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Spatio-temporal variations determine plant–microbe competition for inorganic nitrogen in an alpine meadow

Xingliang Xu[1]*, Hua Ouyang[1], Andreas Richter[2], Wolfgang Wanek[2], Guangmin Cao[3] and Yakov Kuzyakov[4]

[1]*Key Laboratory of Ecosystem Network Observation and Modelling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, 11A Datun Road, Chaoyang District, Beijing 100101, China;* [2]*Department of Chemical Ecology and Ecosystem Research, University of Vienna, Althanstrasse 14, A-1090 Wien, Austria;* [3]*Northwest Institute of Plateau Biology, Chinese Academy of Sciences, 23 Xinning Road, Xining 810008, China; and* [4]*Department of Agroecosystem Research, University of Bayreuth, D-95440 Bayreuth, Germany*

Summary

1. Plant–microbe competition for available nitrogen (N) has been suggested to be an important mechanism controlling N limitation of plants in a variety of ecosystems. However, spatio-temporal patterns of competition between plants and microbes for soil N remain unclear.

2. Short-term ^{15}N tracer experiments were conducted during a growing season (July, August and September) in an alpine meadow on the Tibetan Plateau to unravel spatio-temporal patterns of plant–microbe competition for NH_4^+ and NO_3^-.

3. Alpine plants were poorer competitors than soil microorganisms for inorganic N in July compared with August and September. Occupation of soil volume by roots and root density (high in August and September) played a greater role in plant–microbe competition than air temperature or precipitation (high in July).

4. In topsoils (0–5 cm, highest root density), alpine plants effectively competed with soil microorganisms for N and showed a preference for $^{15}NO_3^-$, while soil microorganisms that preferentially took up $^{15}NH_4^+$ out-competed plants below 5 cm soil depth (lower root density). Competition between plants and soil microorganisms for inorganic N strongly depended on root density ($P < 0.0001$, $R^2 = 0.93$, exponential decay model).

5. *Synthesis*. Plant–microbe competition for inorganic N showed a clear spatio-temporal pattern in alpine meadows depending on (i) root density and therefore soil depth, (ii) inorganic N form, and (iii) different periods during the growing season. These findings have important implications for our understanding of above-ground–below-ground interactions and plant–microbial competition for available N.

Key-words: ^{15}N tracer, alpine *Kobresia* meadow, ammonium, inorganic N, nitrate, plant–microbe competition, plant–soil (below-ground) interactions, rhizosphere interactions, spatio-temporal pattern

Introduction

Nitrogen (N) is a key element controlling primary production in many terrestrial ecosystems (Vitousek & Howarth 1991; Aerts & Chapin 2000; Lebauer & Treseder 2008). Competition for N between plants and soil microorganisms is thought to be an important mechanism controlling N limitation in plants (Kaye & Hart 1997). This indicates that a better understanding of the competition for available N between plants and microorganisms is a prerequisite to unravelling the mechanisms behind N limitation of plant growth in terrestrial ecosystems. However, the exact nature of this competition remains unclear (Kaye & Hart 1997; Hodge, Robinson & Fitter 2000). Whether plants or microorganisms compete more efficiently for available N in terrestrial ecosystems has been intensively debated (Hodge, Robinson & Fitter 2000). A growing body of studies has focused on such competition under both field and controlled conditions (Verhagen, Laanbroek & Wolendorp 1995; Lipson & Monson 1998; Lipson *et al.* 1999; Korsaeth, Molstad & Bakken 2001; Bardgett, Steeter & Bol 2003; Cheng &

*Corresponding author. E-mail: xuxingl@hotmail.com

Bledsoe 2004; Xu *et al.* 2008). These studies showed that soil microorganisms acquire available N effectively in temperate grasslands (Bardgett, Steeter & Bol 2003), arctic tundra (Nordin, Schmidt & Shaver 2004) and subarctic heath ecosystems (Andresen *et al.* 2008).

Soil resources available to plants and microorganisms in ecosystems vary temporally and spatially (Chapin 1980; Gibson 1986; Magid & Nielsen 1992; Hodge *et al.* 2000; Corre, Schnabel & Stout 2002; Zhu & Carreiro 2004; Miller *et al.* 2009). Temporal patterns in demand for soil-available N play an important role in regulating N cycling in cold ecosystems (Bardgett *et al.* 2002, 2007; Weintraub & Schimel 2005). Moreover, the soil microbial community also changes with season in alpine ecosystems. This indicates the difficulty in fully understanding such competition without considering the spatio-temporal context (Hodge, Robinson & Fitter 2000; Bardgett *et al.* 2005). Several studies point to temporal partitioning of N between plants and microorganisms: in an alpine meadow in the Colorado Front Range, USA, Jaeger *et al.* (1999) showed that plants acquire more NH_4^+ (required for growth) during the early growing season, while microorganisms immobilized N only late in the growing season. Similar patterns were found in montane heath communities in Scotland, UK, with greater microbial N immobilization in autumn than early in the growing season (Bardgett *et al.* 2002). A seasonal partitioning was reported in a grazed Arctic salt marsh (Hargreaves, Horrigan & Jefferies 2009). However, NH_4^+ and NO_3^- coexist in soil solution, and plants and soil microorganisms often show different preferences for the two. For instance, many dominant plant species in alpine meadows prefer to take up NO_3^- (Miller, Bowman & Suding 2007; Song *et al.* 2007). Our previous studies also showed that the fate of NO_3^- and NH_4^+ differed distinctly in alpine meadows within 1 year: more of the former was recovered in plants, more of the latter in microbial biomass and soil organic matter (Xu *et al.* 2003, 2004). Moreover, roots (Jama *et al.* 1998; Schenk & Jackson 2002; Tao *et al.* 2006; Zhou, Chao & Zhou 2007; Ma *et al.* 2008), microorganisms (Bardgett *et al.* 1997; Fierer, Schimel & Holden 2003; Wang, Cao & Wang 2007) and nutrients (Gupta & Rorison 1975; Merryweather & Fitter 1995; Jama *et al.* 1998; Farley & Fitter 1999) decrease down the soil profile. The decrease of roots with soil depth, however, is stronger compared with that of microorganisms. A better understanding of spatio-temporal competition for available N therefore requires a simultaneous investigation of NO_3^- and NH_4^+ acquisition by plants and microorganisms with soil depth.

