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Do plant species with different growth strategies vary in their ability to compete with soil microbes for chemical forms of nitrogen?

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Abstract

We used dual labelled stable isotope (^{13}C and ^{15}N) techniques to examine how grassland plant species with different growth strategies vary in their ability to compete with soil microbes for different chemical forms of nitrogen (N), both inorganic and organic. We also tested whether some plant species might avoid competition by preferentially using different chemical forms of N than microbes. This was tested in a pot experiment where monocultures of five co-existing grassland species, namely the grasses *Agrostis capillaris*, *Anthoxanthum odoratum, Nardus stricta*, *Deschampsia flexuosa* and the herb *Rumex acetosella*, were grown in field soil from an acid semi-natural temperate grassland. Our data show that grassland plant species with different growth strategies are able to compete effectively with soil microbes for most N forms presented to them, including inorganic N and amino acids of varying complexity. Contrary to what has been found in strongly N limited ecosystems, we did not detect any differential uptake of N on the basis of chemical form, other than that shoot tissue of fast-growing plant species was more enriched in ^{15}N from ammonium-nitrate and glycine, than from more complex amino acids. Shoot tissue of slow-growing species was equally enriched in ^{15}N from all these N forms. However, all species tested, least preferred the most complex amino acid phenylalanine, which was preferentially used by soil microbes. We also found that while fast-growing plants took up more of the added N forms than slow-growing species, this variation was not related to differences in the ability of plants to compete with microbes for N forms, as hypothesised. On the contrary, we detected no difference in microbial biomass or microbial uptake of ^{15}N between fast and slow-growing plant species, suggesting that plant traits that regulate nutrient capture, as opposed to plant species-specific interactions with soil microbes, are the main factor controlling variation in uptake of N by grassland plant species. Overall, our data provide insights into the interactions between plants and soil microbes that influence plant nitrogen use in grassland ecosystems.

Keywords: Amino acids; Grassland; Organic nitrogen; Inorganic nitrogen; Microbial biomass; Plant-microbial competition; Stable isotopes; Growth strategies; Nitrogen

1. Introduction

There is a growing awareness that the uptake of organic nitrogen (N) by plants constitutes a critical component of the terrestrial N cycle (Schimel and Bennett, 2004; Jones et al., 2005). While it has been known for some time that plants have the capacity to uptake organic N from soil, in the form of amino acids, it is still unclear how important this pathway of N acquisition is compared to the uptake of N from microbial mineralization (Schimel and Bennett, 2004; Jones et al., 2005). Despite this, there is now ample evidence that direct uptake of organic nitrogen, in the form of amino acids, represents a substantial fraction of total plant N uptake in a number of terrestrial ecosystems, especially those that are strongly N limited, such as arctic and alpine tundra (Kielland, 1994; Schimel and Chapin, 1996; Raab et al., 1999; Henry and Jefferies, 2003; Nordin et al., 2004), boreal (Näsholm et al., 1998; Nordin et al., 2001) and temperate forest (Finzi and Berthrong, 2005), and low productivity temperate grassland (Streeter et al., 2000; Bardgett et al., 2003; Weigelt et al., 2003, 2005; Harrison et al., 2007).

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Plant species-specific differences in uptake of different chemical forms of N, both organic and inorganic, have also been shown to occur in strongly N limited ecosystems. For example, different species of plants of arctic tundra have been shown to differ in terms of timing, depth and chemical form of N uptake (McKane et al., 2002). Similarly, co-existing plant species of alpine meadows are reported to differ in their ability to take up different forms of soil N (Miller and Bowman, 2002, 2003). It has been argued that this form of partitioning of N, where individual species are differentiated in their use of a limited range of 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; Reynolds et al., 2003; Miller and Bowman, 2003; Bardgett, 2005). Whether this form of species-specific partitioning of the soil N pool occurs in temperate situations, where rates of N turnover are generally faster (Schimel and Bennett, 2004), is less clear: Pot experiments of individual grassland plant species grown in field soil reveal species-level differences in preferences for different forms of N (Weigelt et al., 2005), suggesting that these species have fundamental niches based on chemical form of N. Also, a ^{15}N-labelling study of a range of grasslands in Germany showed that different plant functional groups relied on different N pools to meet their N demands, suggesting that N uptake patterns across functional groups are driven by different fundamental niches for chemical forms of N (Kahmen et al., 2006). In contrast, ^{15}N-labelling studies in British grasslands showed that while co-existing grassland species varied in uptake rates of different chemical forms of N, they all had a similar preference profile across N forms, in that all species preferentially took up simple N forms, such as inorganic N, over more complex amino acids (Harrison et al., 2007). Collectively, these studies suggest that while plant species and functional groups of grassland have fundamental niches based on chemical forms of N (Weigelt et al., 2005; Kahmen et al., 2006), these are not always realised in nature when co-existing species compete for N (Harrison et al., 2007).

One of the complications in determining species-level uptake of different N forms from soil is the extent that individual plant species experience competition from soil microbes for N, which greatly influences the availability of soil N to plants at the individual plant level (Kaye and Hart, 1997). In fertile situations, such as temperate grasslands, plant roots are considered to be poor competitors against microbes for amino acids in soil (Hodge et al., 1999; Owen and Jones, 2001). This view is supported by in situ ^{15}N labelling studies in grassland, which show that soil microbes take up a greater proportion of ^{15}N-labelled amino acid added to soil than do plants (Bardgett et al., 2003; Harrison et al., 2007). Likewise, pot experiments show that microbes compete more effectively than plant roots for ^{15}N-labelled amino acids and inorganic N, and that the intensity of microbial competition for this N is enhanced by stimulation of microbial biomass, leading to reductions in plant growth (Dunn et al., 2006). Coupled with this is the potential for plants themselves to modify the extent that they are subject to microbial competition for N at the species level, by modifying the size and structure of their associated soil microbial community: It is well established that different plant species of grassland can select for specific microbial communities by altering the quantity and quality of resources entering the soil (Bardgett et al., 1999; Wardle et al., 1999; Porazinska et al., 2003; Innes et al., 2004; Wardle et al., 2004; Bartelt-Ryser et al., 2005; Bardgett, 2005). Therefore, it is plausible that selection for microbial communities at the plant species level will alter microbial sink strength for N, thereby altering plant N availability.

