Nitrogen acquisition strategies used by *Leymus chinensis* and *Stipa grandis* in temperate steppes

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Abstract *Leymus chinensis* and *Stipa grandis* are two important plant species of temperate steppes in Inner Mongolia of North China. They differ in their life forms, e.g., *L. chinensis* is a type of rhizomatous clonal grass, whereas *S. grandis* is a type of tussock grass. Here we hypothesize that both plant species possess distinct nitrogen (N) acquisition strategies for their growth and survival. To test this hypothesis, we conducted a four-factor experimental field study using a short-term (three hours) ¹⁵N labeling technique in two plant communities mono-dominated by *L. chinensis* and *S. grandis* of the temperate steppes over two months (July and August) and at two soil depths. In both of communities, *L. chinensis* and *S. grandis* directly absorbed all three of the common forms of N, including substantial portions of N-derived from glycine (organic and inorganic forms) ranged from 2.7 to 17.8 %, although they absorbed more inorganic N. Nitrogen uptake rates showed significant effects of communities, months, soil depths, and N forms. The uptake rate was higher

in August than in July and at 0–5 cm than at 5–15 cm soil depths. *L. chinensis* and *S. grandis* showed different preference on N form across months. *L. chinensis* shifted its uptake pattern from more nitrate (NO₃[−]) in July to more ammonium (NH₄⁺) in August, whereas *S. grandis* took up comparable NH₄⁺ and NO₃[−] in both months. In general, *L. chinensis* showed a more flexible N acquisition strategy and *S. grandis* performed a more concentrated and relatively more stable N acquisition strategy. The distinct N acquisition strategies used by *L. chinensis* and *S. grandis* varied greatly across different months and soil depths. These findings are more helpful in further understanding the plasticity of nutrient utilization issues of different plant species in response to N-limited conditions of grassland ecosystems.

Keywords Organic nitrogen · Nitrogen acquisition strategies · Life form · Monodominant community · Temperate steppes

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Introduction

The nutrient absorption by plant is a function of plant demand and soil supply (Gillespie 1989). In general, the limitation of plant acquisition for all the nutrients is primarily controlled by the processes in the rhizosphere (Nye 1977). Barber (1962) developed a concept that nutrient availability in soil for plant was principally governed by the movement rate of nutrients through the soil to root surface. Two processes of mass-flow in the water absorbed by plant and diffusion of ions were involved, which could act differently across spatial and temporal scales. Factors, such as soil water content (Danielson and Russell 1957; Mederski and Wilson 1960), transpiration ratio (Brouwer 1954), relative extent of root system, concentration gradient at root interface (Barber 1962)

and soil texture (Schofield and Graham 1960), could strongly affect the uptake of nutrients by plants.

Nitrogen (N) is an essential element but often limits plant maintenance, growth, and reproduction in many terrestrial ecosystems (Vitousek and Howarth 1991; LeBauer and Treseder 2008). There is abundant diversity of chemical N forms in the soil, ranging from simple inorganic N such as ammonium (NH_4^+) and nitrate (NO_3^-) to organic N (mainly as free amino acids and short peptides) (Johnsson et al. 1999; Paungfoo-Lonhienne et al. 2008; Jämtgård et al. 2010; Farrell et al. 2011). These N forms have different properties, e.g., electric property (Finzi and Berthrong 2005; Näsholm et al. 2009), assimilation costs (Engels and Marschner 1995), mobility and toxicity (Krom and Berner 1980; Engels and Marschner 1995; Lambers et al. 1998; Miller and Cramer 2004; Dijkstra et al. 2012), which would influence the N uptake by plants. Since the mid-late 1990s, the organic N uptake has been suggested as “short circuiting” for the N cycle (Abuzinadah and Read 1986, 1989; Finlay et al. 1992; Chapin 1995). More studies had confirmed that plants can access organic N in the form of free amino acids (Weigelt et al. 2005; Näsholm et al. 2009), small peptides (Soper et al. 2011; Anna et al. 2014) and even proteins (Paungfoo-Lonhienne et al. 2008; Hill et al. 2011) from soil solution. From then on, the N cycling has been considered as the depolymerization of N-containing compounds, which could be utilized by either plants or microbes, after cleaved by microbial extracellular enzymes (including mycorrhizal symbiosis) (Schimel and Bennett 2004). Evidence suggests that a variety of plant species have different N uptake patterns across space and time to avoid resource-based competition in alpine tundra (Ashton et al. 2010), alpine and subalpine meadow (Miller et al. 2009; Xu et al. 2011b; Schleuss et al. 2015), alpine wetland (Gao et al. 2014), arctic tundra (Mckane et al. 2002), and temperate grasslands (Bardgett et al. 2003; Stefanie et al. 2009; Kaštovská and Šantrůčková 2011).

