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Preferential uptake of soil nitrogen forms by grassland plant species

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Abstract In this study, we assessed whether a range of temperate grassland species showed preferential uptake for different chemical forms of N, including inorganic N and a range of amino acids that commonly occur in temperate grassland soil. Preferential uptake of dual-labelled (^{13}C and ^{15}N) glycine, serine, arginine and phenylalanine, as compared to inorganic N, was tested using plants growing in pots with natural field soil. We selected five grass species representing a gradient from fertilised, productive pastures to extensive, low productivity pastures (*Lolium perenne, Holcus lanatus, Anthoxanthum odoratum, Deschampsia flexuosa*, and *Nardus stricta*). Our data show that all grass species were able to take up directly a diversity of soil amino acids of varying complexity. Moreover, we present evidence of marked inter-species differences in preferential use of chemical forms of N of varying complexity. *L. perenne* was relatively more effective at using inorganic N and glycine compared to the most complex amino acid phenylalanine, whereas *N. stricta* showed a significant preference for serine over inorganic N. Total plant N acquisition, measured as root and shoot concentration of labelled compounds, also revealed pronounced inter-species differences which were related to plant growth rate: plants with higher biomass production were found to take up more inorganic N. Our findings indicate that species-specific differences in direct uptake of different N forms combined with total N acquisition could explain changes in competitive dominance of grass species in grasslands of differing fertility.

Keywords Amino acids · Grasses · Stable isotopes · *Lolium perenne*

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Introduction

Historically, a central assumption of terrestrial nutrient cycles was that for soil nitrogen (N) to be available for plant uptake it needs to be in an inorganic form. A growing body of evidence now challenges this view, pointing to the importance of dissolved organic nitrogen (DON) in the form of amino acids for plant nutrition. Several studies have shown that plants of both natural and agricultural habitats can take up amino acids directly, by-passing the need for microbial mineralization to produce simpler inorganic N forms. For example, organic N uptake has been demonstrated for plants of sub-Antarctic herbfield, subtropical rainforest and tropical savanna woodland in Australia (Schmidt and Stewart 1999), for plants of the arctic and alpine tundra (Chapin et al. 1993; Kielland 1994; Schimel and Chapin 1996; Lipson and Monson 1998) and salt marsh (Henry and Jefferies 2003a,b), for boreal forest (Näsholm et al. 1998; Nordin et al. 2001) and for alpine and subalpine fen communities (Raab et al. 1999). In agricultural habitats, experiments of grasses (Näsholm et al. 2000; Thornton 2001) and wheat (Näsholm et al. 2001) have shown that plants can take up glycine directly, while others have suggested that due to fast microbial turnover of organic N in these systems the main route for plant uptake of N is as mineral N after microbial mineralization (Hodge et al. 1998, 1999; Owen and Jones 2001). That organic N uptake is of limited importance in agricultural situations is also evidenced by in situ measurements of N uptake by plants in temperate grasslands which show that whilst glycine can be taken up directly by plants, microbial turnover and release of this N into

the plant-soil system is the major pathway for N acquisition (Bardgett et al. 2003). Despite the comparatively low direct uptake of glycine by plants in these grassland systems, these authors did find significant differences between improved *Lolium perenne* grassland and low productivity *Agrostis capillaris–Festuca ovina* grassland, in that more organic N was captured by plants of the unimproved site where amino acids were the dominant soluble N form in soil (Bardgett et al. 2003).

The capacity of plants to take up organic N directly appears to be ubiquitous, but there is emerging evidence that plants do vary in their capacity to take up different chemical forms of N and actually show species-specific preferential uptake of either organic or inorganic N. So far, species-specific differences have been demonstrated in arctic tundra (McKane et al. 2002), in alpine communities (Miller and Bowman 2002, 2003), and in low productivity grasslands (Weigelt et al. 2003). This finding is especially significant since the existence of species-level differences in the preference of plants to take up chemical forms of N might provide a mechanism for plants to efficiently partition a limited soil N pool, thereby facilitating species coexistence and the maintenance of plant diversity (McKane et al. 2002). On the other hand, preferences for different N forms could be a competitive advantage especially on nutrient-limited sites. Many different amino acids are present in soil solution (Kielland 1994; Streeter et al. 2000), but most of the aforementioned work focused on the uptake of glycine or other simple amino acids, whilst the ones testing several amino acids used hydroponic solution (Table 1). In grassland, total free amino acids (TFAA) are more abundant in unimproved compared to improved sites (Bardgett et al. 2003) and within these pools the percentage of more complex amino acids (e.g. arginine, histidine and phenylalanine) is higher in unimproved compared to improved soils while for simple organic N forms (glycine and alanine) it is vice versa (Streeter et al. 2000).

In this study, we assessed under glasshouse conditions whether temperate grassland species displayed any preferences for different chemical forms of N, including inorganic N and amino acids that commonly occur in temperate grassland soil. We hypothesize preferential uptake of inorganic N for fast growing species of fertilised grassland, while typical species of unimproved grassland should more intensively exploit organic N sources. Specifically, we investigated preferential uptake of dual-labelled (^{13}C and ^{15}N) glycine, serine, arginine and phenylalanine, as compared to ammonium-nitrate, by five grass species growing in pots with natural field soil. The grasses were selected to represent those that occur along a fertility gradient ranging from improved, fertilised *Lolium* dominated grassland, to unfertilised, less productive *Agrostis-Festuca* grassland.