The aim of this study was to examine how plant species of temperate grassland vary in their ability to compete with soil microbes for different chemical forms of N, both inorganic and organic. We also tested whether some plant species might actually avoid microbial competition by preferentially using those chemical forms of N that are less favoured by microbes. These objectives were tested in a pot experiment where monocultures of five co-existing grassland species, namely the grasses *Agrostis capillaris*, *Anthoxanthum odoratum, Nardus stricta*, *Deschampsia flexuosa* and the herb *Rumex acetosella*, were grown in field soil from an acid, unfertilised and semi-natural temperate grassland (Bardgett et al., 2001). After seven weeks growth, each plant species was presented with a mixture of four, increasingly complex forms of N: ammonium-nitrate, and the amino acids glycine, serine and phenylalanine. The use of dual labelled (^{13}C and ^{15}N) stable isotope approaches (Streeter et al., 2000) allowed us to detect variability in plant-microbial uptake of the different N forms at the species level. The plant species used cover the range of growth strategies found in British grassland: *D. flexuosa* and *N. stricta* are slow-growing, stress-tolerator grasses, whereas *R. acetosella, A. capillaris* and *A. odoratum* are fast-growing, competitive species (Grime and Hunt, 1975). Our prediction was that fast-growing species will select for a soil microbial community that does not compete effectively with roots for different N forms, thereby allowing these species to maintain high rates of N uptake, irrespective of N form. In contrast, we predict that slower-growing grasses will select for a soil microbial community that competes effectively for N forms, especially more simple forms, thereby reducing the availability of N for plant uptake (Weigelt et al., 2005).

2. Materials and methods

2.1. Experimental setup

Five common grassland species (*A. capillaris*, *A. odoratum, N. stricta*, *D. flexuosa* and the herb *R. acetosella*), which typically coexist in acidic, agriculturally unimproved grassland (UK National Vegetation Classification U4a;

Rodwell, 1992), were grown in field soil. Seeds of each species were germinated at 18 °C on filter paper soaked with distilled water, and three seedlings of each individual species were planted in each foil-covered microcosm (45 mm diameter, 110 mm height) filled with 80 g dry weight equivalent field soil (Weigelt et al., 2005). Microcosms were set up to provide five replicates for each species and for each treatment, yielding a total of 125 microcosms. The soil was collected from the surface 15 cm (Ah horizon) of unimproved *Festuca-Agrostis-Gallium* grassland where these species co-exist (i.e. NVC U4a) at Littledale, in north Lancashire, UK (54°3′N, 2°42′W) (Bardgett et al., 2003). The site is typical of sheep grazed pasture that is ubiquitous to the cool, wet upland fringes of north-west Britain (Rodwell, 1992). The site is located at ~300 m altitude, and had an annual rainfall of approximately 1200 mm and a mean annual maximum temperature less than 25 °C. The soil was acidic, with an average pH of 4.25. Prior to potting, soil was passed through a 6 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 and watered frequently.

2.2. Isotope labelling and harvest

After 7 weeks of growth, a solution (5 ml) containing a mix of N forms of varying complexity, both organic and inorganic, was injected into the root zone of each pot. The solutions contained a mix of the four N forms: ammonium-nitrate, and the amino acids glycine, serine and phenylalanine. Glycine and serine are small molecules and simple in structure, whereas phenylalanine is a relatively large molecule, having both a phenolic ring and a high C:N ratio. Glycine commonly dominates the amino acid profile of these grassland soils, together with aspartate and glutamate (Streeter et al., 2000). However, other amino acids, such as lysine, arginine and serine, are sometimes also present in relatively high concentrations (Streeter et al., 2000; Lipson and Näsholm, 2001). The solution was made up of equal concentrations of the individual N forms (2.5 μg N g dry soil^{-1} for each N form) and the total concentration of N added to each pot was 10 μg g dry soil^{-1}. (This was injected in 1 ml aliquots distributed over the soil surface, using a glass syringe and a luer lock needle, 19 gauge 152 mm length, which was sealed at the tip and had four side ports of 1 mm diameter, located sequentially at 22, 19, 15 and 11 mm from the tip of the needle.) This concentration was based on average concentrations of N from free amino acids typically found in these grassland soils (Streeter et al., 2000; Bardgett et al., 2003; Weigelt et al., 2005; Harrison et al., 2007). While all pots received the same mixed solution of N forms, individual treatments were set-up where only one of each of the four N forms was isotopically labelled with ^{15}N and ^{13}C for amino acids (Glycine–U–$^{13}C_2$ 98%; ^{15}N 98%, Serine–U–$^{13}C_3$–^{15}N, ^{13}C 98%, ^{15}N 98% and Phenylalanine–U–$^{13}C_9$–^{15}N, ^{13}C 98%, ^{15}N 98%, CK Gas Products Ltd.), or as ^{15}N for inorganic N (Ammonium-Nitrate–$^{15}N_2$ ^{15}N 98%+, CK Gas Products Ltd.). One treatment contained no isotopically labelled N forms and was used for natural abundance assessments. This experimental setup, which was replicated 5 times for each treatment, enabled us to test for any preferences in uptake of each of the labelled N forms by plants and the soil microbial biomass. It also allowed us to test whether individual amino acids were taken up directly by plants, as evidenced by enrichment of plant tissue with both ^{13}C and ^{15}N, or as mineral N after microbial mineralization (Näsholm et al., 1998).

Pots were harvested 50 h after labelling to measure short-term plant-microbial competition for N (Kaye and Hart, 1997; Bardgett et al., 2003; Weigelt et al., 2005; Harrison et al., 2007). This short incubation period was also chosen to minimise any effect of isotope pool dilution within the microcosms. At this time, shoots of all pots were clipped and dried at 80 °C for 72 h. Roots were separated from soil and rinsed in 0.5 M $CaCl_2$ before being washed with tap water and dried at 80 °C for 72 h. Soil was passed through a 3 mm sieve and a sub-sample was dried at 80 °C for 72 h, with the remaining soil being stored fresh at 4 °C until further analysis.

