Nitrogen competition between three dominant plant species and microbes in a temperate grassland

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Abstract

Background and aims To test the hypothesis that dominant plant species could acquire different nitrogen (N) forms over a spatial scale and they also have the ability to compete for available N with microbes.

Methods A short-term ^{15}N labeling experiment was conducted in the temperate grassland ecosystem of North China in July of 2013. Three N forms (NO_3^- , NH_4^+ and glycine) labeled with ^{15}N were injected into the two soil depths (0–5 and 5–15 cm) surrounding each plant to explore N acquisition by plants and microbes. Three dominant plant species (*Artemisia frigida*, *Cleistogenes squarrosa* and *Artemisia capillaris*) were investigated.

Results Two hours after ^{15}N labeling, all three dominant plant species absorbed both organic and inorganic N, but different patterns were observed at two soil depths. Uptake of NO_3^- was significantly higher at 0–5 cm than at 5–15 cm soil depth among all the dominant plant

species. ^{15}N recovery by microbes was significantly higher than plants. However, ^{15}N recovery by plants showed different patterns over soil depths.

Conclusions Dominant plant species in the temperate grassland have different patterns in acquisition of N added to soil in organic form and absorption of inorganic N, and microbes were more effectively than plants at competing for N in a short-term period.

Keywords Inorganic N · Microbes · ^{15}N · Root N uptake · Temperate grassland

Introduction

In terrestrial ecosystems, all living organisms rely on the same nutrients, which form the basis for interspecific competition between plants and microbes (Kuzyakov and Xu 2013). Nitrogen (N) is one of the most essential elements limiting plant net primary production (NPP) in most terrestrial ecosystems (Vitousek and Howarth 1991; Lebauer and Treseder 2008), and the strong competition for N often occurs between plants and microbes. Clarifying plant-microbial competition for N will improve our understanding how N controls NPP and plant species coexistence.

In soil, there are diverse chemical forms of N that can be used by plants and microbes (Harrison et al. 2008; Kaštovská and Šantrůčková 2011), such as NH_4^+ , NO_3^- and organic N, including free amino acids and small molecular peptides and proteins (Paungfoo-Lonhienne et al. 2008, 2012; Hill et al. 2011a, 2011b,

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2012; Wilkinson et al. 2014, 2015). Some of studies showed that in short-term experiments, microbes took up more $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ than plants in some of ecosystems such as in the arctic tundra (Marion et al. 1982; Schimel and Chapin 1996) and annual grasslands (Jackson et al. 1989), and some of studies proved that plants took up more inorganic N than soil microbes (Kaye and Hart 1997; Jaeger et al. 1999; Schimel and Bennett 2004; Bardgett et al. 2003; Harrison et al. 2007, 2008). In the past three decades, organic N in the form of amino acids were found that could be directly acquired by plants from the soil, which is a significant portion of the total soluble N pool and an important N source for plants of alpine and subalpine grasslands (Raab et al. 1996, 1999; Owen and Jones 2001; Xu et al. 2006, 2011a; Gao et al. 2014). A large number of studies conducted in the field or laboratory have emphasized on the utilization of available N by plants and microbes (Schimel and Chapin 1996; Bardgett et al. 2002, 2003; Kuzyakov and Xu 2013). Microbes utilize available N more effectively than plants over a short time, although in the long run, plants might be more competitive (Harrison et al. 2007; Kuzyakov and Xu 2013). A temporal niche for N acquisition often occurs between plants and microbes (Jaeger et al. 1999). Initially after N additions, microbes often acquire much more N than plants because soil microbes have a large surface-volume ratio (Kaye and Hart 1997; Wilkinson et al. 2014). With timing, added N gradually enters plants because they show long life (Kuzyakov and Xu 2013). The patterns of N uptake by plants and microbes may be the most important mechanism of avoiding competition for plant species coexistence and plant diversity maintenance (McKane et al. 2002; Miller and Bowman 2003; Wang et al. 2012). The differentiation of chemical niches for N acquisition among dominant plant species might be the result of long-term evolution and adaptation (Lambers et al. 2009), which varies over spatial and temporal scales in many ecosystems. It is well documented that soil N availability varies vertically with soil depth (Zhu and Carreiro 2004; Xu et al. 2011b).