The Tibetan Plateau has been regarded as 'the third pole of the Earth' (Qiu 2008). The low temperature at this high altitude depresses soil organic matter decomposition, but does not affect N immobilization by microorganisms (Song *et al.* 2007). Thus, the inorganic N concentration in these meadows is low, and plant growth is strongly limited by available N (Zhou 2001). Although one of our previous studies showed that organic N may be a significant N source for alpine plants (Xu *et al.* 2006), inorganic N contributed more than 80% to plant N nutrition. Although concentrations of dissolved organic N (DON) were slightly higher than dissolved inorganic N (DIN) in the alpine meadow soils (Table 1), the largest fraction of DON is not directly available for microorganisms and roots (Blagodatskaya *et al.* 2009). We therefore focused only on inorganic N uptake by plants and microorganisms in this study. The inorganic N concentration in the topsoil also showed a clear seasonal pattern, increasing in early July and in mid-August, but decreasing in late July (Zhou 2001). Interactions between plant species can mediate the competition for inorganic N with soil microorganisms (Song *et al.* 2007), indicating strong competition for available N during the growing season in alpine meadows.

Table 1. Characteristics of the upper 10 cm of soils at the study site. Means ± 1 SE are shown (n = 6–8). Dissolved organic N (DON) measured as total dissolved N minus dissolved inorganic N (DIN). Data from Xu *et al.* (2006)

pH (H_2O)	8.0 ± 0.1
Bulk density (g cm^{-3})	0.70 ± 0.05
C:N ratio	12.8 ± 0.2
Soil organic C (%)	7.06 ± 0.37
Total soil N (%)	0.55 ± 0.03
Microbial biomass N (g N m^{-2})	6.5 ± 0.3
DON (g N m^{-2})	1.8 ± 0.1
DIN (g N m^{-2})	1.4 ± 0.4

We here performed a short-term ^{15}N tracer experiment to investigate the temporal and spatial competition for NH_4^+ and NO_3^-. We tested the following three hypotheses, i.e. alpine plants compete more efficiently than soil microorganisms

1 for inorganic N in the topsoil (0–5 cm) compared with the soil layers from 5 to 15 cm (defined here as the subsoil), because root density is much higher in the topsoil than in the subsoil (Zhou 2001; Tao *et al.* 2006);

2 for inorganic N in the middle of the growing season compared with later stages because of higher growth rates and subsequent higher root production in the middle of the growing season related to high temperature and rainfall;

3 for NO_3^- than for NH_4^+, because NO_3^- moves more easily in soil solution than NH_4^+ (Nye & Tinker 1977; Owen & Jones 2001; Miller & Cramer 2004) and roots therefore have a better chance to capture NO_3^-.

Materials and methods

STUDY SITE

The experiment was conducted at the Haibei Alpine Meadow Ecosystem Station of the Chinese Academy of Sciences, Qinghai Province (37°36′60″ N, 101°19′14″ E, 3215 m a.s.l.). The area is located in the typical alpine meadow zone and climate. Average annual temperature and annual precipitation were −1.7 °C and 600 mm, respectively, during the past 25 years. Average July temperature and rainfall were c. 10.0 °C and 110 mm, respectively. The dominant plant species are *Kobresia humilis* Serg., *Stipa aliena* Keng., *Poa* sp., *Festuca ovina* Linn., *Gentiana aristata* Maxim, *Gentiana straminea* Maxim.,

Saussurea superba Anth., and *Gueldenstaedtia diversifolia* Maxim. (Zhou 2001). The soil is classified as Mat-Gryic Cambisol (Chinese Soil Taxonomy Research Group 1995), corresponding to Gelic Cambisol (WRB 1998). An overview over soil properties are presented in Table 1 (Xu *et al*. 2006).