2.3. Soil microbial biomass

Microbial biomass carbon (C) and N were measured using the fumigation-extraction technique of Vance et al. (1987). Briefly, soil samples (15 g fresh weight) were fumigated with $CHCl_3$ for 24 h at 25 °C. After removal of the $CHCl_3$, soluble C was extracted from fumigated and un-fumigated samples with 75 ml 0.5 M K_2SO_4 for 30 min on an orbital shaker. Total organic C in filtered extracts (Whatman No. 1) was determined using a Shimadzu 5000 A TOC analyser. Microbial C flush (difference between extractable C from fumigated and un-fumigated samples) was converted to microbial biomass C using a k_{EC} factor of 0.35 (Sparling et al., 1990). Extractable N in the above extracts was determined by oxidation with $K_2S_2O_8$, using the methodology of Ross (1992), and measurement of the resultant NO_3^--N and NH_4^+-N by autoanalyser procedures. The microbial N flush was converted to microbial biomass N using a K_{EN} factor of 0.54 (Brookes et al., 1985).

2.4. Isotopic analysis of plant and soil material

Measurement of total C and N, as well as isotope analysis of ^{13}C and ^{15}N, of ground shoot and root material was performed on a continuous flow-isotope ratio mass spectrometer (CF-IRMS) using an automated nitrogen/carbon analysis-mass spectrometry sysytem (ANCA-MS) (Europa 20/20, Crewe, UK). Ground wheat flour (with 1.08338 at% ^{13}C and 0.3674 at% ^{15}N) and potassium nitrate (with 1.0, 5.0 and 10.0 at% ^{15}N) were used as the working standard. Values of atom percent and concentrations of C and N were

used to calculate moles excess of ^{13}C and ^{15}N, as described in Harrison et al. (2007). Mean values of ^{15}N and ^{13}C abundances of the unlabelled control plants were used as references for ^{13}C and ^{15}N excess and were calculated separately for shoots and roots of each plant species.

Uptake of ^{13}C and ^{15}N by the soil microbial biomass was determined using the fumigation-extraction procedure, followed by diffusion onto acidified paper discs (MacKowen et al., 1987). For microbial ^{13}C assessment, a subsample of the K_2SO_4 extract was immediately frozen and freeze-dried. Acid traps and freeze-dried samples were then combusted using an ANCA-MS (Europa 20/20) system. Any SO_2 derived from the extract was prevented from entering the IRMS part of the system by adding some silver wool (Elemental Microanalysis, Okehamton, UK) to the combustion tube in the elemental analyser. Values of atom percent and concentrations of C and N (in %) were used to calculate uptake of ^{13}C and ^{15}N on a per unit mass basis (micromoles excess of ^{13}C and ^{15}N per gram).

2.5. Statistical analysis

The effects of adding different forms of N on plant and microbial uptake were analysed using a two-way ANOVA, followed by a Fisher's LSD post-hoc test with the SAS statistical package (GLM PROC, SAS 8.2, 1999). Dependant variables were normalised, if required, prior to analysis using square root transformations for shoot ^{15}N and microbial biomass ^{13}C, and $\log_{10}$ transformations for shoot ^{13}C, root ^{15}N and root ^{13}C. Independent variables were species, which had five values (*A. capillaris, A. odoratum, R. acetosella, D. flexuosa and N. stricta*), and treatment, which had four values (Ammonium-nitrate, glycine, serine and phenylalanine). Shoot biomass was included in the model as a covariate in order to determine whether there was any relationship between plant biomass and uptake of ^{15}N. Proportional data were calculated to yield % ^{15}N recovered in plant (shoots and roots), microbial, soil and undetected fractions. Competition between plants and microbes was tested by carrying out a three-way ANOVA followed by a Fisher's LSD post-hoc test. The dependent variable was % ^{15}N recovered and the independent variables were species (as above), treatment (as above) and fraction, which had two values (plant material or microbes). The effect of adding different forms of N on soil microbial biomass C and N and C:N and shoot and root biomass were also analysed using a two-way ANOVA. Dependant variables did not require transformation prior to analysis and the independent variables were as above, however, treatment also included a control, where no labelled N forms were added. Soil moisture content was included in the model as a covariate to test whether plant species effects on microbial biomass C and N were mediated by plant uptake of water.

3. Results

3.1. Plant and microbial biomass

When data were analysed across all treatments, plant species varied significantly in shoot and root biomass. As expected, the two slow-growing species *D. flexuosa* and *N. stricta* had the lowest shoot biomass at harvest ($F_{4,100} = 4.95$, $P = 0.0011$, Fig. 1(a)). *D. flexuosa* also had the lowest root biomass of all species, followed by *R. acetosella* which had significantly less root biomass than the most productive species *A. capillaris* and *A. odoratum* ($F_{4,100} = 14.56$, $P < 0.0001$, Fig. 1(b)). *N. stricta* was