In regards to the plant-microbial competition for N, a large number of studies have focused on N uptake strategies by plants and microbes in various ecosystems, such as alpine meadows and tundra, subtropical and forest agricultural ecosystems (McKane et al. 2002;

Buckeridge and Jefferies 2007), but fewer investigations have been conducted on the plant species level. The mechanism, utilization and preference of organic and inorganic N by plant species is still unclear, although more work related to the nutrient cycling has been conducted (Wu et al. 2011; Zhang et al. 2013; Kleinebecker et al. 2014). How these temperate grassland plant species acquire N from the soil and what the patterns of N uptake between plants and microbes remain unclear. We designed this experiment to explore these issues.

Artemisia frigida is a plant species involved in the degradation of grasslands. Due to long-term overgrazing and unplanned grassland utilization, *A. frigida* accompanied with *C. squarrosa* and *A. capillaris* can become the dominant species in the temperate steppe ecosystem of the farming pastoral ecotone of northern China. To investigate how these dominant plants and microbes absorb organic and inorganic N in the temperate grassland, we conducted a ^{15}N short-term labeling experiment to test the hypotheses: (1) plants and microbes have different N acquisition strategies to acquire different chemical N forms; and (2) microbes compete for N more efficiently than plants over a short time. From the nutrient cycle standpoint, this study provides useful scientific data and theories on how to reduce degradation and promote restoration of grasslands and further improves our management capacity of degraded grasslands in the farming pastoral ecotone of northern China.

Materials and methods

Study site and target species

The experiment was conducted in a temperate grassland ecosystem in Inner Mongolia of North China (41°35' 50" N, 114°51' 45" E, 1500 m above sea level). The area is located in the southern margin of the farming-pastoral ecotone of North China and is characterized by a semi-arid continental climate. During the past 35 years, the mean annual temperature and annual precipitation were 2.1 °C and 384.4 mm, respectively. The steppe areas are dominated by *Artemisia frigida*, *Cleistogenes squarrosa*, *Artemisia capillaris*, *Stipa krylovii* and *Leymus chinensis* (Xu et al. 2014). We chose the important values of three of the largest plant species, i.e., *A. frigida*, *C. squarrosa* and *A. capillaris*,

as our target plant species. The zonal soils were chestnut soil (Chinese Soil Taxonomy Research Group 2001) and corresponded to Calcic Orthic Aridisols according to the USDA Soil Taxonomy (Soil Survey Staff 1987), which are poor in organic matter (2.7 %) and total N (0.14 %) (Table 1).

Experiment design

A 100 m × 100 m area, uniform in cover and species condition (Xu et al. 2014), was selected in a fenced temperate steppe in July, 2013. A total of 84 circular microplots (10 cm in diameter) within the experimental site were randomly established including 72 labeled microplots (^{15}N -labeled) and 12 control microplots (^{15}N -unlabeled). They were equally divided into three groups: *A. frigida*, *C. squarrosa* and *A. capillaris*. Each group included two soil depth treatments (0–5 cm and 5–15 cm) was labeled with $^{15}\text{NH}_4^+$ ($(^{15}\text{NH}_4)_2\text{SO}_4$, 98.4 atom% ^{15}N), $^{15}\text{NO}_3^-$ (K^{15}NO_3 , 98.2 atom% ^{15}N) or glycine- ^{15}N ($\text{C}_2\text{H}_5^{15}\text{NO}_2$, 95 atom% ^{15}N). ^{15}N tracers were dissolved in distilled water and injected into the soil at 2.5 cm depth for 0–5 cm soil depth treatment and 10 cm for the 5–15 cm treatment with four replicates per treatment. A total of 24.3 mg N of solid N mixed material ($(\text{NH}_4)_2\text{SO}_4$, KNO_3 and glycine, only one N form labeled by ^{15}N) was dissolved in 200 ml of distilled water with equivalent N amount (8.1 mg) of NH_4^+ -N, NO_3^- -N and glycine-N. The total amount of N added to soils was equal to 0.060 g N m^{-2} . To ensure ^{15}N evenly distributed, total 4 ml of labeled solutions were injected in each soil depth of each microplot, around the plant in a triangle (i.e., the plant generally growing in the center of a triangle). When the plant was located at the center of the triangle, we injected the solution at a distance of about 0.5 cm far from the center to avoid injecting in roots. Each 1 ml of ^{15}N solution

was injected on all the three vertexes and in the center of the triangle. The experiment was conducted at 10:00 am on a sunny day in July 2013.