Materials and methods

Experimental setup

We used five common grassland species (*Lolium perenne*, *Holcus lanatus*, *Anthoxanthum odoratum*, *Deschampsia*

Table 1 List of studies on amino acid uptake

Amino acid	Experimental type	Study
Glycine	Intact plants or excised roots in hydrosolution	Turnbull et al. (1996); Raab et al. (1999); Schmidt and Stewart (1999); Thornton (2001)
Glutamic acid	Excised roots in hydrosolution	Blaudez et al. (2001)
Proline	Excised roots in hydrosolution	Schobert et al. (1988)
Glycine, serine	Excised roots in hydrosolution	Schiller et al. (1998)
Aspartic acid, glutamic acid, glycine	Excised roots in hydrosolution	Chapin et al. (1993)[a]; Kielland (1994)
Fifteen different amino acids	Excised roots in hydrosolution	Persson and Näsholm (2001)
Alanine	Seedlings in hydrosolution	Bajwa and Read (1985)
Alanine, glutamic acid, glycine	Intact plants in hydrosolution	Falkengren-Grerup et al. (2000)
Aspartic acid, glutamic acid, glycine, leucine	Intact plants in hydrosolution	Henry and Jefferies (2003a)
Aspartic acid, serine	Pots with sterile substrate	Cliquet et al. (1977)
Alanine, aspartic acid, glutamine, glutamic acid, glycine	Pots with sterile substrate	Stribley and Read (1980)
Arginine, glycine	Pots with non-sterile soil	Öhlund and Näsholm (2001)
Glycine	Pots with field soil or soil mixture (non sterile)	Raab et al. (1996); Näsholm et al. (2000); Miller and Bowman (2003); Weigelt et al. (2003)
Glutamic acid, glycine	Field-like experiment	Lipson et al. (1999)
Glutamic acid, glycine, leucine	Field-like experiment	Henry and Jefferies (2003b)
Glycine	Field experiment	Lipson and Monson (1998); Näsholm et al. (1998); Streeter et al. (2000); Näsholm et al. (2001); McKane et al. (2002); Miller and Bowman (2002); Bardgett et al. (2003)
Aspartic acid, glycine	Field experiment	Schimel and Chapin (1996)

[a]Additional use of mixed solution of equimolar concentrations of glycine, glutamic acid, aspartic acid, alanine and arginine

flexuosa, *Nardus stricta*). These species inhabit temperate grassland, but with changing abundances along gradients of soil fertility. The fast growing species *Lolium* typically dominates fertilised pastures (improved grasslands, National Vegetation Classification [NVC] Mg6 and Mg7, Rodwell 1992) on moist and circumneutral brown soil and is quite abundant in semi-improved grasslands. *Holcus* and *Anthoxanthum* are common in grassland of intermediate fertility on more base-poor mineral soils (semi-improved grassland, NVC U4b), whilst *Nardus* and *Deschampsia* are most abundant in unfertilised extensive pastures and acidic grassland on the upland fringes (unimproved grassland, NVC U4a).

These grasses were tested for preferential uptake of five different nitrogen forms of increasing size and complexity: ammonium-nitrate, glycine, serine, arginine and phenylalanine. Glycine and serine are small and simple in structure. Arginine is the largest molecule but also simple and with a high N content (low C:N ratio), while phenylalanine has a phenolic ring and a high C:N ratio (see Table 2). Glycine commonly dominates the amino acid profile together with aspartate and glutamate, but other amino acids such as lysine, arginine and serine are sometimes also present in relatively high concentrations (see Lipson and Näsholm 2001 and references therein). Earlier work on soil of five different unimproved grassland sites, similar to that chosen for the current experiment, revealed TFAA concentrations of 28–96 μg g dry soil^{-1} (equivalent to 4.8–21 μg amino acid N g dry soil^{-1} ; T.K. Nettleton, unpublished data). Other studies on unimproved grassland measured 5–25 μg g dry soil^{-1} (Streeter et al. 2000; Bardgett et al. 2003) with relative percentages of the four selected amino acids as given in Table 2 (Streeter et al. 2000).

Seeds were germinated at 18°C for 7 days on filter paper soaked with double deionised (DD) water. A pre-experiment showed that three seedlings produced enough biomass for the analyses and grew well in the pots for the time period of the experiment. Therefore, three seedlings of each species were planted in pots (5 cm diameter, 10 cm height) filled with 100 g dry weight equivalent field soil. The soil was collected 2 days before planting from an unimproved grassland site near Bangor University farm, Bangor, UK. As mentioned above, previous studies have used similar grassland soils (Streeter et al. 2000; Bardgett et al. 2003). Prior to potting, soil was passed through a 2.3 mm sieve and watered to field capacity. Pots were placed in a greenhouse with an average (16/8) h day/night cycle of (18/10)°C. Pots were watered with equal amounts of tap water every other day. There was no fertiliser application.