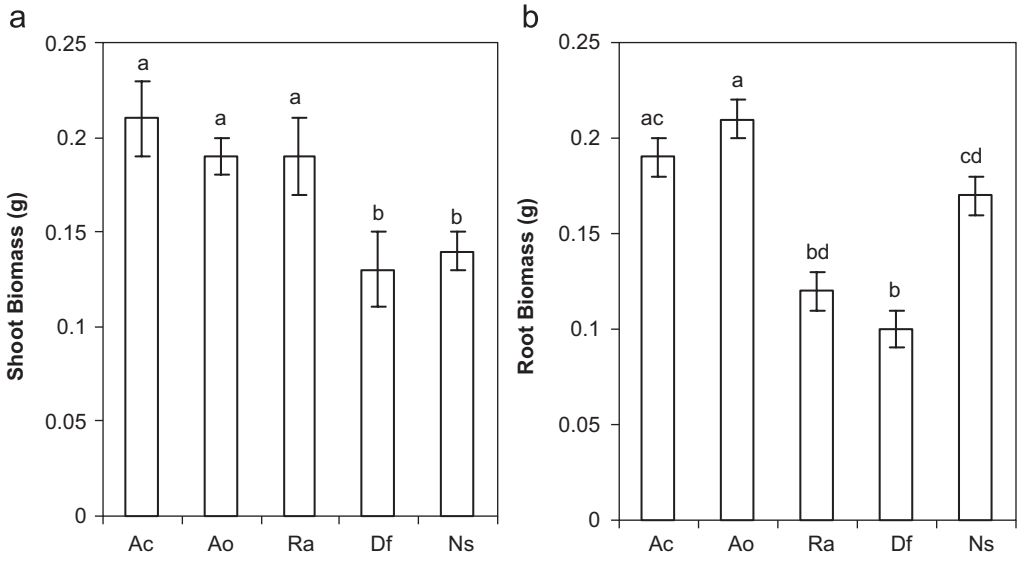

Fig. 1. Biomass of the species *Agrostis capillaris* (Ac), *Anthoxanthum odoratum* (Ao), *Rumex acetosella* (Ra), *Deschampsia flexuosa* (Df) and *Nardus stricta* (Ns) in terms of (a) shoot biomass and (b) root biomass ($n = 25$). Values are means ± SE. Values with the same letter are not significantly different at the $P < 0.05$ level.

intermediate, in that it had a lower root biomass than *A. odoratum*, but not *A. capillaris* (Fig. 1(b)).

Soil microbial biomass C and N, and the microbial C:N ratio, were not affected by any of the N addition treatments (data not presented). However, these measures did differ significantly across plant species: Microbial biomass N was significantly ($F_{4,100} = 6.56$, $P < 0.0001$) greater in soil planted with the two slow-growing grasses (*D. flexuosa* and *N. stricta*) than all other species (Fig. 2(a)), whereas microbial biomass C was significantly ($F_{4,100} = 5.83$, $P = 0.0003$) greater in soils planted with *D. flexuosa* and *N. stricta* than with *A. odoratum* and *R. acetosella*, but not *A. capillaris* (Fig. 2(b)). When soil moisture content was included in the data analysis as a covariate, plant species remained the most significant factor affecting microbial biomass N. This indicates that differences in microbial biomass N between plant species were not a result of changes in soil moisture content resulting from differences in growth rate and water use by plant species. The microbial C:N ratio, a measure of N limitation within the microbial community, was greatest in soils planted with *A. capillaris* ($F_{4,100} = 3.48$, $P = 0.0105$, Fig. 2(c)).

3.2. Plant uptake of different chemical forms of N

All the plant species were able to take up the full range of amino acids presented to them, as shown by ^{15}N and ^{13}C enrichment in shoot tissue (Fig. 3(a) and (b)). According to Näsholm et al. (1998), direct uptake of amino acids can be demonstrated by a significant relationship between excess ^{13}C and ^{15}N. Using this approach, our data indicate direct uptake of the amino acids serine and phenylalanine by some grass species, and glycine and phenylalanine by the