Many plant species vary in their growth form and size, and exhibit inherently different resource-use patterns, which is defined as “generalist” or “specialist” (Duke and Caldwell 2001; Stefanie et al. 2012). Recently, however, whether the spatial-temporal variation on N acquisition are caused by interspecific resource partitioning of diverse plants or plasticity of specific plant species still remain unclear (Kahmen et al. 2006; Stefanie et al. 2012). Most previous studies mainly focused on plant species either coexisting (e.g., Gebauer and Ehleringer 2000; Pornon et al. 2007; Gao et al. 2014) or neighbor removal (e.g., Miller et al. 2007; Ashton et al. 2008), but fewer investigated plant species in monodominant communities where the effects of other species are excluded.

Grassland ecosystems cover 40.5 % of the land surface on the earth (Harper 1977). In the temperate semi-arid steppes of North China, the *Leymus chinensis* community and *Stipa grandis* community are two generally known steppes widely

distributed on the Mongolian plateau (Li 1988). *L. chinensis*, possessing long strong rhizomes and vigorous vegetative propagation, gives rise to extensively spreading clones and often forms monodominant stands (Bai et al. 2009). *S. grandis*, however, is a type of perennial tussock grass completely different from *L. chinensis* (Zhao et al. 2004). Different root architectures of plants, such as rhizomatous and tussock, would produce significant impacts on N uptake by different plants. In this study, we conducted a short-term ^{15}N labeling field experiment in two plant communities mono-dominated by *L. chinensis* and *S. grandis*, respectively, to test their differences in N uptake over months and soil depths. We hypothesized that plant species with different life forms would demonstrate distinct N acquisition strategies across temporal and spatial variations.

By using the in situ ^{15}N labeling technique, this study attempted to quantify the amounts of different N forms absorbed by plants from soils in the two monodominant plant communities. Glycine was used to represent the organic forms of soil N in this study, for it was one of the most abundant amino acid in grasslands soil (Bol et al. 2002; Stefanie et al. 2009). The equal lower doses of three N forms added to the soil in the field were used to determine the N uptake by plant species and ensured the applied N amount would not cause a fertilization effect (Miller et al. 2007).

Materials and methods

Study site

This study was carried out in the ecological protection region of the Maodeng Pasture, fenced since 2003, located in the Xilin River Basin of Inner Mongolia of North China (Wu 2013). Two monodominant plant communities, one dominated by *L. chinensis* (Trin.) Tzvelev (116° 20.72' E, 44° 16.39' N) and the other dominated by *S. grandis* P. Smirn (116° 22.62' E, 44° 11.17' N), were chosen as our target plant communities. The two study sites were approximately 10 km apart and characterized by a semi-arid continental temperate steppe climate. The mean annual air temperature is 2.6 °C, with the highest mean temperature of 21.4 °C in July and the lowest mean temperature of −19.0 °C in January. The mean annual precipitation is 271.4 mm (Gu et al. 2015). Based on the data of 2014 obtained from the Dynamet meteorological station near the study site, in July, the mean temperature during the ^{15}N labeling period was 26.1 °C, the total month precipitation was 34.0 mm and there were no rainfall for 5 days around the experimental date. In August, the mean temperature during the ^{15}N labeling period was 20.6 °C, the total month precipitation was 29.5 mm and there were no rainfall for 8 days around the experimental date. The soils are classified as chestnut soil (Chinese Soil Taxonomy Research Group 2001; Wang et al. 2015) and

correspond to Calcic Orthic Aridisols according to the USDA soil taxonomy (Soil Survey Staff 1987), with low nutrient levels (Table 1) and a low water-holding capacity (Wang et al. 2015).

Experiment layout

Four plots of 5 m × 5 m, uniform in cover, were selected in each monodominant community (*L. chinensis* or *S. grandis* dominant) as four replicates in 2014. The distance between these plots was at least 30 m. We totally set up 32 subplots of 10 cm × 10 cm in these four plots in each month (July or August). We chose July and August to represent mid-growing season and late-growing season. Each plot included four ¹⁵N-labeling groups (none or the addition of NH₄⁺-¹⁵N, NO₃⁻-¹⁵N, or glycine-¹⁵N) and two injected soil depths (0–5 cm and 5–15 cm), respectively.