Table 2 Selected properties of the amino acids used. The field concentration refers to soil taken from unimproved grassland similar to the site of the current experiment and gives the range of three sampling dates (July, August, September; data from Streeter et al. 2000). *Gly* glycine; *ser* serine; *phe* phenylalanine; *arg* arginine

	Gly	Ser	Phe	Arg
Molecular formula	$C_2H_5NO_2$	$C_3H_7NO_3$	$C_9H_{11}NO_2$	$C_6H_{14}N_4O_2$
Molecular weight	75.07	105.09	165.19	174.20
Isoelectric point	5.97	5.68	5.48	11.15
^{13}C:^{15}N	1:1	3:1	9:1	1.5:1
Field concentrations (% of TFAA)	9.4–24.1	5.3–15.2	1.3–1.9	3.3–6.4

Isotope labelling and harvest

After 7 weeks, when plants were well established, all pots received the same amount of five nitrogen forms, varying only in the labelled form of N. One water control and one unlabelled nitrogen control were set up for measurements of natural abundance of ^{13}C and ^{15}N. The water control additionally provided information about soil nutrient concentrations without N addition, but no significant differences were found and therefore only means are presented here. The total of seven treatments for five species with four replicates resulted in 140 pots. Each treatment received either uniformly ^{15}N labelled ammonium-nitrate ($^{15}NH_4^+ + {}^{15}NO_3^-$) or uniformly ^{15}N and ^{13}C dual labelled amino acids (U−^{13}C > 98%; U−^{15}N 96–99% from Promochem, Herts., England) except for glycine where glycine-2−^{13}C−^{15}N was used. The nitrogen control treatment received the same amount of all unlabelled N forms. By using dual labelled amino acids the uptake of intact amino acid can be distinguished from uptake of N from mineralised amino acids. Thus, if both ^{13}C and ^{15}N are detected in plants, this shows that uptake of intact amino acid occurs (Näsholm et al. 2000). Consistent with our previous studies of these soils, we used concentrations that were within the range of natural abundance found in unimproved field sites (see above). In total 12.5 μg N g dry soil^{-1} was added with 2.5 of each nitrogen form resulting in 10 μg N g dry soil^{-1} of amino acids. Five millilitres of solution was added to each pot in 1 ml aliquots with single injections homogeneously distributed over the soil surface. We injected each aliquot with a glass syringe and a luer lock needle (50 mm length, outer diameter 0.63 mm, inner diameter 0.32 mm, side hole/dome style, 23 Gauge) that was slowly withdrawn to ensure uniform spread throughout the profile. After 48 h of incubation, shoots of all pots were clipped and dried at 70°C for 72 h. Subsequently, roots and soil were separated. Roots were first rinsed in 0.5 M $CaCl_2$ and then thoroughly washed under tap water. All roots and a small soil sub-sample were dried to constant weight. The remaining soil was stored at 4°C until further analysis. All replicates per species were labelled in groups to ensure a rapid harvest and processing after the incubation time. The incubation time of 48 h was used in accordance with previous studies that showed maximal uptake of label at this time (Streeter et al. 2000). Subsequently, this label period has been used in other studies (Bardgett et al. 2003; Weigelt et al. 2003), enabling comparison with that work.

We measured dissolved inorganic nitrogen (DIN) and DON by shaking soil samples on an orbital shaker with DD water for 10 min (soil: solution 1:4 w/v). The resulting suspension was centrifuged and filtered through Whatman GF/A paper. The concentration of NO_3^-–N and NH_4^+–N in the extracts was determined colourimetrically in a continuous flow stream using a Bran + Luebbe autoanalyser 3. Total N was determined by oxidation of inorganic and organic nitrogen compounds by sulphate radicals which are produced by the photolytic decomposition of persulphate in an online UV digestor. The resulting NO_3^-–N was measured by autoanalyser procedures. Organic N was then calculated by subtracting the amount of inorganic-N from the total-N in the water extract. For pots with *A. odoratum* TN data is only available for controls due to an autoanalyser failure ($n = 8$ for DON). For soil pH soil samples were shaken with DD water (soil: solution 1:2.5 w/v) and left to settle for 12 h before measurement.

Measurements of total C and N as well as isotope analysis of ^{13}C and ^{15}N of shoots, roots and soil was performed on a continuous flow-isotope ratio mass spectrometry (CF-IRMS), using an automated nitrogen/carbon analysis-mass spectrometry (ANCA-MS) system (Europa 20/20, Crewe, UK; precision 0.0003 atom% ^{15}N and 0.00002 atom% ^{13}C). Ground wheat flour (with 1.08338 atom% ^{13}C and 0.3674 atom% ^{15}N) was used as the working standard. Values of atom percent and concentrations of C and N were used to calculate moles of ^{13}C and ^{15}N in excess of the atomic standard as described in Näsholm et al. (2000). Mean values of ^{15}N and ^{13}C abundances of the unlabelled control plants (water and nitrogen controls) were used as references for ^{13}C and ^{15}N excess and were calculated separately for shoots and roots of each grass species. During incubation time control and labelled pots were mixed randomly in a tray to control for the possibility of refixation of respired $^{13}CO_2$, which would have been detected as enrichment of ^{13}C in plants of the unlabelled control.