EXPERIMENTAL LAYOUT

A 25 × 25 m area, uniform in cover and species composition, was selected in a *K. humilis* meadow in 2004. In July, August and September, 90 circular microplots (diameter 10 cm) were randomly set up. These microplots were equally divided into two groups: $^{15}NH_4^+$ (injected with $^{15}NH_4NO_3$) and $^{15}NO_3^-$ (injected with $NH_4{}^{15}NO_3$). Each group included three soil depth treatments (0–5, 5–10 and 10–15 cm) and three sampling times (4, 24 and 48 h after ^{15}N addition), with five replicates per treatment. ^{15}N tracers (98.2% ^{15}N enrichment for $^{15}NO_3^-$ and 98.4% enrichment for $^{15}NH_4^+$) were dissolved in H_2O and injected at 2.5 cm depth for the 0–5 cm soil depth treatment, at 7.5 cm for the 5–10 cm soil depth, and at 12.5 cm for the 10–15 cm soil depth. Each microplot was injected with 7 mL ^{15}N solution, and the added amount corresponded to 0.32 g N m^{-2}. In total, a four-factor design was constructed: the first factor was N form (^{15}N in NO_3^- or NH_4^+); the second factor was season (July, August or September); the third factor was sampling time after ^{15}N injection (4, 24 or 48 h); and the fourth factor was soil depth of ^{15}N injection (2.5, 7.5 or 12.5 cm).

SAMPLING AND ANALYSES

Four, 24 and 48 h after ^{15}N tracer injection, above-ground plant parts were collected using scissors. Soil samples were collected down to 15 cm depth and cooled immediately to 4 °C. Roots were carefully separated from soils and rinsed first with tap water, then for 30 min with 0.5 mmol L^{-1} $CaCl_2$ solution and again with distilled water. Above-ground plant parts and roots were dried at 75 °C for 48 h, weighed for dry mass and ground to a fine powder using a ball mill (MM2, Fa. Retsch, Haan, Germany) for measuring N content, and ^{15}N:^{14}N ratios. Fresh soil was sieved to 2 mm and stored at −20 °C for later measurements. Aliquots (2 mg) of plant materials were weighed into tin capsules to analyse total N, C and ^{15}N:^{14}N ratios by continuous-flow gas isotope ratio mass spectrometry (MAT253, Finnigan MAT, Bremen, Germany), coupled by ConFlo III device (Finnigan MAT, Bremen, Germany) to an elemental analyser (EA 1112, CE Instruments, Milan, Italy).

^{15}N incorporation into microbial biomass was determined by chloroform fumigation (Brookes *et al*. 1985). Twenty grams of fresh soil were fumigated with chloroform for 24 h and then immediately extracted with 60 mL 0.5 M K_2SO_4. An additional soil sample was extracted without fumigation.

CALCULATION AND STATISTICS

^{15}N atom% excess (APE) was calculated as the atom% ^{15}N difference between treated and control plants. ^{15}N recovery by plants was calculated by multiplying the N content in the pool by its mass per square meter and APE, divided by total added ^{15}N per square meter. ^{15}N recovery by microbial biomass was calculated as the difference in ^{15}N mass between fumigated and non-fumigated soil samples, divided by total added ^{15}N per square meter (Zogg *et al*. 2000). In this study we did not apply a K_{EN} factor to correct microbial ^{15}N uptake for incomplete extraction of microbial N in the chloroform-fumigation samples for two main reasons. First, Jenkinson (1988) suggested that the correction factor should not be used when N immobilization controlling microbial activity is variable. In the alpine meadows N immobilization was the dominant process of soil N cycling, exceeding N mineralization by far (Song *et al*. 2007), and N immobilization varied between seasons. Secondly, as has been argued before (Hofmockel, Schlesinger & Jackson 2007), a recovery coefficient should not be applied in such studies due to the uncertainties associated with temporal variations in the extractability of N and the variability in incorporation efficiency into the cytoplasmic (soluble) vs. structural (insoluble) components (Bremer & van Kessel 1990). Therefore, the results given here represent a conservative estimate of the microbial biomass pool and isotope content. To consider a possible bias and for purpose of comparison with the conservative estimate, we also calculated the plant–microbe competition using the K_{EN} factor of 0.54 (Brookes *et al*. 1985) to correct microbial ^{15}N uptake for incomplete extraction (see Discussion section; termed 'speculative estimate', Fig. 4). The competition between plants and soil microorganisms for N was referred to as the ratio of ^{15}N recovery by microbial biomass to ^{15}N recovery by plants.

The standard errors of means are presented in figures and tables as a variability parameter. Multivariate ANOVA was calculated to estimate the effects of N form, season, sampling time, soil injection depth, and their interactions on ^{15}N recovery by microbial biomass, ^{15}N recovery by plants and the ratio of ^{15}N recovery by microbial biomass to ^{15}N recovery by plants using the SPSS 16.0 software package (SPSS Inc., Chicago, IL, USA). The contribution of the factors and their interactions to the total variance was calculated by dividing the respective type III sum of squares by the total sum of type III sum of squares from multifactorial ANOVA. All differences were tested at $P < 0.05$. Results of regression analysis of root biomass (or microbial biomass) vs. ratios of ^{15}N recovery by microbial biomass to ^{15}N recovery by plants were calculated by the SPSS 16.0 software package.