Sampling and analysis

Because of the rapid turnover of amino acids in the soil (Jones and Kielland 2002), plant and soil samples were immediately harvested 2 h after the ^{15}N labeling. Soil cores (10 cm in diameter) were dug up to 15 cm depth for all labeled or unlabeled microplots with a soil probe. All plant materials were carefully picked up from soil cores and classified into aboveground and belowground parts. Roots were rinsed with tap water and soaped in 0.5 mM CaCl_2 solution for 30 min and again rinsed with distilled water to remove ^{15}N absorbed on the root surface (Xu et al. 2011a). Both aboveground plants and roots were dried at 70 °C for 48 h. Dried samples were weighed and ground into fine powder using a ball mill (MM2, Fa Retsch). All soil from the core was put into a plastic bag and immediately taken into the laboratory within 1 hour.

The fresh soil was processed through 2 mm mesh sieve to remove coarse fragments for measuring pH, moisture content, microbial biomass N, inorganic N (NH_4^+ and NO_3^-), glycine, microbial ^{15}N , total N and soil organic carbon (SOC). The soil pH was measured using a glass electrode method with a 1:2.5 soil-to-water ratio. The soil moisture content dried at 105 °C was measured by oven drying method. Total N was measured by Kjeldahl digestion with a salicylic acidification (Pruden et al. 1985). SOC was measured with wet oxidation by acid dichromate solution described by Kalembasa and Jenkinson (1973). The microbial biomass N was analyzed by a chloroform fumigation-direct extraction technique (Brookes et al. 1985). Two set of subsamples of 40 g of fresh soil from cooled soil samples were placed in beakers. One set of subsample was placed in a desiccator and fumigated with alcohol-free CHCl_3 for 24 h. After fumigation, the subsamples were immediately extracted with $0.05 \text{ mol L}^{-1} \text{ K}_2\text{SO}_4$ (4:1 ratio of solution to dry soil weight) (Werth and Kuzyakov 2010). The extracts were shaken for 1 h with 150 r min^{-1} of shaker and then filtered through Whatman No. 1 paper. And meanwhile, another set of subsamples was also extracted with $0.05 \text{ mol L}^{-1} \text{ K}_2\text{SO}_4$ (Werth and Kuzyakov 2010). All of the filtered extracts were analyzed for NH_4^+ and NO_3^- using an auto-analyzer (AA3, Bran-Luebbe, Germany). The soil

Table 1 Characteristics of the upper 15 cm soil in the temperate grassland of North China (means ± SE; $n = 16$)

Soil organic matter (mg g^{-1})	27 ± 2
Total nitrogen (mg g^{-1})	1.4 ± 0.09
C/N	11.3 ± 0.05
pH	8.17 ± 0.03
NH_4^+ -N ($\mu\text{g N g}^{-1}\text{d.w.soil}$)	3.05 ± 0.09
NO_3^- -N ($\mu\text{g N g}^{-1}\text{d.w.soil}$)	7.12 ± 0.09
glycine-N ($\mu\text{g N g}^{-1}\text{d.w.soil}$)	1.39 ± 0.09

glycine concentration was measured in the extracts (Näsholm et al. 1987) by high performance liquid chromatography (Waters 515, Waters Inc., USA).

Aliquots (2 mg) of plant materials were weighed into tin capsules to analyze ^{15}N atom% by continuous-flow gas isotope ratio mass spectrometry (MAT253, Finnigan MAT, Bremen, Germany), coupled to a ConFlo III device (Finnigan MAT, Bremen, Germany) and to an elemental analyzer (EA 1112, CE Instruments, Milan, Italy). ^{15}N incorporation into the microbial biomass was determined by chloroform fumigation (Brookes et al. 1985).