Statistical analysis

Analysis of variance and post-hoc Tukey (HSD) tests were used to detect significant differences between the five grass species or N forms. The analyses were performed on untransformed data for total biomass and ^{15}N of roots and shoots, while other data were reciprocal (^{13}C), log (root: shoot ratio) or square root (total ^{15}N per plant) transformed to meet the assumptions of normality and homogeneity of variances. For all non-normally distributed data (pH, DIN, DON, ^{13}C:^{15}N ratio of root and shoot), Kruskal–Wallis Anova and multiple (two-tailed) comparisons were used to test for significant differences between species or N forms. Analysis of covariance (ANCOVA) was used to test if uptake rates and preferences between organic and inorganic N forms were related to plant biomass, using total dry weight as covariate and total ^{15}N uptake per plant as dependent variable. All statistical analyses were performed with Statistica for Windows (Version 6.1, Statsoft, USA.).

Results

Soil analysis and total plant biomass

After 7 weeks of growth, soil analyses revealed significant differences in pH, DIN and DON among species. Soil pH was significantly lower in soil grown with *H. lanatus* and *A. odoratum* compared to the three other species (Table 3). DIN was significantly greater in pots of *N. stricta* and *D. flexuosa* compared to *H. lanatus*, *A. odoratum* and *L. perenne*, but DON concentration was significantly greater in soil planted with *D. flexuosa* and *H. lanatus* than *N. stricta*, *A. odoratum* and *L. perenne* (Table 3). Total plant biomass indicated two groups of species: one group of slow growing species with *N. stricta* and *D. flexuosa*, which are characterized by low root/shoot ratios, and a second group of fast growing species with *H. lanatus*, *A. odoratum* and *L. perenne* having significantly higher root/shoot ratios (Table 3).

Isotope analysis

The marked differences in relative growth rate of plant species complicate the interpretation of results. The higher growth rate of some species will inevitably lead to a higher accumulation of N in shoot tissue. Differences in uptake of different N forms are therefore based on root measurements and given as amount of labelled compounds per unit root biomass rather than the total amount of label per plant. This definition of uptake enables us to quantify inter-species differences which are not primarily based on different growth rates. Data on the amount of label per shoot and total plant biomass, however, allow inferences about internal transport and the overall efficiency of uptake for different N forms (Näsholm and Persson 2001). Here we treat the two fractions separately.

Table 3 Soil *pH*, *DIN* and *DON* (μg g dry weight soil^{-1}), total plant *Dry weight* (mg) and root:shoot ratio (*R:S ratio*) given as means (± SE) for all species over control and other treatments. Different letters indicate significant differences between species, calculated for each parameter separately ($P < 0.05$, $n = 28$)

	pH[e]	DIN[e]	DON[e]	Dry weight[f]	R:S ratio[f]
N. stricta	6.3 (0.0)^{b}	14.6 (0.8)^{b}	1.9 (0.1)^{b}	75.9 (2.2)^{a}	0.4 (0.0)^{a}
D. flexuosa	6.3 (0.0)^{bc}	13.8 (0.8)^{b}	17.8 (1.6)^{c}	56.1 (2.9)^{a}	0.4 (0.0)^{a}
H. lanatus	6.0 (0.0)^{a}	1.0 (0.1)^{a}	8.4 (0.5)^{c}	276.6 (10.2)^{c}	1.5 (0.1)^{d}
A. odoratum	6.1 (0.0)^{a}	0.9 (0.1)^{a}	6.2 (0.2)^{ab}	254.1 (5.5)^{c}	1.2 (0.0) ^{c}
L. perenne	6.4 (0.0)^{c}	2.4 (0.3)^{a}	0.0 (0.2)^{a}	209.4 (5.6)^{b}	0.9 (0.0)^{b}

[e]Kruskal-Wallis ANOVA, multiple 2-tailed comparison
[f]ANOVA, Tukey (HSD), $n = 28$

According to Näsholm et al. (1998), direct uptake of amino acids can be demonstrated by a significant relationship between excess ^{13}C and excess ^{15}N. The linear relationship between root ^{13}C and ^{15}N was significant for 50% of the plant species versus N form combinations, despite the small number of replicates ($n=4$), thus giving evidence that a proportion of the supplied tracer was absorbed as intact amino acid (Fig. 1). The slopes of the regression lines of ^{13}C and ^{15}N in plant material are commonly used to give estimates of the fraction of N taken up as intact amino acid. Relative to the ^{13}C:^{15}N ratio of the applied amino acids, the significant slopes accounted for 21.2, 12.2, 3.8 and 53.6% direct uptake of glycine, serine, arginine and phenylalanine, respectively. The regression slopes also indicate differences between plant species, in that *D. flexuosa* always showed the highest and *L. perenne* the lowest fraction of intact amino acid uptake (Fig.1). This result is also supported by values of ^{13}C:^{15}N ratio of root material (Table 4).

