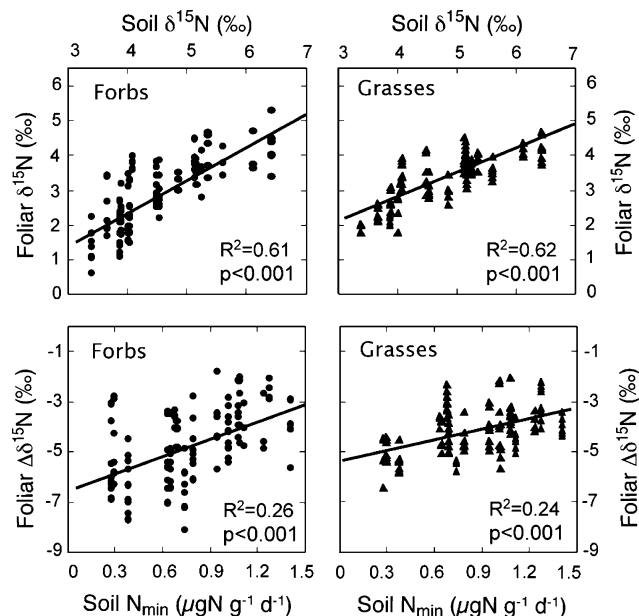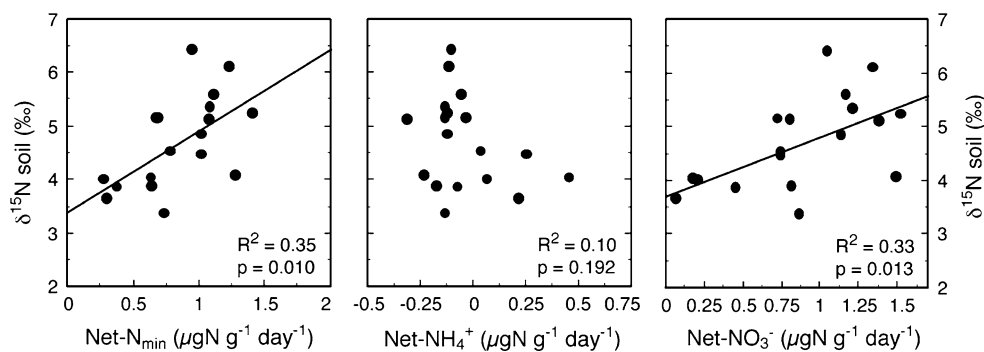
[b] $\Delta\delta^{15}N$ is calculated as $\delta^{15}N$ plant $- \delta^{15}N$ soil. Values for $\Delta\delta^{15}N$ of individual species predicted by the model are presented in Fig. 4

(<0.01 μg g^{-1}) to 3.00 μg g^{-1} and from 3.09 to 6.99 μg g^{-1}, respectively. Net N mineralization was dominated by net nitrification, which exceeded net ammonification in most sites. Net nitrification and net ammonification ranged from 0.08 to 1.53 μg g^{-1} day^{-1} and from -0.31 to 0.46 μg g^{-1} day^{-1}, respectively (Table 1).

Soil $\delta^{15}N$ values across the 18 sites increased significantly with net N mineralization and net nitrification in linear regression analyses. However, soil $\delta^{15}N$ values showed a negative but non-significant trend with net ammonification (Fig. 1). Plant leaves from both forbs and grasses were all depleted in ^{15}N compared to bulk soils and showed a positive relationship with bulk soil $\delta^{15}N$ in linear regression analyses (Fig. 2). $\Delta\delta^{15}N$ values ($\delta^{15}N$ leaf $- \delta^{15}N$ soil) were negative for all plant species. Despite the correction for the bulk soil background $\delta^{15}N$ signal, $\Delta\delta^{15}N$

Fig. 1 Simple linear regression analyses of $\delta^{15}N$ of bulk soil and daily net mineralization (N_{min}), ammonification (NH_4^+) and nitrification (NO_3^-) rates in 18 different temperate grasslands

Fig. 2 Simple linear regression analyses between $\delta^{15}N$ of bulk soil and leaf $\delta^{15}N$ for different forb and grass species as well as the relationship between daily net mineralization (N_{min}) and foliar $\Delta\delta^{15}N$ ($\delta^{15}N$ plant $- \delta^{15}N$ soil) for different forb and grass species in 18 temperate grasslands. d^{-1} Day^{-1}

Table 2). Interestingly, the effects of NH_4^+ uptake and the ratio between NO_3^- and NH_4^+ uptake were independent of the functional group identity of plants, i.e., the interaction between the factor "functional group" and the covariate "N uptake" were not significant in any of the three models (Table 2).