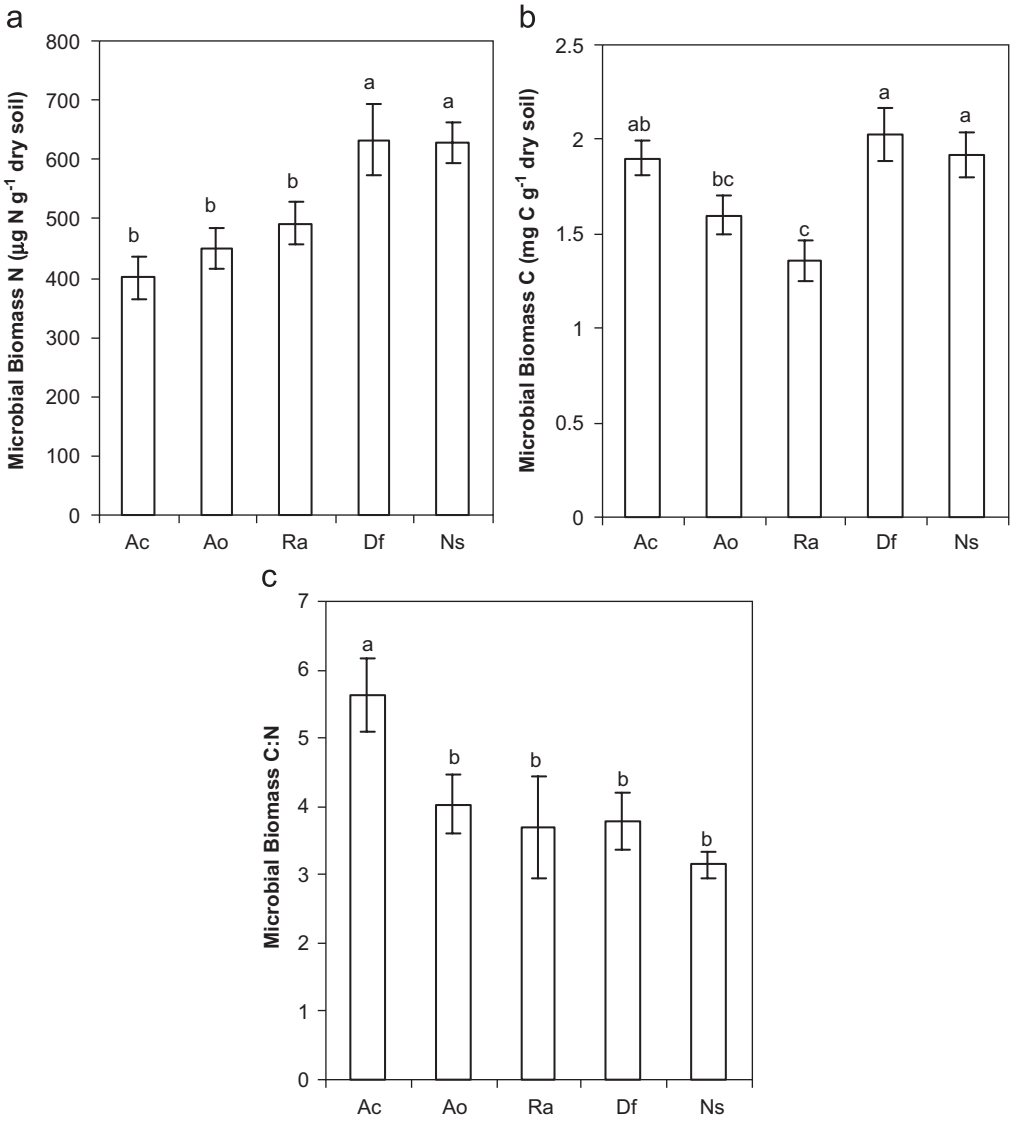

Fig. 2. Microbial biomass (a) N, (b) C and (c) C:N in soil collected from pots containing the species *Agrostis capillaris* (Ac), *Anthoxanthum odoratum* (Ao), *Rumex acetosella* (Ra), *Deschampsia flexuosa* (Df) *and Nardus stricta* (Ns) ($n = 25$). Values are means ± SE. Values with the same letter are not significantly different at the $P < 0.05$ level.

herb *R. acetosella*. Significant linear relationships between ^{13}C and ^{15}N content of shoot tissue were detected for *A. capillaris* ($P = 0.0019$, $r^2 = 0.97$ and $P = 0.0473$, $r^2 = 0.78$ for serine and phenylalanine, respectively) and *N. stricta* ($P = 0.0073$, $r^2 = 0.93$ and $P = 0.0108$, $r^2 = 0.92$ for serine and phenylalanine, respectively); for root tissue, significant linear relationships between ^{13}C and ^{15}N were only detected for *R acetosella* for the amino acids glycine ($P = 0.001$, $r^2 = 0.99$) and phenylalanine ($P = 0.001$, $r^2 = 0.99$) ($n = 5$ for all regressions).

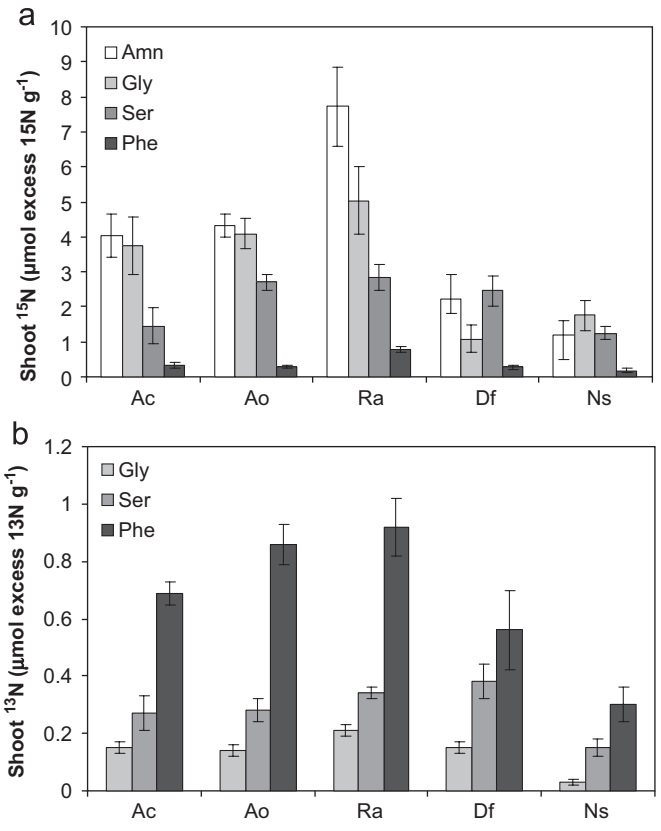

Fig. 3. Shoot (a) ^{15}N and (b) ^{13}C content for the plant species: *Agrostis capillaris* (Ac), *Anthoxanthum odoratum* (Ao), *Rumex acetosella* (Ra), *Deschampsia flexuosa* (Df) *and Nardus stricta* (Ns) when presented with the $^{15}N/^{13}C$ labelled N forms: ammonium-nitrate, glycine, serine and phenylalanine ($n = 5$). Values are means ± SE.

Across all species, ^{15}N enrichment of shoot tissue was greatest for inorganic N and the simplest organic N form glycine, than the more complex amino acids serine and phenylalanine; for all plant species, ^{15}N enrichment of shoot tissue was least when presented with labelled phenylalanine ($F_{3,80} = 63.09$, $P < 0.0001$; Table 1). A similar pattern emerged for ^{15}N enrichment of root tissue: When data were analysed across all species, ^{15}N enrichment of root tissue was significantly ($F_{3,80} = 82.33$, $P < 0.0001$) greater for ammonium-nitrate and glycine, than for the more complex amino acids serine or phenylalanine (Table 1). ^{13}C data showed the opposite pattern, in that both shoot ($F_{3,58} = 107.3$, $P < 0.001$) and root ($F_{3,59} = 92.85$, $P < 0.001$) tissue were most enriched in ^{13}C from phenylalanine and least enriched from glycine (Table 1). Despite these significant overall effects, some plant species-specific differences in uptake of different N forms were detected. In particular, a significant treatment x plant species interaction ($F_{12,58} = 1.99$, $P = 0.001$) for shoot ^{15}N indicated that *A. capillaris*, *A. odoratum* and *R. acetosella* took up, and translocated, more ^{15}N from ammonium-nitrate and glycine to shoots than from the other N forms, whereas the slow-growers *D. flexuosa* and *N. stricta* were equally enriched with ^{15}N from ammonium-nitrate, glycine and serine (Fig. 3(a)). Despite this pattern, analysis of ^{15}N enrichment of root tissue provided no evidence of differential uptake of N forms at the plant species level (data not presented).