Another indication of direct uptake of all amino acid N forms is the significant enrichment of both ^{15}N and ^{13}C in root tissue for all five grass species tested (Table 4). A comparison of the mean root ^{15}N content over all N forms revealed significant species-specific differences (Fig. 2). *L. perenne* showed the greatest root ^{15}N content, followed by *A. odoratum* and *N. stricta*, whilst *H. lanatus* and *D. flexuosa* had the lowest root ^{15}N values. For root ^{13}C, the variability in uptake was generally higher resulting in no significant differences between species. Taking a closer look at the root ^{15}N content for each species and N form separately reveals no preferential uptake of any N form for *D. flexuosa*, *H. lanatus* and *A. odoratum* (Table 4). *N. stricta* had a significantly greater uptake capacity for serine than the other N forms and *L. perenne* showed a significant preference for uptake of inorganic N compared to the most complex amino acid phenylalanine.

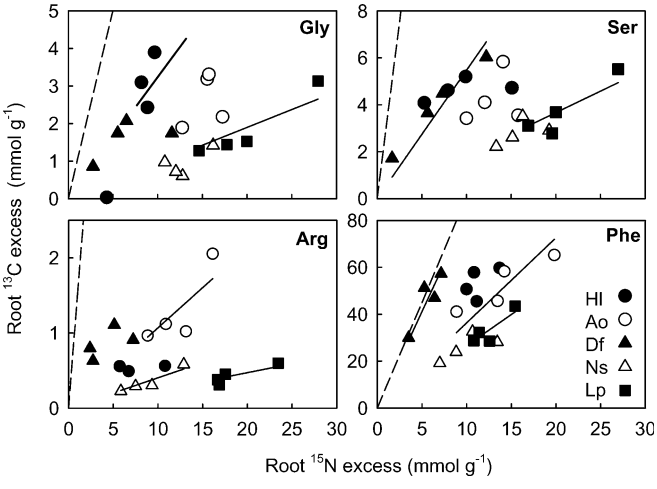

Fig. 1 The relationship between root ^{13}C and ^{15}N in excess of natural abundance for the four different amino acids tested: glycine (*Gly*), serine (*Ser*), arginine (*Arg*) and phenylalanine (*Phe*). Given are single measurements for *H. lanatus* (*filled circles*), *A. odoratum* (*open circles*), *D. flexuosa* (*filled triangles*), *N. stricta* (*open triangles*) and *L. perenne* (*filled squares*). Each symbol represents one pot. *Broken lines* show the molar ^{13}C:^{15}N ratios for the nitrogen sources injected (gly 1:1, ser 3:1, arg 1.5:1 and phe 9:1). *Solid lines* show significant regressions between the excess of both isotopes in plant root material

In shoot tissue, as expected, the slow growing species *N. stricta* and *D. flexuosa* accumulated significantly less ^{15}N than the fast growing species *H. lanatus*, *A. odoratum* and *L. perenne*, although concentration in *L. perenne* was significantly reduced compared to the other fast growing species (Fig. 2). Patterns of shoot ^{13}C content were similar with greater enrichment in fast growing species, but significantly so only for *H. lanatus* and *A. odoratum* (Fig. 2). Compared to ^{15}N concentrations in roots, the slow growing species *Nardus* and *Deschampsia* had greater values of ^{15}N in root than shoot tissue, whereas for *Holcus*, *Anthoxanthum* and *Lolium* shoot ^{15}N concentration was two to three times greater than in roots. These differences are clearly revealed from root: shoot ratios of ^{15}N content (Table 4). Moreover, this ratio demonstrates the differences in transport of different N forms; all species except *A. odoratum* show lower ratios for ammonium-nitrate than amino acids with significant differences between inorganic N and phenylalanine for three species (Table 4). Inorganic N is transported significantly faster from roots to shoots. The overall ^{15}N uptake (sum of root and shoot ^{15}N concentration) was significantly greater in fast growing compared to slow growing species, although it also differed between the slow growing *Nardus* and *Deschampsia* (Fig. 3). Additionally, Fig. 3 reveals a much greater enrichment of ^{15}N for inorganic N compared to the mean of amino acids in the fast growing species, albeit not significant for *H. lanatus*. In contrast, *Nardus* and *Deschampsia* had equal amounts of ^{15}N in inorganic and organic N treatments. This suggests that uptake rates and preferences between inorganic and organic N forms are related to plant growth rate such that plants with higher biomass production take up more inorganic N. Further evidence for this suggestion comes from an analysis of covariance that showed significant differences between ammonium-nitrate and every amino acid if total plant biomass was used as a covariate [ANCOVA, post hoc Tukey (HSD) test, $P<0.05$, $n=20$].