Finally, we tested in an additional ANOVA model if the relationship between net N mineralization and $\Delta\delta^{15}N$ of individual species across the 18 sites was significantly affected by functional group identity, species identity, the abundance of a species, or by any interactions of the above parameters. We found that net N mineralization, functional group identity, species identity and the abundance of plants all had significant effects on $\Delta\delta^{15}N$. More importantly, however, the effects of functional group identity on $\Delta\delta^{15}N$ as well as the effects of species identity on $\Delta\delta^{15}N$ depended on the net N mineralization rate of a site (i.e., significant interaction between the factor "functional group" or "species identity" and the covariate "N mineralization") and the abundance of a particular species (i.e., significant interaction between the factor "species identity" and the covariate "abundance").

Discussion

We found substantial variability in soil and plant $\delta^{15}N$ values across the 18 investigated grasslands. For soil $\delta^{15}N$ values part of this variability was explained by net N mineralization (Fig. 1). Previous studies have suggested that increasing bulk soil $\delta^{15}N$ values may reflect increasing rates of soil N cycling which are associated with losses of ^{15}N-depleted mineral N that lead to a gradual ^{15}N enrichment of the remaining bulk soil N (Mariotti et al. 1981; Amundson et al. 2003; Pardo et al. 2006). Although the grasslands investigated here are extensively managed and have not been fertilized for at least 10 years, previous studies have shown that NO_3^- in such grasslands is prone to losses, either via leaching or via microbial denitrification to N_2O and N_2 (Scherer-Lorenzen et al. 2003; Tilsner et al.

of forbs and grasses still increased significantly with net N mineralization (Fig. 2).

$\Delta\delta^{15}N$ values of both, forbs and grasses revealed substantial within and across site variability (Fig. 2). In order to explain what drives this observed within-site variability among different plant species and functional groups, we tested the effects of NO_3^- and NH_4^+ uptake on $\Delta\delta^{15}N$ values of forbs and grasses from sites 2, 4 and 7 in an ANOVA (Fig. 3; Table 2). In all three models site-specific soil N fluxes had a significant effect on $\Delta\delta^{15}N$ matching our observations from all 18 sites (Fig. 2). The analyses also revealed that NO_3^- uptake had no significant effect on $\Delta\delta^{15}N$ of forbs and grasses. More importantly, however, NH_4^+ uptake had a significantly negative effect on $\Delta\delta^{15}N$ and the ratio between NO_3^- and NH_4^+ uptake a significantly positive effect $\Delta\delta^{15}N$ of forbs and grasses (Fig. 3;

2003b). Our study therefore fits nicely into the context of previous work that has linked increasing bulk soil $\delta^{15}N$ values to losses of mineral N and to the openness of the N cycle (Högberg 1990; Högberg 1991; Johannisson and Högberg 1994; Austin and Vitousek 1998; Emmett et al. 1998; Korontzi et al. 2000; Brenner et al. 2001; Pardo et al. 2002, 2006; Amundson et al. 2003; Choi et al. 2003; Houlton et al. 2006; Templer et al. 2007).

In order to compare $\delta^{15}N$ values of plants across sites irrespective of soil $\delta^{15}N$, we corrected plant $\delta^{15}N$ values for soil $\delta^{15}N$ and calculated $\Delta\delta^{15}N$, the deviation of $\delta^{15}N$ plants from soil $\delta^{15}N$ (Amundson et al. 2003). $\Delta\delta^{15}N$ was negative across all 18 sites for all species (Fig. 2). Negative $\Delta\delta^{15}N$ values are typically considered to result from mineral soil N uptake, which is depleted in ^{15}N compared to bulk soil N or soil organic N (Mariotti et al. 1981; Robinson 2001). It has been shown that plants can discriminate against ^{15}N during N acquisition, either directly, or indirectly via associations with mycorrhizal fungi (Evans et al. 1996; Michelsen et al. 1996; Hobbie et al. 2000; Hobbie and Colpaert 2003). Direct discrimination against ^{15}N during N uptake is, however, considered not to be relevant in ecosystems where N is a critical and limiting resource such as in the extensively managed temperate grasslands investigated in this study (McKee et al. 2002). Also, isotopic fractionation associated with N uptake via mycorrhizal fungi has typically been observed in ecto- or ericoid mycorrhizal plants (Emmerton et al. 2001). In the grasslands investigated here, only arbuscular mycorrhizal fungi (AMF) infections of roots were found (Börstler et al. 2006), and AMF plants are assumed to discriminate only marginally against ^{15}N during N uptake (Handley et al. 1993; Azcon-G-Aguilar et al. 1998). Consequently, we conclude that $\Delta\delta^{15}N$ values of plant material in our study are in fact driven by the uptake of ^{15}N-depleted inorganic N and that plant material reflects the $\delta^{15}N$ signature of plant available soil N.