Calculation and statistics

Atom% ^{15}N excess (APE) was calculated as the atom% ^{15}N difference between labeled and control plant by the following equation (Hertenberger and Wanek 2004):

$$\text{APE} = \text{atom}\%_{\text{plant-labeled}} - \text{atom}\%_{\text{plant-control}} \quad (1)$$

where $\text{atom}\%_{\text{plant-labeled}}$ refers to the atom% ^{15}N from the ^{15}N -labeled plant, $\text{atom}\%_{\text{plant-control}}$ refers to the atom% ^{15}N from the same control plant.

^{15}N Uptake by individual plant species was calculated by multiplying N content ($\mu\text{g N g}^{-1}\text{d.w. root}$), APE and biomass (g m^{-2}) (McKane et al. 2002) as following:

$$U_{\text{labeled}} = N_{\text{content}} \times \text{APE} \times \text{Biomass} \quad (2)$$

Uptake of available N form by individual plant species ($\mu\text{g N g}^{-1}\text{d.w. root}$) corresponding to the ^{15}N -labeled N form was calculated by the following equation (McKane et al. 2002):

$$U_{\text{unlabeled}} = U_{\text{labeled}} (m_{\text{unlabeled}} / m_{\text{labeled}}) \quad (3)$$

where m_{labeled} refers to the total mass (g m^{-2}) of ^{15}N -labeled N injected per quadrat, and $m_{\text{unlabeled}}$ refers to the mass of available N forms measured in soils. U_{labeled} (g m^{-2}) refers to uptake of ^{15}N from the source m_{labeled} , and $U_{\text{unlabeled}}$ refers to uptake of available N from the source $m_{\text{unlabeled}}$.

$U_{\text{unlabeled}}$ divided by root biomass (g m^{-2}) and ^{15}N labeling time (h) (Gioseffi et al. 2012), we would get N uptake rate by plants ($\mu\text{g N g}^{-1}\text{d.w. root h}^{-1}$) (Recovery is not necessarily a direct function of uptake rate in the following section).

^{15}N recovery in plants (R_{plant} , %) was calculated by following equation (Clemmensen et al. 2008):

$$R_{\text{plant}}(\%) = \frac{U_{\text{labeled}}}{^{15}\text{N}_{\text{added}}} \times 100 \quad (4)$$

Where U_{labeled} refers to the ^{15}N mass uptake by plants, $^{15}\text{N}_{\text{added}}$ refers to the total ^{15}N mass added to the soil per square meter.

^{15}N recovery in microbial biomass (R_{MB} , %) was calculated by the following equation (Zogg et al. 2000):

$$R_{\text{MB}}(\%) = (R_{\text{fum}} - R_{\text{non-fum}}) / ^{15}\text{N}_{\text{added}} \times 100 \quad (5)$$

Where R_{fum} refers to the ^{15}N mass recovered in fumigated soil extracts, $R_{\text{non-fum}}$ refers to the ^{15}N mass recovered in non-fumigated soil extracts, $^{15}\text{N}_{\text{added}}$ refers to the total ^{15}N mass added to the soil per square meter.

Here we do not use any Ken in the calculation of R_{MB} . The added labeling ^{15}N is in a state of disequilibrium in short-term ^{15}N labeling experiment which could have underestimated microbial ^{15}N uptake (Xu et al. 2011a).

The standard errors of the means are presented in the figures and tables as a variability parameter. A one-way ANOVA followed by Duncan tests was used to examine biomass and N uptake rate for the three dominant species. The significance of difference in ^{15}N recovery between the microbes and the plants and ^{15}N content among different plant species, different N forms and different labeling injection depths was examined using a multivariate ANOVA. The analysis was conducted with the SPSS 20.0 software package (SPSS Inc., Chicago, IL, USA). All the significant differences were tested at $P < 0.05$.