For the ¹⁵N labeling treatment, the total amount of N added to each subplot was 0.6 g N m⁻², with the same amount (0.2 g N m⁻², corresponding to 1.08 μg N g⁻¹ d.w. soil in a 10 cm × 10 cm × 15 cm soil block) of NH₄⁺-N, NO₃⁻-N, and glycine-N, but only one form of N was labeled with ¹⁵N (NH₄⁺-¹⁵N (99.14 %), NO₃⁻-¹⁵N (99.19 %) or glycine-¹⁵N (99.04 %)). A total of 60 mg N of solid N mixed material was dissolved in 450 ml of distilled water with equivalent N amount (20 mg) for each of NH₄⁺-N, NO₃⁻-N, and glycine-N. Forty five milliliter solutions were injected into each subplot, respectively. To maintain a more even distribution of the ¹⁵N solutions, each subplot (10 cm × 10 cm) was equally divided into nine quadrats and 5 ml of ¹⁵N solutions were injected in the center of each quadrat (As the plant grew near the center of the subplot, the ¹⁵N solutions were injected at a distance of about 0.5 cm far from the center to avoid injecting in the plant roots). We injected ¹⁵N tracers at 2.5 cm depth for the 0–5 cm soil depth treatment and at 10 cm depth for the 5–15 cm

treatment using a syringe needle. To avoid clogging of the needle, holes at 2.5 cm and 10 cm soil depths were drilled with 5.5-mm-thick drilling steel before ¹⁵N injection (Stefanie et al. 2009).

In total, we constructed a four-factor experimental design including two communities (*L. chinensis* or *S. grandis* dominant), three N forms (NH₄⁺-¹⁵N, NO₃⁻-¹⁵N, or glycine-¹⁵N), two months (July or August), and two injected soil depths (0–5 cm or 5–15 cm).

Sampling and analysis

Biomass plots

Four plots of 25 cm × 25 cm were randomly set up in each plant community for measuring aboveground biomass and belowground biomass. Aboveground biomass was collected with scissors. For root biomass, soil blocks of 25 cm × 25 cm × 5 cm were excavated to collect roots at 0–5 cm soil depth, and then excavated down to 15 cm to collect roots at the 5–15 cm soil depth. Aboveground biomass and belowground biomass were dried at 65 °C for 48 h, and weighed to measure dry mass. Based on this measured biomass to illustrate the distribution characteristics of root biomass in 0–15 cm soil depth, and also could figure out the root biomass ratio from 0–5 cm depth to 5–15 cm depth.

Treatment plots

Plant and soil samples were harvested 3 h after the injection of ¹⁵N solutions. Samples were carefully excavated as a block, 10 cm wide by 10 cm long by 15 cm deep, from each subplot. The aboveground plant parts with their roots were separated from the soils and rinsed with tap water to remove soil and then soaked in a 0.5 mM CaCl₂ solution for 30 min and rinsed again with distilled water (Gao et al. 2014). CaCl₂ solution

Table 1 Characteristics of upper 15 cm soils in two temperate steppes of North China (means ± SE; n = 4)

	<i>Leymus chinensis</i>		<i>Stipa grandis</i>	
	July	Aug	July	Aug
Mean soil water content (%)	17.39 ± 0.43	18.39 ± 0.32	17.50 ± 0.61	18.77 ± 0.42
Soil organic C (%)	2.09 ± 0.02	2.01 ± 0.03	1.59 ± 0.01	1.57 ± 0.04
Total N (%)	0.23 ± 0.01	0.21 ± 0.01	0.17 ± 0.01	0.17 ± 0.01
C/N	9.08 ± 0.23	9.45 ± 0.04	9.20 ± 0.16	9.21 ± 0.17
pH (H ₂ O)	8.04 ± 0.10	8.22 ± 0.05	8.02 ± 0.09	7.99 ± 0.05
NH ₄ ⁺ -N (μg N g ⁻¹ d.w. soil)	3.76 ± 0.04 ^A	6.76 ± 0.06 ^{Ba}	7.59 ± 0.11 ^a	7.14 ± 0.12 ^a
NO ₃ ⁻ -N (μg N g ⁻¹ d.w. soil)	3.88 ± 0.05 ^A	6.33 ± 0.08 ^{Ba}	5.94 ± 0.06 ^{Aa}	15.25 ± 0.18 ^{Bb}
Glycine-N (μg N g ⁻¹ d.w. soil)	2.34 ± 0.43	1.94 ± 0.55 ^b	1.16 ± 0.12 ^b	1.12 ± 0.11 ^c