Across the 18 sites, $\Delta\delta^{15}N$ values of grasses and forbs were not constant, but became less negative (less ^{15}N depleted compared to soils) with increasing net N mineralization in the soil (Fig. 2). Assuming that average plant $\delta^{15}N$ of a site reflects the $\delta^{15}N$ signature of the plant's

N source, i.e., mineral N, this pattern indicates that soil mineral N becomes increasingly enriched in ^{15}N with increasing net N mineralization. This finding matches our interpretation of bulk soil $\delta^{15}N$, which becomes enriched with increasing soil N mineralization (Fig. 1) since accelerating losses of ^{15}N-depleted NO_3, N_2O and N_2 with increasing net N mineralization result in ^{15}N enrichment of the remaining inorganic N pool. Changes in average $\Delta\delta^{15}N$ values across different ecosystems therefore reflect rates of N mineralization in soils.

Despite net N mineralization explaining some of the variation in overall plant $\Delta\delta^{15}N$, there remains a large variability among different species within each site (Fig. 2). Based on the assumptions that: (1) the observed $\Delta\delta^{15}N$ values of plants largely reflect the $\delta^{15}N$ signature of their N source; and (2) the fact that NO_3^- and NH_4^+ can vary substantially in their $\delta^{15}N$ signature depending on the relative rates of mineralization, nitrification and denitrification (Shearer et al. 1974; Mariotti et al. 1981; Handley and Raven 1992), we tested if differences in NO_3^- versus NH_4^+ uptake might explain the variability in $\Delta\delta^{15}N$ values among plant species within a site. Interestingly, NO_3^- uptake showed no significant effect on leaf $\Delta\delta^{15}N$ values (Fig. 3; Table 2). Although not specifically tested in this study, the location of NO_3^- assimilation (roots or foliage) can vary among plants. Due to strong isotope effects during plant NO_3^- assimilation, any difference in the location of NO_3^- assimilation could obscure a potential $\delta^{15}N$ signal derived from soil NO_3^- in plant tissues, and could thus explain the lack of a direct influence of NO_3^- uptake on leaf $\delta^{15}N$ (Pate et al. 1993).

Other than NO_3^-, NH_4^+ uptake and the ratios of NO_3^- to NH_4^+ uptake significantly affected $\Delta\delta^{15}N$ of plants, with increasing NH_4^+ uptake leading to decreased $\Delta\delta^{15}N$ values (Fig. 3; Table 2). We are aware of only two other studies that have directly tested the effects of NO_3^- and NH_4^+ uptake on foliar $\delta^{15}N$ values (Miller and Bowman 2002; Falkengren-Grerup et al. 2004). Interestingly, both studies found that plant $\delta^{15}N$ values decreased with increasing $NO_3^-:NH_4^+$ uptake ratio, a relationship that is opposite in direction to what we found in our experiment (Fig. 3). These contrasting results suggest that ^{15}N enrichment of NH_4^+ compared to NO_3^- can vary significantly in different