Results

Biomass and root to shoot ratio

The aboveground biomasses were similar among the three dominant plant species (Fig. 1). However, there were significant differences in belowground biomass among *C. squarrosa*, *A. frigida* and *A. capillaris* (Fig. 1). The root to shoot ratio of *A. frigida* (6.21 ± 0.96) was significantly higher than the other two species, while *C. squarrosa* and *A. capillaris* were approximately the same. The root to shoot ratio of the three dominant species were greater than one (Fig. 1).

Fig. 1 The biomass and root to shoot ratio (values) of the dominant plant species in the temperate grasslands of North China. The error bars show mean $\pm$ SE ($n = 16$). Different letters show significant differences among aboveground biomass and belowground biomass of the dominant plant species, and the values upon the bars were the root to shoot ratios

Diversity of the N uptake rate by plants

There were remarkable effects of species, chemical N forms and soil depth on the N uptake rate by plants (Fig. 2). The N uptake rates by *C. squarrosa* and *A. capillaris* were significantly higher than that of *A. frigida* ($P < 0.001$) in NO_3^- more than in NH_4^+ and glycine ($P < 0.001$) and at depth of 0–5 cm more than at 5–15 cm depth ($P < 0.001$) (Fig. 2). This indicated that different dominant plant species could absorb different N forms at various soil depths.

There was an interactive effect of species $\times$ soil depth on the N uptake rate ($P < 0.001$). Generally, glycine N and NO_3^- uptake rates by the three dominant species decreased as the soil depth increased. This was especially true in that the decline of *A. capillaris* was faster than *C. squarrosa* and *A. frigida*. This indicated that there was spatial heterogeneity of N uptake by the dominant plants. Additionally, species $\times$ N forms and N forms $\times$ soil depth have significantly interactive effects on the N uptake rate by plants ($P < 0.001$). Organic and inorganic

nitrogen uptake rates by *C. squarrosa* and *A. capillaris* were significantly higher than by *A. frigida*, but *A. frigida* absorbed more NO_3^- (Fig. 2). At 0–5 cm depth, plants absorbed NO_3^- faster than glycine and NH_4^+ , and the uptake rate of NO_3^- by plants decreased with increasing soil depth. There was no difference between *C. squarrosa* and *A. capillaris* uptake NO_3^- and NH_4^+ (Fig. 2). There was a significantly interactive effect of species $\times$ N forms $\times$ soil depth (Table 2), which implied that N forms and soil depth greatly affect the N uptake by different plants.

Variation of inorganic- ^{15}N recovered between the microbial biomass and the plants

There was a significant effect of species on N uptake by microbes in the steppe ecosystem. The patterns of ^{15}N recovery by microbial biomass differed in the soil that the plants grew (Table 2, Fig. 3). The N uptake by microbes was significantly higher in the soil where *A. frigida* grew compared to the soil where *C. squarrosa* and *A. capillaris* grew (Fig. 3). There were also significantly interactive effects of species $\times$ N forms and species $\times$ N forms $\times$ soil depth on the microbes' N acquisition (Table 2, Fig. 3). $^{15}\text{NO}_3^-$ uptake by microbes in the soil where *A. frigida* grew was higher than where *C. squarrosa* and *A. capillaris* were grown at both soil depths (Fig. 3a and b). $^{15}\text{NH}_4^+$ uptake by microbes in the soil at 0–5 cm depth was much greater where *A. frigida* and *A. capillaris* grew than where *C. squarrosa* grew (Fig. 3c). At 5–15 cm depth, microbes in the soil where *A. frigida* grew absorbed more $^{15}\text{NH}_4^+$ than where *C. squarrosa* and *A. capillaris* grew (Fig. 3d).

There were significant effects of species, N forms and soil depth on ^{15}N recovery by plants (Table 2, Fig. 3). Overall, ^{15}N recovery was significantly higher in *A. frigida* and *A. capillaris* than in *C. squarrosa*, in $^{15}\text{NO}_3^-$ than in $^{15}\text{NH}_4^+$ and at 0–5 cm than at 5–15 cm soil depth. There were also significantly interactive effects of species $\times$ soil depth, species $\times$ N forms, N forms $\times$ soil depth and species $\times$ N forms $\times$ soil depth on ^{15}N recovery by plants (Table 2, Fig. 3). ^{15}N recovery was higher with the addition of $^{15}\text{NO}_3^-$ than $^{15}\text{NH}_4^+$ at both 0–5 cm and 5–15 cm soil depths. ^{15}N recovery of $^{15}\text{NO}_3^-$ by *A. frigida* or *A. capillaris* was higher than *C. squarrosa*. However, *C. squarrosa* and *A. capillaris* took up more $^{15}\text{NH}_4^+$ than *A. frigida* at 0–5 cm soil