Stable isotope data revealed significant differences in N uptake across plant species. When data were integrated across N forms, a significant ($F_{4,80} = 21.59$, $P < 0.0001$) pattern emerged: *R. acetosella* took up and translocated more ^{15}N to shoots than all other plant species, as evidenced by greater ^{15}N enrichment of shoot tissue, whereas the slow growing species *D. flexuosa* and *N. stricta* took up the least ^{15}N into shoot tissue (Table 2). The grasses *A. odoratum* and *A. capillaris* were intermediate in terms of shoot ^{15}N enrichment (Table 2). When included in the analysis as a covariate, we found that shoot biomass did not have a significant affect on ^{15}N enrichment of shoot tissue ($F_{1,79} = 0.39$, $P = 0.5318$), suggesting that there was no relationship between plant uptake and plant biomass

Table 1
Shoot, root and microbial biomass ^{15}N and ^{13}C content over all species when presented with the N forms ammonium-nitrate, glycine, serine and phenylalanine ($n = 25$)

Property	Treatment			
	Ammonium-nitrate	Glycine	Serine	Phenylalanine
Shoot ^{15}N (μmol excess ^{15}N g^{-1})	3.899 ± 0.537^{a}	3.143 ± 0.4056^{a}	2.135 ± 0.1986^{b}	0.369 ± 0.0512^{c}
Shoot ^{13}C (μmol excess ^{13}C g^{-1})	–	0.139 ± 0.0139^{a}	0.283 ± 0.02440^{b}	0.665 ± 0.0582^{c}
Root ^{15}N (μmol excess ^{15}N g^{-1})	3.325 ± 0.3507^{a}	3.587 ± 0.3409^{a}	2.064 ± 0.1728^{b}	0.422 ± 0.0406^{c}
Root ^{13}C (μmol excess ^{13}C g^{-1})	–	0.334 ± 0.036^{a}	0.618 ± 0.0670^{b}	1.913 ± 0.1536^{c}
Microbial biomass ^{15}N (μmol excess ^{15}N g^{-1} dry soil)	0.005 ± 0.0014^{a}	0.006 ± 0.0006^{a}	0.005 ± 0.0005^{a}	0.006 ± 0.0003^{a}
Microbial biomass ^{13}C (μmol excess ^{13}C g^{-1} dry soil)	–	0.011 ± 0.0010^{a}	0.017 ± 0.0020^{b}	0.032 ± 0.0016^{c}

Values are means ± SE. Values with the same letter are not significantly different at the $P < 0.05$ level.

Table 2
Shoot, root and microbial biomass ^{15}N and ^{13}C content over all N forms for the species *Agrostis capillaris, Anthoxanthum odoratum, Rumex acetosella, Deschampsia flexuosa* and *Nardus stricta* ($n = 25$)

Property	Species				
	A. capillaris	*A. odoratum*	*R. acetosella*	*D. flexuosa*	*N. stricta*
Shoot ^{15}N (μmol excess $^{15}N\,g^{-1}$)	2.393 ± 0.4451^{b}	2.851 ± 0.3910^{b}	4.0908 ± 0.6889^{a}	1.5118 ± 0.2899^{c}	1.087 ± 0.1958^{c}
Shoot ^{13}C (μmol excess $^{13}C\,g^{-1}$)	0.367 ± 0.0663^{bc}	0.425 ± 0.0878^{ac}	0.487 ± 0.0883^{a}	0.336 ± 0.0663^{ab}	0.171 ± 0.0368^{d}
Root ^{15}N (μmol excess $^{15}N\,g^{-1}$)	1.904 ± 0.3242^{b}	2.341 ± 0.3331^{ab}	3.304 ± 0.6013^{a}	2.105 ± 0.3530^{b}	2.080 ± 0.3081^{b}
Root ^{13}C (μmol excess $^{13}C\,g^{-1}$)	0.868 ± 0.159^{a}	0.883 ± 0.2067^{ac}	1.171 ± 0.2730^{a}	1.233 ± 0.2849^{a}	0.675 ± 0.1475^{bc}
Microbial biomass ^{15}N (μmol excess $^{15}N\,g^{-1}$ dry soil)	0.006 ± 0.0014^{a}	0.006 ± 0.0007^{a}	0.005 ± 0.0008^{a}	0.006 ± 0.0007^{a}	0.005 ± 0.0005^{a}
Microbial biomass ^{13}C (μmol excess $^{13}C\,g^{-1}$ dry soil)	0.019 ± 0.0027^{ac}	0.025 ± 0.0036^{a}	0.019 ± 0.0027^{ac}	0.019 ± 0.0044^{bc}	0.020 ± 0.0031^{ac}

Values are means ± SE. Values with the same letter are not significantly different at the $P < 0.05$ level.

in this study. A pattern also emerged for root tissue: *R. acetosella* was more enriched with ^{15}N than all other species across all N forms ($F_{4,79} = 4.74$, $P = 0.0018$) (Table 2). Data for plant ^{13}C were less consistent (Table 2), although both shoot and root ^{13}C were lowest for *N. stricta* (Table 2).

3.3. Microbial competition for different forms of N

There was no significant difference in uptake of ^{15}N from different N forms by the soil microbial biomass ($F_{3,75} = 1.17$, $P = 0.3255$; Table 1), nor did plant species identity significantly affect uptake of ^{15}N by the microbial biomass ($F_{4,75} = 1.21$, $P = 0.3141$; Table 2). However, uptake of ^{13}C by the microbial biomass did vary significantly ($F_{3,55} = 53.59$, $P < 0.001$) between N forms, being greatest for phenylalanine, followed by serine, and least for glycine (Table 1). Microbial ^{13}C also varied with plant species identity ($F_{4,55} = 3.84$, $P = 0.008$); when data were integrated across all N forms, microbial ^{13}C was greater in soil planted with *A. odoratum* than *D. flexuosa* (Table 2).