Fig. 3 The relationship between NO_3^- or NH_4^+ uptake and predicted $\Delta\delta^{15}N$ ($\delta^{15}N$ plant $-$ $\delta^{15}N$ soil) for 30 plant individuals from seven forb species (14 individuals) and eight grass species (16 individuals) as predicted by the ANOVA models in Table 2. N uptake was determined in site 2, site 4 and site 7
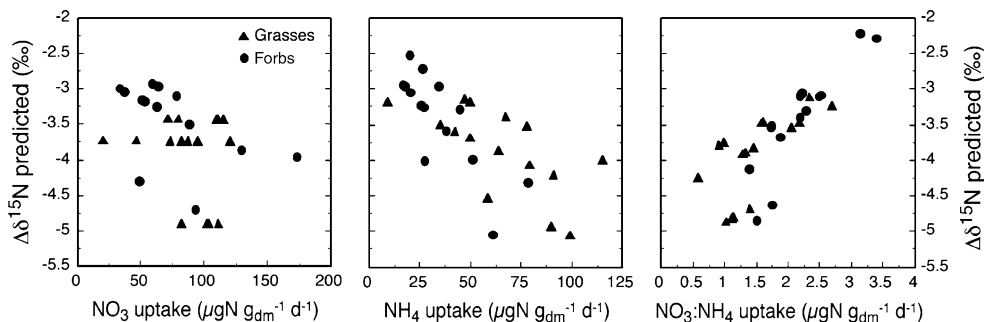
Fig. 4 The relationship between daily net mineralization (N_{min}) and $\Delta\delta^{15}N$ ($\delta^{15}N$ plant $-$ $\delta^{15}N$ soil) for 20 different plant species from 18 different temperate grasslands. For details of the statistical analyses see Table 3. *Open symbols* represent observed values, *closed symbols* values predicted by the ANOVA model presented in Table 3
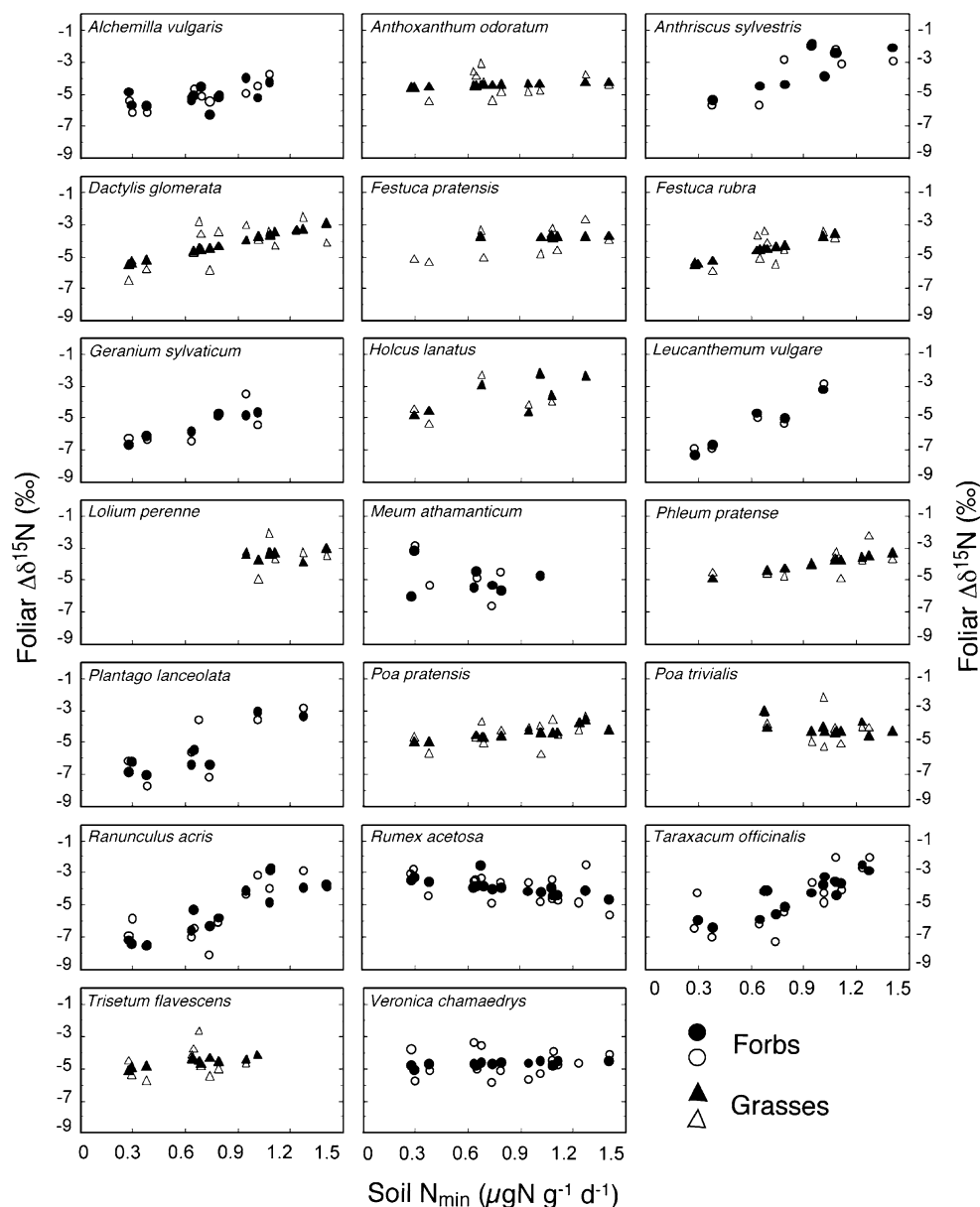
ecosystems, depending on the nature of the N cycle. In an ecosystem with a closed N cycle, inorganic N compounds become increasingly depleted in ^{15}N along the N cycle, i.e., NO_3^- is ^{15}N depleted compared to NH_4^+, and therefore plants should also become depleted with increasing NO_3^-:NH_4^+ uptake (Mariotti et al. 1981; Nadelhoffer and Fry 1994; Robinson 2001). However, NO_3^- can become enriched in ^{15}N compared to NH_4^+ in ecosystems where denitrification rates are high (Mariotti et al. 1981; Piccolo et al. 1994; Robinson 2001; Houlton et al. 2006, 2007; Pörtl et al. 2007). For extensively managed temperate grasslands such as investigated in this study denitrification has been shown to be an important path of N loss (Tilsner et al. 2003a, b). Consequently, denitrification causing NO_3^- to become ^{15}N enriched compared to NH_4^+ could