Fig. 2 The effects of species, N forms and soil depth on the N uptake rate by the dominant plant species in the temperate grasslands in northern China. The bars and error bars show means $\pm$ SE ($n = 4$). The uppercase and lowercase letters

represent the difference between plant species and N forms, respectively. The bars sharing the same letter are not significantly different at $P = 0.05$

depth. At 5–15 cm soil depth, *C. squarrosa* absorbed more $^{15}\text{NH}_4^+$ than *A. capillaris* and *A. frigida*.

Competition of N uptake between plants and microbes

The N competition between plants and microbes is obvious. ^{15}N recovery by microbial biomass is significantly higher than by plants (Table 2, Fig. 3). There were significant effects and strong interactions of species, N forms and soil depth on N uptake by plants and microbes.

Discussion

Numerous studies have demonstrated that a variety of plant species can directly take up organic N from soil solution (Näsholm et al. 2009; Hill et al. 2011a;

Wilkinson et al. 2015). In this study, we confirmed that plant species in temperate grasslands also have the capacity to take up the added N in organic form, but its contribution to plant N nutrition is very lower than alpine plant species (Xu et al. 2006, 2011a). Our results revealed that the dominant plant species in the temperate grassland absorbed a substantial amount of organic N (Fig. 1), agreed well with previous research in the temperate grasslands (Owen and Jones 2001; Weigelt et al. 2005) and alpine wetland (Gao et al. 2014). The percentage of glycine-N uptake by plants (6 to 25 %) in temperate grasslands of this study (Fig. 4) was in accordance with that on the Tibetan Plateau by plants in alpine meadow (2 to 24 %) (Xu et al. 2011a), less than in alpine wetland with 40 % (Wang et al. 2012). The results suggest that organic N may be an additional

Table 2 ANOVA results for the effects of plant species, N forms, soil depth and their interactions on ^{15}N recovery by microbes and plants

Sources of variation	^{15}N recovery by microbes		^{15}N recovery by plants	
	F value	P values	F values	P values
Species	62.717	<0.001	8.525	<0.001
N forms	5.279	0.028	92.972	<0.001
Soil depth	0.001	0.971	106.39	<0.001
Species $\times$ Soil depth	0.836	0.442	5.086	<0.011
Species $\times$ N forms	8.955	<0.001	17.038	<0.001
N forms $\times$ Soil depth	0.516	0.477	29.740	<0.001
Species $\times$ N forms $\times$ Soil depth	3.290	0.049	3.274	0.049

Fig. 3 ^{15}N recovered by microbes and plants (% of added ^{15}N) from $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ 2 h after ^{15}N injection at two soil depths in the temperate grasslands in northern China. The bars and error

bars show means $\pm$ SE ($n = 4$). The bars sharing the same letter are not significantly different at $P = 0.05$

important N source for the plant growth in temperate grasslands, and the plants would mainly utilize organic N to meet their basic nutrient demands in the soil of lower available inorganic N.

The three dominant plants acquired more inorganic N and glycine-N from 0 to 5 cm soil depth than from 5 to 15 cm depth (Fig. 2), which showed different spatial patterns of N uptake among dominant plants. This might be related to the fact that most roots are typically located close to the soil surface (Table 3, Fig. 5) in all the ecosystems (Jackson et al. 1989; Kuzyakov and Xu 2013). The total N uptake rates (the sum of N uptake rates of three N forms) by *A. capillaris* and *C. squarrosa* were significantly higher than by *A. frigida*, although the total biomass and root to shoot ratio of *A. frigida* were the highest of all the plant species (Figs. 1 and 2). This result agrees well with previous findings that *A. frigida* is usually a dominant plant species that survives in the degraded grasslands (Zhan et al. 1999). Our results from the short time ^{15}N -labeling experiment revealed that in the long-term overgrazing grassland