Across all treatments, a significantly lower proportion of ^{15}N was detected in the microbial biomass (25%) than plant material (shoots and roots, 34%) ($F_{1,155}$, $= 21.25$, $P < 0.0001$). However, when data were analysed on the basis of individual N forms, there was a high degree of variation in the proportion of ^{15}N uptake by plants and microbes: Plants took up proportionally more of the added ^{15}N than microbes from ammonium-nitrate, glycine and serine, but the opposite occurred for phenylalanine, where a significantly greater proportion of added ^{15}N was detected in the microbial biomass (44%) than plant material (13%) ($F_{3,155} = 53.03$, $P < 0.0001$ for the fraction x treatment interaction) (Fig. 4(a)). Plant species-specific differences in uptake of different N forms, expressed as a proportion, were also detected: A significantly greater proportion of ^{15}N from all N forms was detected in plant tissue than microbes for the fast-growing plants *A. capillaris, A. odoratum and R. acetosella*. But, there was no significant difference in the proportion of ^{15}N detected in these two pools for the slow-growing grasses *D. flexuosa* and *N. stricta* ($F_{4,155} = 8.24$, $P < 0.0001$ for the species × fraction interaction) (Fig. 4(b)).

4. Discussion

Our data show that plant species covering a spectrum of growth strategies commonly found in temperate grassland are able to take up a range of chemical forms of N, both inorganic and organic. The uptake of amino acids was confirmed by the detection of enrichment of shoot and root tissue of all plant species with both ^{15}N and ^{13}C after labelling soil with dual labelled (^{13}C, ^{15}N) amino acids. However, as discussed by many authors (e.g.; Jones et al., 2004), we cannot rule out the possibility that a significant portion of the labelled organic N was mineralised by soil microorganisms to inorganic N prior to plant uptake. This view is supported by the finding that we found significant linear relationships between excess ^{15}N and ^{13}C in shoot and root tissue for only a limited number of plant species and amino acids; direct uptake of amino acids is thought to be confirmed by a significant relationship between excess ^{13}C and ^{15}N (Näsholm et al., 1998), but absolute confirmation of this would require gas chromatography–mass spectrometry (GC–MS) or gas chromatography–combustion–isotope ratio mass spectrometry (GC–C–IRMS) analysis of plant tissue to detect isotopically labelled amino acids inside the plant (Bol et al., 2002, 2004; Nordin et al., 2004). This uncertainty aside, our data do support the notion that plant species, including those of grassland, are ubiquitous in their ability to uptake a variety of chemical forms of N (Näsholm et al., 2000; Lipson and Näsholm, 2001; Schimel and Bennett, 2004; Jones et al., 2005; Weigelt et al., 2005; Harrison et al., 2007).

When root and shoot ^{15}N data were integrated across all plant species, there was a greater uptake of ^{15}N from labelled inorganic N and glycine, than from the more complex amino acids serine and phenylalanine (Table 1). However, when data were considered at the individual species level, some, albeit small, differences in uptake patterns were apparent and appeared to be related to growth strategy: Shoot tissue of the fast-growing species *A. capillaris, A. odoratum* and *R. acetosella* was more

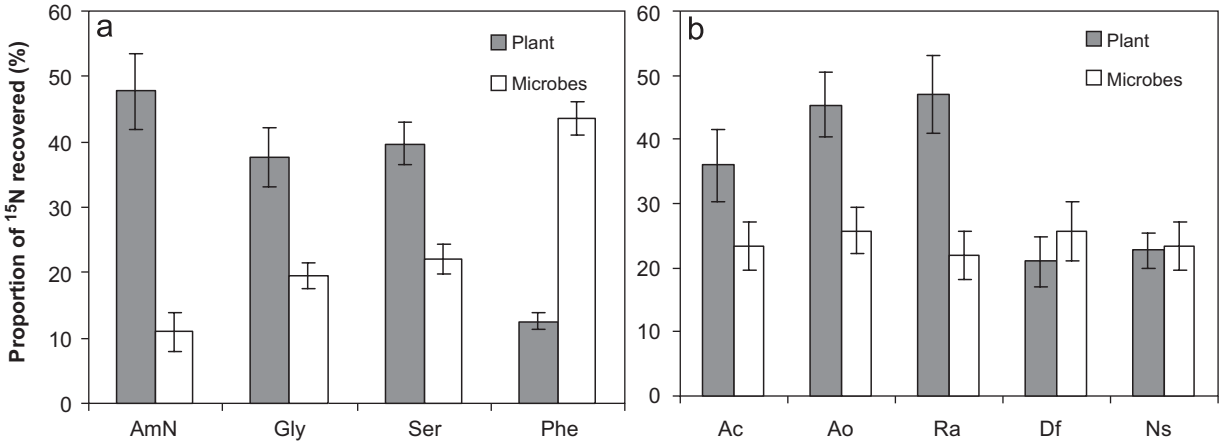

Fig. 4. Proportion (%) of ^{15}N detected in plant material (roots and shoots) and microbial biomass over (a) all N forms in the plant species: *Agrostis capillaris* (Ac), *Anthoxanthum odoratum* (Ao), *Rumex acetosella* (Ra), *Deschampsia flexuosa* (Df) *and Nardus stricta* (Ns) ($n = 25$) and (b) all plant species presented with ammonium nitrate, glycine, serine and phenylalanine ($n = 25$). Values are means ± SE.

enriched with ^{15}N from ammonium-nitrate and glycine than from other N forms, whereas shoot tissue of the slow-growing grasses *D. flexuosa* and *N. stricta* was equally enriched with ^{15}N from ammonium-nitrate, glycine and serine. These findings, which are broadly similar to those of Weigelt et al. (2005), might indicate that while fast-growing species have a preference for inorganic N and simple organic forms over more complex amino acids, slow-growing species are more equal in terms of their uptake and translocation to shoots of different chemical forms of N. However, the absence of more marked differences in preference for N forms across species, and the lack of similar species-specific differences in uptake of ^{15}N in roots, provides little support the notion that plant species of grassland have fundamental niches based on chemical form of N, as has been suggested for other ecosystems (*sensu* McKane et al., 2002; Miller and Bowman, 2003; Reynolds et al., 2003 Kahmen et al., 2006). This conclusion is consistent with the results of Harrison et al. (2007), who found, using in situ labelling techniques in the field, that co-existing plant species of temperate grassland do not have realised niches based on chemical forms of N; in their study, all co-existing species tested showed a preference for inorganic N over organic N forms, and simple amino acids over more complex one.