Different capital letters indicate significant differences ($P < 0.05$) for each N form contrast between the two months while different lowercase letters show significant differences ($P < 0.05$) for three N forms contrasts in the same months

was used to clear the labeled ^{15}N forms absorbed on the surface of roots (Wei et al. 2014). After that, the aboveground parts and belowground parts were carefully separated using scissors and dried at 65°C for 48 h, weighed to measure dry mass and ground to a fine powder using a ball mill (MM2, Fa. Retsch, Haan, Germany). A subsample of 2 mg powder was weighed into tin capsules to measure N content, atom% ^{15}N of shoots and roots by PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

Soil samples were transferred quickly to the laboratory within 30 min and cooled at 4°C , and processed immediately (Gao et al. 2014; Jacob and Leuschner 2014). The concentrations of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and glycine-N in the native soil (control), extracting for 1 h with 2 M KCl solution (Dijkstra et al. 2012), were measured by SEAL Auto-Analyzer 3 HR and HPLC-MS/MS (Ultimate3000-API 3200 Q TRAP).

Calculation and statistics

^{15}N atom% excess (atom%ex) was calculated as excess compared with the atom% of the control treatment, according to the equation (Fry 2006):

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

where $\text{atom\%}_{\text{plant-labeled}}$ indicates atom percent in the treated plant, and $\text{atom\%}_{\text{plant-control}}$ indicates atom percent in the same control plant.

Total amount of ^{15}N uptake by plant ($\mu\text{g N m}^{-2}$) was calculated by following equation (Mckane et al. 2002):

$$U_{\text{labeled}} = \text{atom\%ex} \times N_{\text{content}} \times B \quad (2)$$

where U_{labeled} indicates total amount of ^{15}N uptake by plant ($\mu\text{g N m}^{-2}$), atom%ex indicates atom percent excess, N_{content} indicates plant N content (%), and B indicates plant biomass (g m^{-2}).

We corrected total amount of ^{15}N uptake by plant (U_{labeled}) with soil-native concentrations of specific N forms by following equation (Mckane et al. 2002; Jacob and Leuschner 2014):

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

Where $U_{\text{unlabeled}}$ indicates total amount of N uptake by plant ($\mu\text{g N m}^{-2}$), m_{labeled} indicates total amount of ^{15}N added to soil in each subplot ($\mu\text{g N g}^{-1}$ d.w. soil), and $m_{\text{unlabeled}}$ indicates soil-native concentration of specific N form in controlled subplots ($\mu\text{g N g}^{-1}$ d.w. soil).

N uptake rate by plant ($\mu\text{g N g}^{-1}$ d.w. root h^{-1}) was calculated as $U_{\text{unlabeled}}$ divided by root biomass (g m^{-2}) and labeling time (h) (Giuseffi et al. 2011; Jacob and Leuschner 2014).

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

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

Where R_{plant} indicates ^{15}N recovery in plants (%), U_{labeled} indicates total amount of ^{15}N uptake by plant, and $^{15}\text{N}_{\text{added}}$ indicates total amount of ^{15}N added to the soil per square meter.

The standard errors of the means are shown in figures and tables as a variability parameter. We used one-way ANOVA followed by Duncan tests to examine biomass and root-to-shoot ratios between the two communities, native soil N content in each N form by two communities between two months, ^{15}N recovery in each N form by two communities between two months and soil depths. The interaction effects of plant N uptake rates by community (*L. chinensis* or *S. grandis* dominant), added N form (^{15}N in NH_4^+ , NO_3^- or glycine), month (July or August), and soil depth (0–5 cm or 5–15 cm) were tested by four-way ANOVA. Analyses were conducted with the SPSS 20.0 software package (SPSS Inc., Chicago, IL, USA). All differences were tested at $P < 0.05$.

Results

Biomass and root-to-shoot ratio

The aboveground biomass was significantly higher in *S. grandis* than that in *L. chinensis* across both months ($P < 0.001$). From July to August, The aboveground biomass in each community was increased, but neither of them showed significant difference. The belowground biomass in *L. chinensis* was higher than that in *S. grandis* in July, while the belowground biomass in *L. chinensis* in August was lower than that in *S. grandis*. From July to August, the belowground biomass in the two communities, especially in *S. grandis*, significantly increased ($P < 0.001$) (Fig. 1). Comparing the two communities, the root to shoot ratio in *L. chinensis* community was significantly higher than that in *S. grandis* community across two months ($P < 0.001$). In each community, the root to shoot ratios both increased from July to August, and showed significant differences ($P < 0.01$) (Fig. 2).