ecosystems of North China, the most dominant plant *A. frigida* has its own unique N acquisition strategy. Based on the highest biomass and shoot to root ratio (Fig. 1), absorbing relatively less N (Fig. 3) in a short time and much more ^{15}N recovered in soil microbes than in *A. frigida* (Fig. 4) for *A. frigida*, we inferred that N recovered in microbes would enter the soil and utilized by *A. frigida* as a most important N source and pool in the long run. A possible interpretation for this would be that a temporal niche for N acquisition occurs between plants and microbes. Initially, microbes often acquire more N than plants (Andresen et al. 2008) for soil microbes have larger surface to volume ratio. As time going, added N gradually released to soil and absorbed by plants because they show long life (Kuzyakov and Xu 2013). Mycorrhizal colonization status and host specificities of different plant species could probably regulate N acquisition by plants might be another reasonable explanation (Huang et al. 2009; Osés et al. 2008; Wu et al. 2013). In other words, this would further

Fig. 4 The contribution of NH_4^+ , NO_3^- and glycine to the total N uptake (as %) of the dominant plant species at two soil depths ($n = 4$). The bars and error bars show means $\pm$ SE ($n = 4$)

promote *A. frigida* to be more dominant in the N-limited grassland ecosystem.

Inorganic-N uptake by three dominant plants from NH_4^+ and NO_3^- also showed a very different spatial pattern (Fig. 4). At 0–5 cm soil depth, NO_3^- uptake by plants was more than NH_4^+ , perhaps because the diffusion rate of NO_3^- was faster than that of NH_4^+ in topsoil (0–5 cm) (Nye and Tinker 1977; Owen and Jones 2001; Miller and Cramer 2005). At 5–15 cm soil depth, *A. capillaris* absorbed more NO_3^- than *C. squarrosa* but less NH_4^+ than *C. squarrosa*. One possible explanation for this N uptake pattern is that certain plant species would be more like to absorb NH_4^+ , and certain plant species might be more like to acquire more NO_3^- in the same soil conditions. This reflects that different plants differs in the nutrient element absorbing process, and the nature of dominant plant species in utilizing different N forms is the result of their long-term evolution and adaptation. Much more results indicated that plants absorbed more NO_3^- than NH_4^+ (Song et al. 2007; Ashton et al. 2010), while still there were some researches proved plants tended to take up more NH_4^+ (Finzi and Berthrong 2005; Näsholm et al. 2009) with

short time labeling (<72 h). The N uptake patterns of plants varied with seasons and soil depths (McKane et al. 2002; Xu et al. 2011a). And which could be affected by plant species, habitat conditions, mycorrhizal status, different physi-chemical and biological characteristics of N (Kaye and Hart 1997; Sorensen et al. 2008; Clemmensen et al. 2008; Näsholm et al. 2009; Wu et al. 2013).

In the N-limited grasslands of North China, the spatial variations of plant-microbial competition for absorbing inorganic N are important to gain a better understanding of plant-soil interactions in temperate grasslands (Fig. 2). NO_3^- and NH_4^+ uptake by the three dominant plants declined with increasing soil depth (Fig. 2). In contrast, there was no clear trend for microbial uptake within the soil depths (Fig. 2). The result was in accordance with previous observations in alpine grasslands (Xu et al. 2011a) but was different than plant-microbial competition for inorganic N in topsoil (0–5 cm). Microbes are more competitive (Andresen et al. 2008) than plants at both soil depths even though the highest root density occurs in topsoil (Table 3, Fig. 5). A significantly greater amount of added inorganic-N was captured by the microbial biomass in the topsoil and subsoil. This result suggests that fast microbial turnover and the release of inorganic N into the plant-soil system is an important pathway for plant N capture in temperate grassland ecosystems. The microbial sequestration of added N might be an important mechanism of N retention in grasslands (Bardgett et al. 2003), and the result was similar with the research in the low-productivity systems in Lancashire, United Kingdom (Bardgett et al. 2003). Other research shows that soil microbes are more competitive for absorbing inorganic N in short-term labeling (Jackson et al. 1989;