Discussion

Evidence for the uptake of intact amino acids came from the examination of linear relationships between the enrichment of ^{13}C and ^{15}N in root tissue (Näsholm et al. 1998, 2000; Nordin et al. 2001). We found highly significant correlations for 50% of the plant species versus N form combinations, indicating direct uptake of organic N of varying complexity despite the presence of inorganic N. This is the first evidence for direct uptake of serine and phenylalanine by plants growing in field soil. So far, potential uptake for different amino acids has been tested mainly on excised roots and in hydro-

Table 4 Root and shoot excess ^{15}N, ^{13}C:^{15}N ratio and root:shoot ratio of excess ^{15}N for all species studied. Values are means (± SE) for the five treatments of *AmN* ammonium nitrate; *gly* glycine; *ser* serine; *arg* arginine; *phe* phenylalanine. Different letters indicate significant differences calculated separately for each species ($P < 0.05$, $n = 4$)

	Root		Shoot		R:S ratio of ^{15}N[d]
	^{15}N (μmol g^{-1})[d]	^{13}C:^{15}N ratio[e]	^{15}N (μmol g^{-1})[d]	^{13}C:^{15}N ratio[e]	
Nardus stricta					
AmN	8.0 (1.4)[a]	–	4.5 (0.7)	–	1.8 (0.2)
Gly	13.0 (1.2)[ab]	0.1 (0.0)[ab]	8.0 (2.2)	0.2 (0.1)[ab]	2.0 (0.2)
Ser	16.0 (1.3)[b]	0.2 (0.0)[ab]	7.4 (1.6)	0.4 (0.1)[ab]	2.6 (0.7)
Arg	8.9 (1.5)[a]	0.0 (0.0)[a]	4.0 (0.7)	0.1 (0.0)[a]	2.3 (0.3)
Phe	10.0 (1.4)[a]	2.7 (0.2)[b]	4.4 (0.7)	2.3 (0.4)[b]	2.3 (0.3)
Deschampsia flexuosa					
AmN	3.2 (0.2)	–	4.2 (1.0)	–	0.8 (0.2)[a]
Gly	6.6 (1.8)	0.3 (0.0)[a]	4.2 (1.3)	0.2 (0.1)[a]	1.8 (0.5)[ab]
Ser	6.7 (2.2)	0.7 (0.1)[ab]	3.8 (1.4)	1.5 (1.0)[ab]	2.0 (0.3)[ab]
Arg	4.4 (1.1)	0.2 (0.0)[a]	2.7 (0.7)	0.3 (0.1)[ab]	1.7 (0.2)[ab]
Phe	5.6 (0.8)	8.4 (0.5)[b]	2.4 (0.5)	4.0 (1.4)[b]	2.4 (0.2)[b]
Holcus lanatus					
AmN	6.8 (0.3)	–	75.6 (3.6)[b]	–	0.1 (0.0)[a]
Gly	7.8 (1.2)	0.3 (0.1)[ab]	51.4 (6.4)[a]	0.1 (0.0)[ab]	0.2 (0.0)[ab]
Ser	9.5 (2.1)	0.6 (0.1)[ab]	44.6 (0.8)[a]	0.2 (0.0)[b]	0.2 (0.1)[ab]
Arg	7.8 (1.3)	0.1 (0.0)[a]	45.5 (2.4)[a]	0.0 (0.0)[a]	0.2 (0.0)[ab]
Phe	11.4 (0.8)	4.7 (0.3)[b]	38.1 (2.8)[a]	3.5 (0.2)[b]	0.3 (0.0)[b]
Anthoxanthum odoratum					
AmN	13.0 (0.7)	–	73.9 (5.5)[c]	–	0.9 (0.0)[a]
Gly	15.3 (0.9)	0.2 (0.0)[ab]	49.8 (2.1)[b]	0.1 (0.0)[a]	0.3 (0.0)[ab]
Ser	13.0 (1.3)	0.3 (0.0)[ab]	42.8 (2.2)[ab]	0.1 (0.0)[ab]	0.3 (0.0)[ab]
Arg	12.3 (1.6)	0.1 (0.0)[a]	34.5 (2.5)[a]	0.1 (0.0)[a]	0.4 (0.1)[ab]
Phe	14.1 (2.3)	3.9 (0.3)[b]	29.1 (2.1)[a]	3.1 (0.3)[b]	0.5 (0.1)[b]
Lolium perenne					
AmN	25.4 (1.7)[b]	–	79.2 (2.7)[c]	–	0.3 (0.0)[a]
Gly	20.1 (2.8)[ab]	0.1 (0.0)[ab]	36.1 (1.1)[b]	0.0 (0.0)[a]	0.6 (0.1)[ab]
Ser	20.9 (2.2)[ab]	0.2 (0.0)[ab]	36.8 (2.2)[b]	0.1 (0.0)[ab]	0.6 (0.1)[b]
Arg	18.6 (1.6)[ab]	0.0 (0.0)[a]	30.3 (1.9)[ab]	0.1 (0.0)[ab]	0.6 (0.1)[b]
Phe	12.6 (1.0)[a]	2.6 (0.1)[b]	25.0 (2.1)[a]	1.6 (0.1)[b]	0.5 (0.0)[ab]