Variations in nitrogen uptake rate

Some of the ^{15}N -data before converted by a factor soil and a factor root were shown in Table 3 as ^{15}N recovery by plants. From July to August, ^{15}N in each N form recovered by plants and total recovery were increased in relevant soil depths and communities (Table 3), and some of them showed significant differences ($P < 0.05$). As for contrasts of recovery between

Fig. 1 Aboveground and belowground biomass of the two communities in temperate steppes of North China. Bars and error bars show means $\pm$ SE ($n = 16$). Different capital letters indicate significant differences ($P < 0.05$) for month contrasts (the same community between two months), different lowercase letters show significant differences ($P < 0.05$) for community contrasts (two communities in the same month)

soil depths, there was only significant difference in glycine- ^{15}N in July by *S. grandis* ($P = 0.013$).

Four-way ANOVA showed significant effects of community, month, N form added, and soil depth on N uptake rate (Table 2). Overall, N uptake rate was higher in *S. grandis* than *L. chinensis*, in August than July, at 0–5 cm vs. 5–15 cm soil depth, in NH_4^+ and NO_3^- compared to glycine (Figs. 3 and 4). These results indicate that plant N uptake differed greatly across communities, months, soil depths, and N forms.

In addition to these direct effects, interactions between the four factors also highly affected N uptake rate. There were significant two-way interaction effects of community $\times$ depth on N uptake rate (Table 2). Higher N uptake rates were found at 0–5 cm than 5–15 cm soil depth in both *L. chinensis* and *S. grandis*. This trend was more apparent for *L. chinensis* in July

and *S. grandis* in August (Figs. 3 and 4). The significant effect of community $\times$ N form on N uptake rate was also tested (Table 2). *S. grandis* took up NH_4^+ -N and NO_3^- -N more efficiently than *L. chinensis* (Fig. 3). There were also significant effects of month $\times$ N form on N uptake rate (Table 2). Plants took up more NH_4^+ -N and NO_3^- -N in August than in July (Fig. 3).

There was a significant three-way interaction effect of community $\times$ month $\times$ N form on N uptake rate (Table 2). *L. chinensis* preferred to take up more NO_3^- -N in July, and then turned to absorb more NH_4^+ -N in August. The significant differences of NH_4^+ -N uptake rate was not only exist between months but also among N forms in August (Fig. 3a, b). *S. grandis* absorbed roughly equal amounts of NO_3^- -N and NH_4^+ -N in July and in August

Fig. 2 Root to shoot ratio in July and August of the two communities in temperate steppes of North China. Bars and error bars show means $\pm$ SE ($n = 16$). Different capital letters indicate significant differences ($P < 0.05$) for month contrasts (the same community between two months), different lowercase letters show significant differences ($P < 0.05$) for community contrasts (two communities in the same month)

Table 2 Four-way ANOVA analysis for the effects of community, month, N form, soil depth, and their interactions on N uptake rate by plants

Effect	df	F	P
Community	1	0.980	<0.001
Month	1	80.848	<0.001
Depth	1	7.182	0.001
N form	2	114.744	<0.001
Community $\times$ month	1	12.340	0.110
Community $\times$ depth	1	4.526	0.043
Community $\times$ N form	2	1.116	0.001
Month $\times$ depth	1	0.010	0.449
Month $\times$ N form	2	39.421	<0.001
Depth $\times$ N form	2	0.337	0.398
Community $\times$ month $\times$ depth	1	2.395	0.123
Community $\times$ month $\times$ N form	2	4.960	<0.001
Community $\times$ depth $\times$ N form	2	0.892	0.887
Month $\times$ depth $\times$ N form	2	1.270	0.247
Community $\times$ month $\times$ depth $\times$ N form	2	1.186	0.314

P values for significant effects and interactions are shown in italics

Fig. 3 The effects of community, N form, soil depth, and month on N uptake rate of the two dominant species in temperate steppes of North China. Different Latin capital letters indicate significant differences ($P < 0.05$) for month contrasts (same N form between two months), different Latin lower case letters show significant differences ($P < 0.05$) for N form contrasts (three N forms in the same month). Bars and error bars show means $\pm$ SE ($n = 4$)

(Fig. 3c, d). For the absorption of glycine-N, there were significant increases from July to August at 5–15 cm soil depth in both communities (Fig. 3b, d).