Results

SPATIO-TEMPORAL PATTERNS OF ^{15}N RECOVERY BY MICROBIAL BIOMASS FROM $^{15}NH_4^+$ AND $^{15}NO_3^-$

There was a spatio-temporal effect on ^{15}N uptake by microorganisms from $^{15}NH_4^+$ and $^{15}NO_3^-$ in alpine meadows on the Tibetan Plateau (Table 2, Fig. 1 and also see Fig. S1 in Supporting Information): ^{15}N recovery by microbial biomass showed a different pattern with increasing soil depth of ^{15}N injection at different times during the growing season. In July, ^{15}N recovery by microbial biomass from $^{15}NH_4^+$ and $^{15}NO_3^-$ showed similar, but lower values in the two upper soil layers (0–5 cm and 5–10 cm depth), but higher values at 10–15 cm depth (Fig. 1; $P < 0.05$ for $^{15}NH_4^+$ and $P < 0.05$ for $^{15}NO_3^-$). In August, ^{15}N recovery from $^{15}NO_3^-$ remained constant with increasing soil depth ($P = 0.19$), whereas recovery from $^{15}NH_4^+$ declined with increasing soil depth of ^{15}N injection ($P < 0.005$). Recovery from $^{15}NH_4^+$ and $^{15}NO_3^-$ differed significantly in the two top soil layers (Fig. 1; $P < 0.05$). In September, recovery by microbial biomass from $^{15}NO_3^-$ did not significantly change with increasing injection depth ($P = 0.56$), while recovery from $^{15}NH_4^+$ exhibited a pattern similar to that shown in July (Fig. 1; $P < 0.05$). In topsoil, recovery from $^{15}NH_4^+$ was significantly higher than from $^{15}NO_3^-$ (Fig. 1; $P < 0.05$).

Table 2. Multifactorial analysis of variance for the effects of ^{15}N form added, sampling time, season, soil injection depth and their interactions on ^{15}N recovery by microbial biomass and by plants. The competition for ^{15}N between plants and soil microorganisms is presented as ratio of ^{15}N recovery by microbial biomass to ^{15}N recovery by plants. *P* values for significant effects and interactions are in bold

	^{15}N recovery by microbial biomass		^{15}N recovery by plants		Ratio of ^{15}N recovery by microbial biomass to ^{15}N recovery by plants	
Source of variation	*F* values	*P* values	*F* values	*P* values	*F* values	*P* values
Time	2.19	0.12	33.03	**< 0.001**	3.93	**0.02**
Season	28.64	**< 0.001**	73.30	**< 0.001**	50.43	**< 0.001**
Depth	7.99	**< 0.001**	416.45	**< 0.001**	114.16	**< 0.001**
Forms	36.93	**< 0.001**	127.13	**< 0.001**	64.98	**< 0.001**
Time × Season	1.34	0.26	6.17	**< 0.001**	1.13	0.35
Time × Depth	0.78	0.54	4.29	**0.002**	1.62	0.17
Time × Forms	1.07	0.35	1.33	0.267	2.03	0.13
Season × Depth	7.76	**< 0.001**	9.14	**< 0.001**	7.91	**< 0.001**
Season × Forms	3.26	**0.04**	2.96	0.054	1.19	0.31
Depth × Forms	0.77	0.47	4.29	**0.015**	10.51	**< 0.001**
Time × Season × Depth	2.64	**0.009**	5.73	**< 0.001**	7.88	**< 0.001**
Time × Season × Forms	5.58	**< 0.001**	0.36	0.836	3.00	**0.02**
Time × Depth × Forms	0.30	0.88	2.00	0.097	1.67	0.16
Season × Depth × Forms	5.83	**< 0.001**	2.52	**0.043**	0.96	0.43
Time × Season × Depth × Forms	1.33	0.23	2.21	**0.028**	1.76	0.09

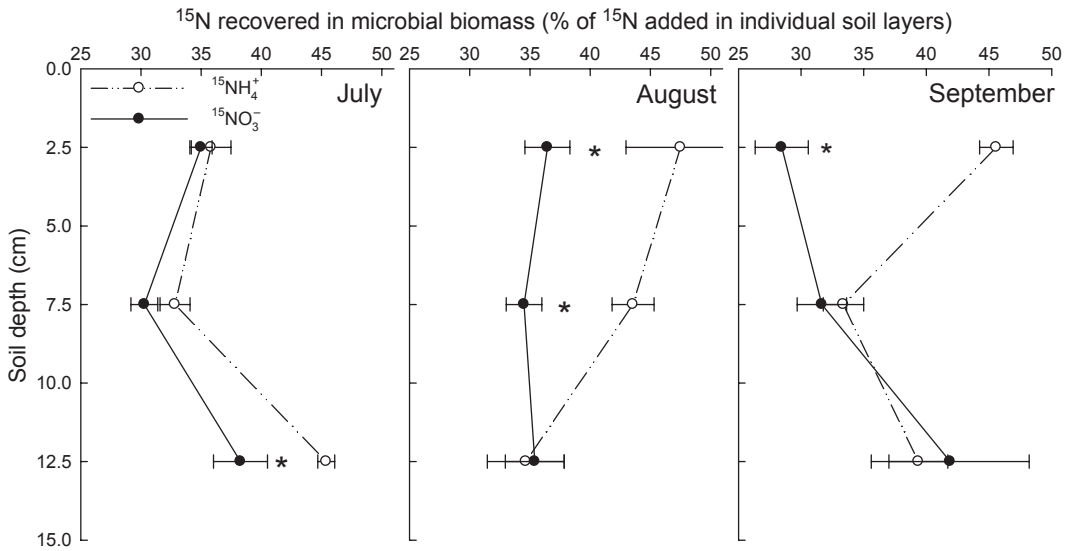

Fig. 1. ^{15}N recovery by microbial biomass (% of added ^{15}N) from $^{15}NH_4^+$ and $^{15}NO_3^-$ 4 h after ^{15}N injection at different soil depths during the growing season (July, August and September). Values are means (± 1 SE) of five replicates. Asterisks indicate significant differences between the ^{15}N recovery by microbial biomass from $^{15}NH_4^+$ and $^{15}NO_3^-$ injections.