[d]ANOVA, Tukey (HSD)
[e]Kruskal-Wallis ANOVA, multiple 2-tailed comparison

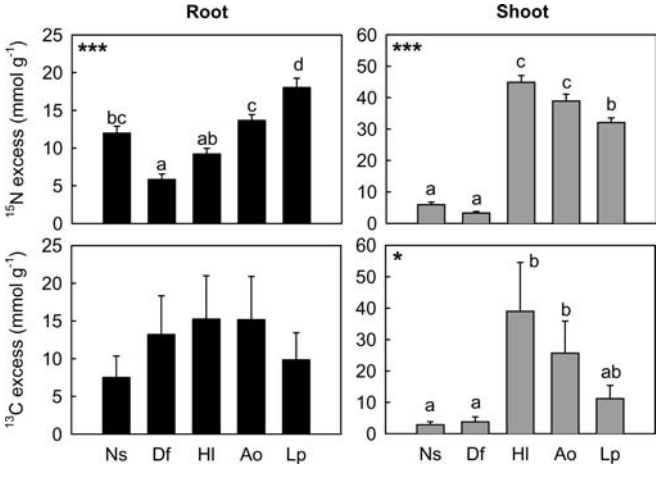

Fig. 2 Excess ^{15}N and ^{13}C values of *N. stricta* (*Ns*), *D. flexuosa* (*Df*), *H. lanatus* (*Hl*), *A. odoratum* (*Ao*) and *L. perenne* (*Lp*) measured separately for root and shoot tissue of amino acid treatments (AmN not included). Values are means with standard errors. *Different letters* indicate significant differences between species at the indicated level except for shoot ^{15}N between Ao and Lp where $P < 0.05$ [ANOVA, post-hoc Tukey (HSD) test, $n = 16$: $^{*}P < 0.05$, $^{***}P < 0.001$]

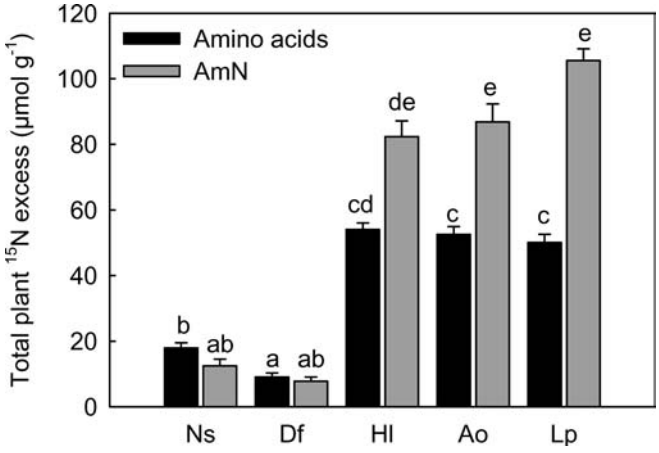

Fig. 3 Total (root + shoot) plant excess ^{15}N of *N. stricta* (*Ns*), *D. flexuosa* (*Df*), *H. lanatus* (*Hl*), *A. odoratum* (*Ao*) and *L. perenne* (*Lp*) for ammonium-nitrate (*AmN*) and the mean of all amino acid treatments. Values are means with standard errors. *Different letters* indicate significant differences between bars (ANOVA, post-hoc Tukey (HSD) test for unequal n, $n = 4$ for AmN and $n = 16$ for amino acids, $P < 0.05$)

ponic solution (Table 1) in the absence of significant competition from soil microbes for these N forms (Stribley and Read 1980; Chapin et al 1993; Owen and Jones 2001). In comparison to field soil, the total amount of amino acids added in this experiment was within the range of TFAA typically found in unimproved temperate grassland sites (Streeter et al. 2000; Bardgett et al. 2003). However, to test preferential uptake of different N forms of varying complexity the addition of equal amounts of N sources was necessary. Therefore, the concentration of individual amino acids added to soils in this experiment was greater than commonly found in natural field soil, where at least phenylalanine and serine contribute less than 25% to the pool of TFAA, while glycine and arginine can be present in relatively high concentrations (Kielland 1995; Raab et al. 1996; also T.K. Nettleton, unpublished data). The amount of direct uptake of phenylalanine and serine might therefore be overestimated relative to its importance in field conditions.

The percentage uptake as calculated from the slopes of linear regression varied from 3.8, 12.2 and 21.2 to 53.6% for arginine, serine, glycine and phenylalanine, respectively. There was, however, pronounced interspecies variability in uptake e.g. for glycine from 10% for *L. perenne* to 32% for *H. lanatus* and similarly so for the other amino acids. The estimated proportional uptake of glycine was within the range of that reported in other studies where 19–23% (Näsholm et al. 2000) 20% (Näsholm et al. 2001) or 32–52% (Bardgett et al. 2003) of intact glycine uptake was found for various graminoids. A study on intact amino acid uptake by tree seedlings, however, reported a much larger fraction of 67–100% for glycine and arginine (Öhlund and Näsholm 2001). The reason for the low slopes mainly of arginine and serine was the low enrichment of ^{13}C relative to ^{15}N, because isotopes were measured in bulk tissue rather than in the soluble N fraction; a problem encountered in several studies (Henry and Jefferies 2003b; Miller and Bowman 2003).