explain why plants with greater NO_3^- over NH_4^+ uptake are enriched in ^{15}N. Our data therefore confirm the studies of Miller and Bowman (2002) and Falkengren-Grerup et al. (2004) showing that within an ecosystem the variability of foliar $\Delta\delta^{15}N$ values gives important information on the plants' NO_3^-:NH_4^+ uptake ratio. However, our study also shows that the direction of the relationship between foliar $\Delta\delta^{15}N$ values and the plants' NO_3^-:NH_4^+ uptake ratio can vary, depending on the dynamics of the N cycle of the ecosystem under consideration.

Interestingly, the effect of NO_3^- to NH_4^+ uptake on $\Delta\delta^{15}N$ in our study was independent of a plant's functional group identity (Table 2). This suggests that leaf $\Delta\delta^{15}N$ is not affected by fractionation patterns during N uptake specific to functional groups nor by functional

group-specific differences in internal N allocation. Consequently, the δ^{15}N signature of the plant's N source is a critical driver of variability in $\Delta\delta^{15}$N among plant species within an ecosystem.

We tested if the observed patterns of within-site variability in $\Delta\delta^{15}$N among species are consistent across the 18 sites or vary in a functional group- or species-specific manner (Table 3). Both, functional group- and species-specific effects on $\Delta\delta^{15}$N were detected, but the effects were dependent on net N mineralization rates (Fig. 4; Table 3). In addition, depending on the identity of a species, the abundance of a particular species also had a significant effect on $\Delta\delta^{15}$N. In summary, functional groups as well as species within these functional groups differed significantly in $\Delta\delta^{15}$N, but the patterns of these differences varied across sites with N mineralization rates and abundance. There are two possible explanations for changes in a species' foliar $\Delta\delta^{15}$N across sites (see also Houlton et al. 2007, addressing similar mechanisms in tropical rainforest species). First: along an environmental gradient, plants might shift their N source as a result of changes in their competitive strength (abundance) compared to other plants and microorganisms. Assuming that the δ^{15}N values of NO_3^- and NH_4^+ remain constant along the gradient, changes in plants' foliar $\Delta\delta^{15}$N values would then reflect a shift in N sources. Second: δ^{15}N values of NO_3^- and NH_4^+ shift with changing environmental conditions and changes in plants' foliar $\Delta\delta^{15}$N values would reflect these changes in soil N dynamics assuming that plants do not change their N source. Unfortunately we cannot distinguish between the two possible explanations in this study since we did not determine the δ^{15}N values of NO_3^- and NH_4^+ given the uncertainties and complexity of the methods involved in these analyses and the vast number of samples that would have to be processed to account for the seasonal and spatial variability in isotope composition of plant-available N pools for 18 different sites. Method development towards an easier assessment of isotopic ratios in soil NO_3^- and NH_4^+ (and soluble organic N) are urgently needed so that ecologists can exploit the full potential of contrasting foliar δ^{15}N values among plant species within and across different sites. This may not only allow a more detailed assessment of ecosystem N dynamics but also the determination of shifts in plant–plant and plant–microbe interactions when competing for different soil N sources along environmental gradients.

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