SPATIO-TEMPORAL PATTERNS OF ^{15}N RECOVERY BY PLANTS FROM $^{15}NH_4^+$ AND $^{15}NO_3^-$

^{15}N uptake by plants from $^{15}NH_4$ and $^{15}NO_3^-$ also showed a clear spatio-temporal pattern (Figs. 2 and S2; Table 2). While ^{15}N recovery decreased with increasing soil depth ($P < 0.05$), the decrease differed during the growing season (Table 2). In July, ^{15}N uptake was significantly higher at 0–5 cm depth compared with deeper soil layers ($P < 0.001$), and was significantly higher for $^{15}NH_4^+$ versus $^{15}NO_3^-$ (Fig. 2; $P < 0.05$). In August these values decreased with increasing soil depth (Fig. 2; $P < 0.005$). Throughout the growing season, uptake from $^{15}NO_3^-$ was higher than from $^{15}NH_4^+$ (Figs 2 and S2, Table 2). ^{15}N recovery in plants increased significantly with time after tracer injection, from 4 to 48 h after labelling (Table 2).

SPATIO-TEMPORAL CHANGES IN RATIOS OF ^{15}N RECOVERY BY MICROBIAL BIOMASS TO ^{15}N RECOVERY BY PLANTS

Multifactorial ANOVA indicated significant effects of soil depth, ^{15}N form added, season and sampling time on the ^{15}N recovery ratio between microbial biomass and plants (Figs 3 and S3,

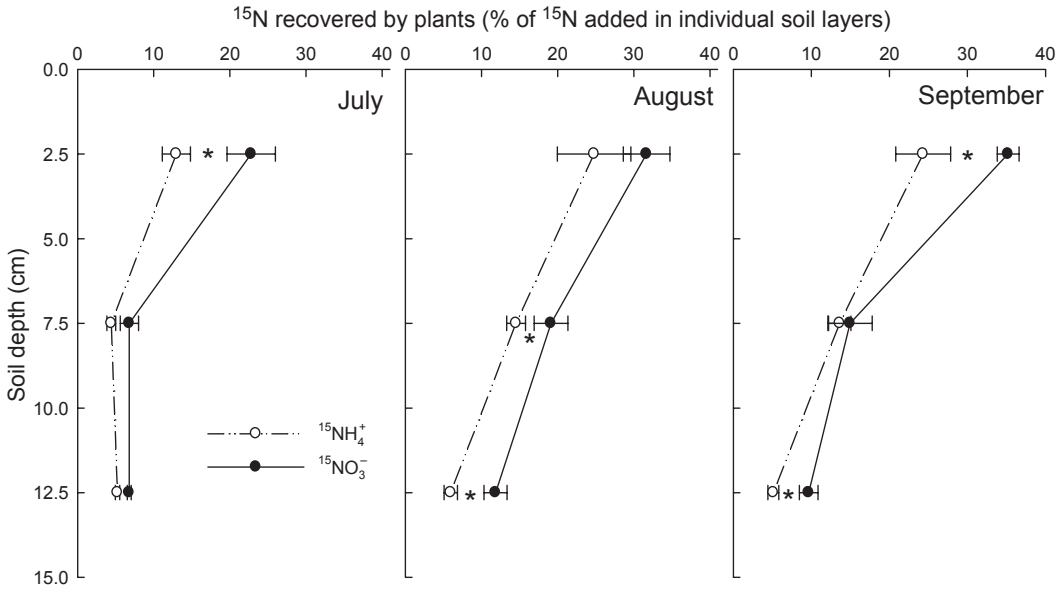

Fig. 2. ^{15}N recovery by plants (% of added ^{15}N) from $^{15}NH_4^+$ and $^{15}NO_3^-$ 4 h after ^{15}N injection at different soil depths during the growing season (July, August and September). Values are means (± 1 SE) of five replicates. Asterisks indicate significant differences between the ^{15}N recovery by plants from $^{15}NH_4^+$ and $^{15}NO_3^-$ injections.