One methodological problem when using dual-labelled amino acids is the dilution of ^{13}C, which is around two orders of magnitude greater than for ^{15}N. This is caused by the high concentration of C in plants and the relatively high level of natural ^{13}C (Näsholm and Persson 2001). A greater ^{13}C addition to soil, therefore, greatly increases the probability of detecting a significant ^{13}C uptake; the much higher ^{13}C:^{15}N ratio and steeper slope of phenylalanine uptake might therefore reflect, in part, this methodological effect. A second reason for the pronounced difference in ^{13}C uptake between phenylalanine and the three other amino acids might be differences in rates of decarboxylation and mineralisation in the soil. Except for glycine, we used amino acids labelled at all C positions, and the loss of the carboxy group during incorporation could explain a rapid shift in ^{13}C:^{15}N ratios e.g. from 3:1 to 2:1 for serine (Näsholm et al. 1998; Lipson and Näsholm 2001). However, aromatic rings are harder to degrade by microbes and would be more stable in the soil compared to the other, simpler molecules. In contrast to phenylalanine, arginine is rapidly ammonified in soil and should therefore be less available than other amino acids, which would explain its low intact uptake (Alef and Kleiner 1986). Moreover, arginine belongs to the group of basic amino acids while glycine, serine and phenylalanine are all neutral amino acids. Hence, binding of the positively charged amino acid arginine to soil particles may have rendered it less available to plants.

Differences in uptake capacity across all N forms are revealed in the concentration of ^{15}N in root tissue, which varied significantly between species, forming a gradient from *Lolium*, which typically dominates high fertility grassland and was most enriched, to *Anthoxanthum* and *Holcus*, which both inhabit soils of moderate fertility and showed intermediate ^{15}N enrichment, to *Deschampsia*, a plant of low productivity grasslands at the low side of root ^{15}N. As an exception, *Nardus*, which typically dominates low fertility grasslands, showed an intermediate root ^{15}N concentration. It is well known that kinetic parameters of amino acid uptake vary for different plant species, as well as fungi and soil microbes (Lipson and Näsholm 2001). These physiological capacities, however, have been determined with excised roots and in hydroponic culture and might differ considerably from direct uptake of amino acids in field soil. Experiments with different species in the field, or in pots with field soil, also found inter-specific differences for the uptake of glycine (Näsholm et al. 2000; Streeter et al. 2000; McKane et al. 2002; Miller and Bowman 2002; Weigelt et al. 2003). Here, we present evidence for species-specific differences in the overall uptake of amino acids including not only glycine, but also other organic N sources against inorganic N.

A more detailed analysis of the root ^{15}N data revealed that *L. perenne* was relatively more effective than the other species at using simple inorganic forms of N compared to more complex amino acids. *N. stricta* took up more serine compared to all other N forms while all other species showed no significant differences in uptake of different N forms, meaning that both inorganic and organic N sources were exploited equally. In addition to the differences in root uptake, there were pronounced differences in shoot and total plant ^{15}N values between species and N forms. These differences between plant species appear to be related to relative growth rate, where a higher total biomass production leads to greater uptake of N and its subsequent transport into shoots. Shoot ^{15}N and ^{13}C content (Fig. 2) therefore mirror total plant dry weight (Table 3). Interestingly, preferences between inorganic and organic N forms are also related to plant growth rate. The fast growing species *H. lanatus*, *A. odoratum* and *L. perenne* took up significantly more inorganic N than their slower growing counterparts during the experimental period. However, the preference for inorganic N was most pronounced for *L. perenne* although this species did not show the highest growth rate. This underlines the importance of species-specific differences for preferential uptake of

inorganic N rather than indicating a simple relationship to plant biomass production.

We believe that the species-specific differences in direct uptake of different N forms, combined with the differences in internal plant transport depending on relative growth rate, could provide some explanation for the increased competitive dominance of *Lolium* on fertilized, high productivity grasslands, where inorganic N is the dominant N form available in soil. On unfertilized, low productivity grasslands, however, slow growing species like *Deschampsia* or *Nardus*, which may take up equal amounts of inorganic and organic N can persist, due to the dominance of organic N as soluble N in those soils. On intermediate fertility or management regimes, other fast growing species like *Holcus* or *Anthoxanthum* might have a competitive advantage, because they are also capable of using a wide range of N sources despite their high biomass production. However, it is important to note that the absolute capacity to take up most N forms, expressed as total amount per gram root dry weight, was still greatest in *Lolium*; in other words, this species, whilst showing a preference for inorganic N over complex organic N forms, is still superior at acquiring N from different N forms present in soil. Any competitive advantage conferred by preference of other species for organic N forms in less fertile grasslands would therefore only result from some kind of interplay between uptake of organic N and other parameters that affected competitive interactions of grasses. Here, it is important to stress that our findings result from plants grown individually or in monoculture, while nothing is known about possible changes of organic N uptake for plants growing in competition.

In conclusion, our data show that five different grass species take up organic N sources of greater complexity than glycine. We also provide evidence of inter-species differences in preferential use of chemical forms of N of varying complexity by some grasses, in that *Lolium* is relatively more effective at using inorganic N compared to amino acids. The faster uptake of inorganic N by plants with greater total biomass production does intensify this effect but does not necessarily overrule species-specific differences. Further studies are necessary to decide whether such species-specific preferences for different nitrogen forms occur in field sites of changing fertility, and whether they are instrumental in enabling species co-existence in temperate grassland.

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