Our main hypothesis was that fast-growing plant species would be better able to compete with the soil microbes for N than slow-growing species. While we found that the three fast-growing plants (*R. acetosella*, *A. odoratum* and *A. capillaris*) took up a greater amount of ^{15}N across all N forms than did the slow-growing species (*D. flexuosa* and *N. stricta*), this variation appeared to be independent of microbial competition for N; indeed, we detected no difference in microbial uptake of ^{15}N, expressed as an absolute amount or proportion of that added, between fast- and slow-growing plant species (Fig. 4(b)), although microbial biomass N was greater in soil planted with slow-growing than fast-growing species (Fig. 2(a)). These findings suggest, therefore, that plant traits related to resource capture, as opposed to plant species-specific interactions with soil microbes (Wardle et al., 2004; Bardgett et al., 2005; Dunn et al., 2006), are likely to be the main factor controlling variation in uptake of N by grassland plant species. It has been proposed that the uptake of ^{15}N from different N forms is related to relative growth rate (Weigelt et al., 2005). However, we did not detect a relationship between uptake of ^{15}N into shoots and total plant biomass production. On the contrary, while plant species had strongly significant effects on uptake of ^{15}N, shoot biomass did not affect this measure. Furthermore, uptake of ^{15}N into roots and shoots was greatest for the herb *R. acetosella,* despite having a lower total biomass than the two fast-growing grasses, and similar total biomass to the slow growing grass *N. stricta*. These findings suggest that plant traits other than relative growth rate are responsible for inter-specific variation in uptake of N from soil. We did not examine differences between species in terms of plant traits related to resource capture, but traits such as root proliferation (Hodge, 2004), root length density (Fransen and de Kroon, 2001; Aanderud et al., 2003) and specific root length (Eissenstat, 1991) are likely to be important.

It is generally thought that microbes are more effective competitors for organic N than are plants in relatively fertile grasslands (Hodge et al., 1999; Owen and Jones, 2001; Bardgett et al., 2003; Jones et al., 2005), largely due to the complete coverage of the soil volume by microbes and their high surface-to-volume ratio which places them in a preferential position to filter amino acids more rapidly than plants (Owen and Jones, 2001). This view has been confirmed by field studies of temperate grasslands, which have used field labelling approaches to show that microbes uptake a greater proportion of added ^{15}N from a variety of chemical forms of N than do plants (Bardgett et al., 2003; Harrison et al., 2007). Here, we found the opposite to be true: a significantly greater proportion of added ^{15}N from ammonium, glycine and serine was detected in plant

material (shoots and roots) than in the microbial biomass, suggesting that grassland plant species, at least under greenhouse conditions, were more effective in acquiring added N than were soil microbes. This was not the case, however, for the more complex amino acid phenylalanine, which was taken up more effectively by microbes than plants. This could be due to the size of this amino acid, as it has been shown that some plants take-up some amino acids, for example glycine, faster than heavier amino acids such as phenylalanine (Kielland, 1994; Lipson et al., 1999). Also, the diffusion rates of phenylalanine, which is a heavy, relatively non-polar compound, are likely to be quite slow in soils, preferentially benefiting the ubiquitous microbial biomass over more localised plant roots. Conversely, degradation by soil microbes may also play an important role, because simple amino acids such as glycine are degraded by microbes at a lower rate than more carbon-rich amino acids. This means that the simpler amino acids might be more available to plant roots because they are poorer C-sources for microbes in comparison to larger, C-rich molecules such as phenylalanine (Lipson and Näsholm, 2001). This view is supported by the microbial ^{13}C data, which was greatest for phenylalanine, followed by serine, and least for glycine, and is consistent with the findings of Harrison et al. (2007) who also found in the field that microbes took up more significantly more ^{13}C from phenylalanine than from other N forms. Collectively, these finding support our hypothesis that plants and microbes might avoid competition for soil N on the basis of differential use of chemical forms.

Previous authors have highlighted the need for caution over the use of labelled compounds to determine organic N use by plants (Jones et al., 2004). As discussed previously (Weigelt et al., 2005; Harrison et al., 2007) one potential limitation of our approach is differential dilution of N forms added to soil. This issue arises from the fact that we added equal amounts of the N sources to test for preferential uptake of the different N forms, while keeping the total amount of amino acids constant and within the range of the seasonal mean concentration for total free amino acids experienced in the field (Streeter et al., 2000; Harrison et al., 2007). As a result, the concentration of individual amino acids added to soil will have been greater than found in soil, where phenylalanine and serine typically contribute less than 25% to the free amino acid pool, and glycine can be present in relatively high concentrations (Kielland, 1995; Raab et al., 1996; Streeter et al., 2000). The amount of uptake of phenylalanine and serine might therefore be overestimated relative to its importance. This, however, does not alter our conclusions that plant species use a variety of N forms, both organic and inorganic, but they showed little differentiation in the use of different N forms at the species level.

In sum, and in line with previous studies (Weigelt et al., 2005) our data show that grassland plant species with different growth strategies are able to compete effectively with soil microbes for most N forms presented to them, including inorganic N and amino acids of varying complexity. Contrary to what has been found in strongly N limited ecosystems (e.g.; McKane et al., 2002), we did not find evidence of differential use of different chemical forms of N, other than that fast-growing plant species translocated to shoots more ^{15}N from ammonium-nitrate and glycine, than from more complex amino acids, whereas shoot tissue of slow-growing species was equally enriched in ^{15}N from all these N forms; all species tested, however, least preferred the most complex amino acid phenylalanine, which was preferentially used by soil microbes. Another key finding of our study was that while fast-growing plants took up more of the added N forms than slow-growing species, this variation did not appear to be related to differences in the ability of plants to compete with microbes for N forms, as was hypothesised. On the contrary, we detected no difference in microbial biomass or microbial uptake of ^{15}N between fast- and slow-growing plant species, suggesting that plant traits that regulate nutrient capture, as opposed to plant species-specific interactions with soil microbes, are the main factor controlling variation in uptake of N by grassland plant species.

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