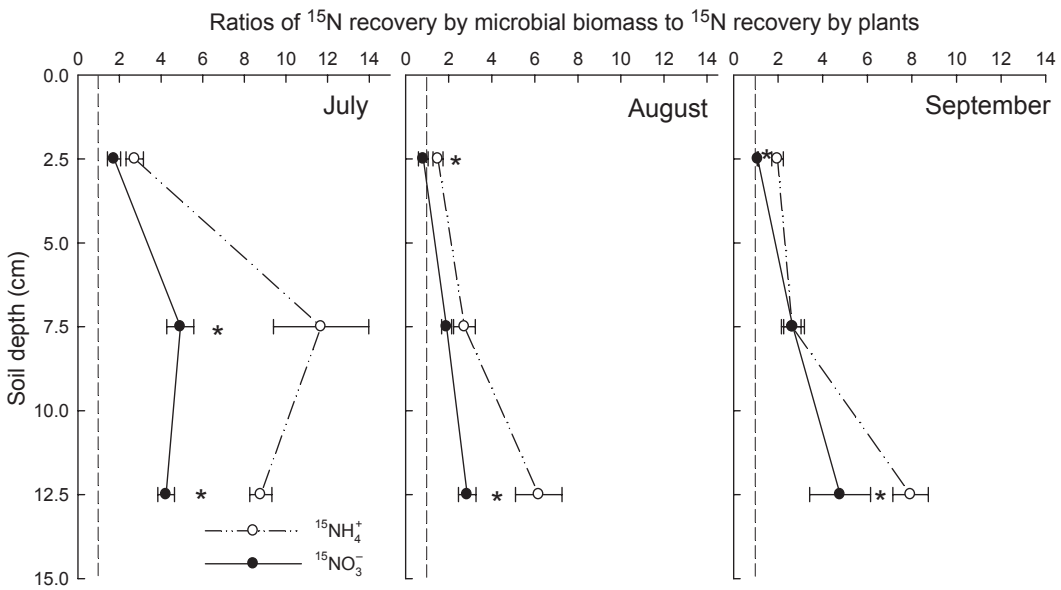

Fig. 3. Ratios of ^{15}N recovery by microbial biomass to ^{15}N recovery by plants from $^{15}NH_4^+$ and $^{15}NO_3^-$ 4 h after ^{15}N injection at different soil depths during the growing season (July, August and September). Values are means (± 1 SE) of five replicates. Asterisks indicate significant differences between the ratios of ^{15}N recovery by microbial biomass to ^{15}N recovery by plants from $^{15}NH_4^+$ and $^{15}NO_3^-$. The vertical dashed line (ratio = 1) corresponds to identical ^{15}N recovery by roots and soil microorganisms. Values above 1 indicate more efficient uptake by microorganisms.

Table 2). Besides these direct effects, the interactions between the four factors also significantly affected these ratios (Table 2). In July, recovery ratios were lower in the topsoil but higher at both 5–10 cm to 10–15 cm depth (Fig. 3; $P < 0.05$). The values at both subsoil layers were similar for $^{15}NH_4^+$ and $^{15}NO_3^-$, but significantly higher for the former than for the latter in the same soil layer (Fig. 3; $P < 0.05$). In August, the ratios increased with soil depth ($P < 0.005$) and were significantly higher for $^{15}NH_4^+$ than $^{15}NO_3^-$ at both 0–5 cm and 10–15 cm (Fig. 3; $P < 0.05$). In September, ratios were similar to those in August (Fig. 3; $P < 0.05$). At all stages of the growing season, recovery ratios were higher for $^{15}NH_4^+$ than for $^{15}NO_3^-$ (Fig. 3; $P < 0.05$). In both August and September, the values were around 1 in topsoils (Fig. 3). The recovery

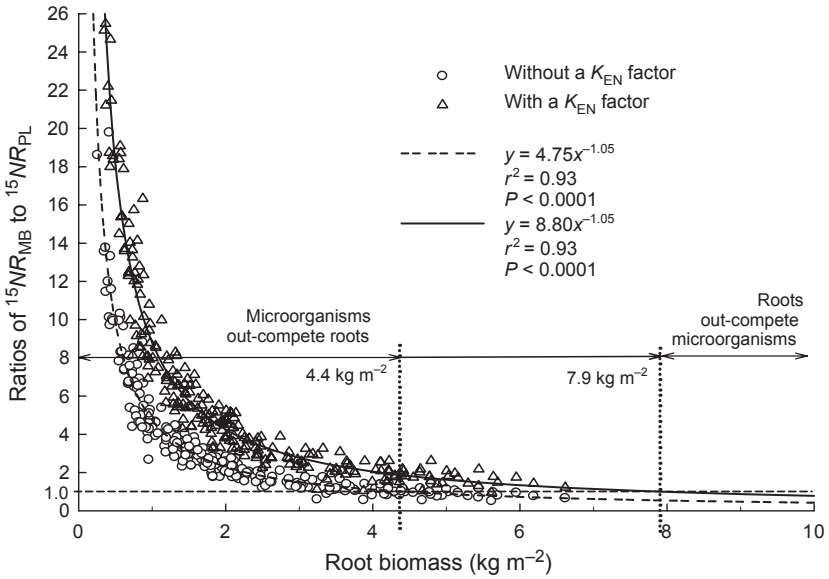

Fig. 4. Correlation between root biomass and the ratio of ^{15}N recovery by microbial biomass to ^{15}N recovery by plants in an alpine meadow. The dashed curve represents plant–microbe competition for inorganic N without using an extraction factor (K_{EN}) to correct microbial ^{15}N uptake for incomplete extraction, here referring to conservative estimate of ^{15}N uptake by microorganisms. Root biomass below the threshold of 4.4 kg m^{-2} indicates the condition where microorganisms out-competed roots, while at a root biomass above 4.4 kg m^{-2} plants out-competed microorganisms in this meadow. The solid curve represents plant–microbe competition for inorganic N applying a K_{EN} factor of 0.54 to correct microbial ^{15}N uptake. In this case at any root biomass of less than 7.9 kg m^{-2} microorganisms out-competed roots.

ratios decreased with time passed after ^{15}N injection (4, 24 and 48 h), due to increasing ^{15}N recoveries in plants, while time did not affect microbial ^{15}N recovery.