Table 3 Root biomass of three dominant plant species at two rooting depths

Rooting depth (cm)	Root biomass (g m^{-2})		
	<i>Cleistogenes squarrosa</i>	<i>Artemisia frigida</i>	<i>Artemisia capillaris</i>
0–5	146.0	331.1	80.1
5–15	93.1	205.6	19.7
0–5/5–15 ratio	1.6 ± 0.35	1.6 ± 0.17	4.1 ± 0.27

Fig. 5 The dominant N form of uptake by three different plant species in specific depths in temperate grasslands of North China

Hodge et al. 2000). This might be because microbes have faster growth rates and higher surface-to-volume ratios compared with the roots of plants (Rosswall 1982). Thus, even in soils with a higher density of roots (Table 3, Fig. 5), for example in the upper 0–5 cm depth in the grassland, most N would be taken up by microbes shortly after N addition. This reduces N leaching losses as a result of the limited uptake capacity of roots. The various N acquisition strategies are a benefit for ecosystem development and species evolution. The rapid allocation of inorganic N in microbes (Jones and Kielland 2002; Bardgett et al. 2003; Fischer et al. 2010) is ascribed to their inherent uptake capacity. This is an important adaptation of the ecosystem against possible N loss by leaching (Bardgett et al. 2003; Kuzyakov and Xu 2013). Additionally, this fast preliminary microbial immobilization of mineral N and amino acids probably affected the plant species competition. In particular, enhanced rhizodeposition provides a strong selection pressure for those microbes retained close to the root surfaces and further promote the development of symbiotic and mutually beneficial effects. There were some results revealed soil microbes effectively out-competed plants for added N within 50 h after ^{15}N addition, however, in the longer term, plant out-competed microbes for added N after 33 days due to microbial turnover releasing N back to the plant-soil system and subsequent plant uptake in temperate grasslands

(Zogg et al. 2000; Hodge et al. 2000; Harrison et al. 2007). This might be the reason of the temporal dynamic of nutrient partitioning between microbes and plants (Xu et al. 2011b; Kuzyakov and Xu 2013; Gao et al. 2014).

Plant-microbial competition for N strongly depends on spatio-temporal variations and root density (Xu et al. 2011b). Most previous studies showed that microbes out-competed plants within minutes to hours due to a large surface-to-volume ratio of microbes and rapid growth rates compared with plant roots (Owen and Jones 2001; Wilkinson et al. 2014). With timing, plants gradually acquire more N due to their long life and are super competitor in a long run (Kuzyakov and Xu 2013). Over soil depth, plants showed strong competition ability in the surface soil while microbes perform better in subsoil (Xu et al. 2011b). Additionally, the distribution of roots and soil microbes and the mobility of the different N forms are important factors controlling competition for inorganic N between plants and microbes (Jackson et al. 1989). All three dominant plants absorbed more $^{15}\text{NO}_3^-$ than $^{15}\text{NH}_4^+$ (Fig. 3), which is consistent with previous studies in tundra and alpine meadows (Miller et al. 2007; Song et al. 2007; Xu et al. 2011a). Our results demonstrated that microbes had a higher recovery of NO_3^- , and one reasonable explanation was that the higher rate of microbial NO_3^- uptake might be the result of the occurrence of microsites where NH_4^+ is depleted (Jackson et al. 1989).

We conclude that the dominant plant species in the degraded grassland ecosystem of North China have different uptake patterns of organic and inorganic N at two soil depths, and N uptake by plants was more at 0–5 cm than at 5–15 cm. Competition for NH_4^+ and NO_3^- between plants and microbes demonstrates that microbes have advantages over plants for inorganic absorption in these short-term ^{15}N experiments. There are spatial patterns of inorganic and organic plant uptake and the competition between plants and microbes. Based on the results, we can partly explain why these dominant plant species can coexist in degraded grasslands due to overgrazing.

For this study, we did not determine the glycine- ^{15}N concentration and the competition for organic N (glycine) between plants and microbes. In light of this study, this is a most important issue for us to be concentrated on the organic N acquisition between plants and microbes in the future.

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