Discussion

Temporal frameworks are important to better understand relationships between above- and below-ground communities (Paterson 2003; Bardgett *et al.* 2005). Plant–microbe competition for inorganic N in an N-limited alpine meadow on the Tibetan Plateau showed that spatio-temporal variations are important for a better understanding of plant–soil interactions in alpine meadows.

$^{15}NO_3^-$ and $^{15}NH_4^+$ uptake by alpine plants significantly declined with increasing soil depth (Figs 2 and S2). In contrast, there was no clear trend for microbial uptake within the soil profile, although we found a clear seasonal pattern (Figs 1 and S1).

The first hypothesis that alpine plants compete more effectively with soil microorganisms for inorganic N in the topsoil but not in the subsoil was not fully supported by our results. Our conservative estimate showed that alpine plants took up a similar amount of ^{15}N as the microbial biomass, (e.g. an equal amount of NO_3^- 4 h and 24 h after ^{15}N injection in August and 48 h after ^{15}N injection from July to September). Even more ^{15}N was immobilized by microbial biomass especially from NH_4^+ (Figs 3 and S3). Nonetheless, alpine plants acquired more inorganic N from the topsoil than from the subsoil (Figs 2 and S2). This was related to higher root biomass in the topsoil, providing a spatial advantage for uptake of available soil N by roots over microorganisms. The distribution of roots and soil microorganisms as well as the mobility of the different N forms are important factors controlling competition for inorganic N between plants and microorganisms (Jackson, Schimel & Firestone 1989). In alpine *Kobresia* meadows, more roots were found in the topsoil compared with the subsoil (Zhou 2001; Tao *et al.* 2006). The ratio of root-to-soil volume (root volume did not include rhizosphere volume) was estimated to be around 0.62 in the top 0–10 cm soil layer, declining to about 0.26 in the 10–20 cm soil layer in the same meadow type close to our research site (G. Cao, unpublished data). We further found strong evidence that plant–microbe competition for available N strongly shifted in favour of plants as root biomass increased (Fig. 4), i.e. alpine plants out-competed soil microorganisms when root biomass exceeded 4.4 kg m^{-2}. When a correction factor (K_{EN}) of 0.54 (Brookes *et al.* 1985) was used to correct for incomplete extraction, alpine plants acquired more inorganic N than soil microorganisms with root biomass greater than 7.9 kg m^{-2}. In contrast, microbial biomass showed a weak correlation with ratios of ^{15}N recovery by microbial biomass to ^{15}N recovery by plants ($y = 0.18x^{1.03}$, $R^2 = 0.11$, $P < 0.001$), but no correlation with root biomass (data not shown). This indicates that roots modify microbial uptake of inorganic N and their competition for inorganic N with plants. Soil depth as a proxy for root density therefore has been identified as a main factor defining plant–microbe competition for N uptake (Fig. 5).

Several studies suggested that plants acquire more of the N required for growth during the early growing season, while soil microorganisms immobilize more N late in the growing season after plant senescence (Jaeger *et al.* 1999; Bardgett *et al.* 2002). In this study, we were unable to observe such a pattern, because roots exploited more soil volume in both August and September. Our second hypothesis, i.e. that alpine

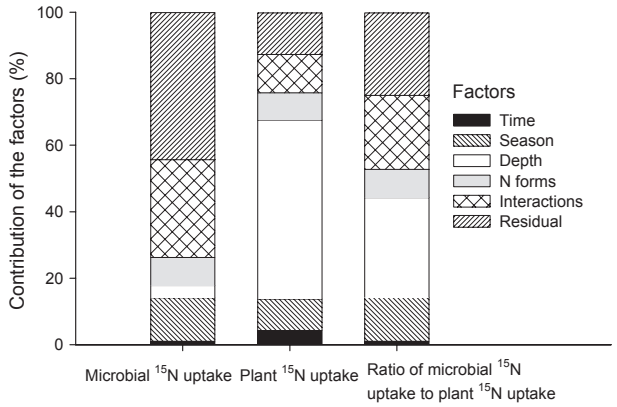

Fig. 5. Contributions of the factors: time after ^{15}N injection, season, soil depth and N form to the variance in microbial ^{15}N uptake, plant ^{15}N uptake and their ratios. Interactions show the sum of interactions among all the factors. Residual depicts the variance unexplained by either factor or factor interactions.

plants compete more effectively with soil microorganisms for inorganic N in the middle versus the late growing season, was therefore not supported. Above-ground biomass in alpine meadows is known to increase fast in July because of higher temperature and precipitation (Zhou 2001), but our study showed that at this time of the year they were poorer competitors for inorganic N compared with microorganisms. Leaf senescence already starts in September in alpine meadows on the Tibetan Plateau, but the competitive strength of plants for inorganic N in September was similar to that in August (Fig. 3). This reflects differences in below-ground biomass during the growing season: Pu *et al*. (2005) showed that the below-ground biomass of alpine plants was low in July despite fast above-ground biomass accumulation, but that their below-ground biomass was high both in August and September. In this study, ratios of shoots to roots were higher in July (0.21) than in August (0.18) and September (0.19). This indicates more root accumulation during the late growing season, thereby effectively allowing roots to compete for available N with soil microorganisms. However, we did not apply a conversion factor (K_{EN}) commonly used in the chloroform-fumigation extraction technique to account for incomplete extraction (Jenkinson, Brooks & Powlson 2004) and thus to correct microbial ^{15}N uptake. The reason is that soluble ^{15}N and insoluble ^{15}N are in disequilibrium in short-term ^{15}N uptake experiments, which could have underestimated microbial ^{15}N uptake (Fig. 4).

A growing body of evidence shows that soil microorganisms are superior competitors for inorganic N in the short term, i.e. hours to days (Jackson, Schimel & Firestone 1989; Kaye & Hart 1997; Hodge, Robinson & Fitter 2000; Bardgett, Steeter & Bol 2003; Nordin, Schmidt & Shaver 2004; Grogan & Jonasson 2005; Buckeridge & Jefferies 2007; Harrison, Bol & Bardgett 2007; Sorensen *et al*. 2008; Månsson *et al*. 2009). This is because they exhibit rapid growth rates and high surface-to-volume ratios compared with plant roots (Rosswall 1982). Several studies, however, reported contrary results. In a mesocosm experiment, for example, graminoids out-competed microbes for $^{15}NH_4^+$ within 48 h after ^{15}N tracer injection (Barnard, Barthes & Leadley 2006). In another experiment, plant roots competed effectively with soil microorganisms for added N within 50 h after ^{15}N addition in temperate grasslands (Harrison, Bol & Bardgett 2008). These contrasting results of plants out-competing microorganisms or *vice versa* after *c*. 2 days may not only be due to quick microbial turnover, but may also be a function of root biomass (and soil depth) as demonstrated in this study (Table 2, Fig. 4). In the topsoil, alpine plants competed effectively with microorganisms and showed a preference for $^{15}NO_3^-$, while microorganisms out-competed plants in the subsoil, with a preference for $^{15}NH_4^+$. This indicates that the exploitation of soil volume by roots has a major impact on plant–microbe competition for available N. Greater $^{15}NO_3^-$ and $^{15}NH_4^+$ uptake by plants in the topsoil compared with subsoil could reflect the decreasing root abundance with soil depth.

In support of the third hypothesis, the ratios of ^{15}N recovery by microbial biomass to ^{15}N recovery by plants from NO_3^- were lower than from NH_4^+ (Figs 3 and S3). One explanation for this uptake pattern is that specific plant species preferentially take up NO_3^- while other species prefer NH_4^+. For example, shrubs preferentially acquired $^{15}NH_4^+$, while *Carex* species took up more $^{15}NO_3^-$ than $^{15}NH_4^+$ in subarctic tundra ecosystems (Sorensen *et al*. 2008), while several other studies showed that certain plant species preferentially took up NO_3^- in alpine meadows (Miller, Bowman & Suding 2007; Song *et al*. 2007). We therefore suggest that the high mobility of NO_3^- in soils (Nye & Tinker 1977; Owen & Jones 2001; Miller & Cramer 2004) and the importance of NO_3^- in balancing cation uptake can help explain the high uptake of $^{15}NO_3^-$ by plant roots.

Compared with previous studies, we investigated simultaneously spatio-temporal patterns of plant–microbe competition for NH_4^+ and NO_3^- in the relatively unexplored alpine meadows on the Tibetan Plateau using a short-term ^{15}N experiment. Our results demonstrate that spatio-temporal variations determine plant–microbe competition for inorganic N in alpine meadows and that root biomass is a critical factor modifying plant–microbe competition for inorganic N (Fig. 5). Root biomass below the threshold of 4.4 kg m^{-2} indicates that microorganisms compete more effectively than alpine plants without using the K_{EN} factor. Alpine plants showed a preference for NO_3^-, and the factor season influenced plant–microbe competition for inorganic N mainly through affecting the distribution of root biomass in alpine meadows. Overall, our findings have important implications for the understanding of above-ground–below-ground interactions and plant–microbial competition for available N.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Figure S1. ^{15}N recovery by microbial biomass (% of added ^{15}N) from $^{15}NH_4^+$ and $^{15}NO_3^-$ 24 and 48 h after ^{15}N injection at different soil depths during the growing season.

Figure S2. ^{15}N recovery by plants (% of added ^{15}N) from $^{15}NH_4^+$ and $^{15}NO_3^-$ 24 and 48 h after ^{15}N injection at different soil depths during the growing season.

Figure S3. Ratios of ^{15}N recovery by microbial biomass to ^{15}N recovery by plants from $^{15}NH_4^+$ and $^{15}NO_3^-$ 24 and 48 h after ^{15}N injection at different soil depths during the growing season.

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