These results suggest that variations in the uptake of different N forms differ between communities and also depend upon months and soil depths.

Discussion

Using ^{15}N dilution approach, we investigated the organic and inorganic N acquisition strategies by both of *L. chinensis* and *S. grandis* in temperate steppes of Northern China.

Both *L. chinensis* and *S. grandis*, in two monodominant temperate grasslands, could directly absorb quite substantial amounts of N-derived from glycine (organic and inorganic forms), agreeing well with previous findings in subtropical

forest (Wei and Yu 2014; Xu et al. 2014), alpine (Gao et al. 2014), wet grassland (Kaštrová and Šantrůčková 2011) and temperate grassland (Weigelt et al. 2005). These results suggest that N-derived from glycine could be an important additional N source for plant growth and reproduction in the temperate steppes. Without dual labeling method, our results still provided judicious estimates of maximal uptake of N-derived from glycine by two temperate steppe sites, ranging from 2.70 to 17.75 % (Fig. 5). The proportion was the same as the findings in two temperate grassland species (10 to 32 %) (Weigelt et al. 2005) and in alpine meadow (2 to 24 %) (Xu et al. 2011a). However, this result was lower than that of the temperate grassland (32 to 52 %) (Bardgett et al. 2003) and alpine wetlands (20 to 40 %) (Gao et al. 2014). One possible explanation may be due to the lower soil native concentrations of glycine-N in our study site for the rapid mineralization rate. A relatively lower soil C/N ratio in our study site indicates that soil

Fig. 4 Total N uptake rate in July and August of two communities in temperate steppes of North China. Different Latin capital letters indicate significant differences ($P < 0.05$) for soil depth contrasts; different Latin lower case letters show significant differences ($P < 0.05$) for month contrasts. Bars and error bars show means $\pm$ SE ($n = 4$)

Fig. 5 Contribution of glycine, ammonium, and nitrate to total N uptake (as %) of two communities in temperate steppes of North China. The area enclosed by black solid line refers to July, whereas the shadow area refers to August. Values are means $\pm$ SE ($n = 4$)

microbes were C limited (Table 1). To meet their basic needs of C for the growth and maintenance respiration, the microbes might quickly immobilize the amino acids released by proteolysis in soil and lead to a much faster turnover. The activities of proteolysis in soils were positively correlated with soil organic N supply and could also be influenced by additional factors such as pH and soil temperature (Raab et al. 1999).

L. chinensis and *S. grandis* took up more N in the later than the middle growing season (Table 3, Figs. 3 and 4), in agreement with a previous study (Xu et al. 2011b). The roots of *L. chinensis* and *S. grandis* observed in this study exploited more soil volumes in August than July (Fig. 1), and the root-to-shoot ratios increased significantly in August compared to July (Fig. 2). The above results indicated that more root accumulation occurred in the later, rather than the earlier growing season, thereby allowing *L. chinensis* and *S. grandis* to more effectively absorb the available N in the later growing season. On the other hand, the soil water content increased in August

might lead to an increase of soil nutrient availability (Table 1), and the diffusion coefficient probably increased by reducing the tortuosity of the diffusion path and increasing cross-sectional area for diffusion (Barber 1962). The increase of nutrient uptake by plant at higher moisture levels might be possibly due to the thicker moisture films through which the ions diffused (Danielson and Russell 1957).

With respect to the spatial patterns of plant N uptake, plants acquired more N at 0–5 cm than at 5–15 cm soil depth (Figs. 3 and 4), which was in line with previous results of a comparative estimation of grassland (Stefanie et al. 2009). Based on our 25 cm $\times$ 25 cm plant biomass plots, we determined that the root biomass in topsoil (0–5 cm) were nearly 2.6 times of that in subsoil (5–15 cm) in *L. chinensis*, and approximately 1.9 times in *S. grandis*. The higher water and nutrient availability (Schenk and Jackson 2002) and the lower probability of oxygen deficiency (Schenk 2006) closer to the soil surface are part of the reason that plants favored root placement in

Table 3 ^{15}N recovery (%) by plants in two temperate steppes of North China (means $\pm$ SE; $n = 4$)

Recovery (%)	<i>Leymus chinensis</i>				<i>Stipa grandis</i>			
	July		August		July		August	
	0–5 cm	5–15 cm	0–5 cm	5–15 cm	0–5 cm	5–15 cm	0–5 cm	5–15 cm
Glycine – ^{15}N	0.26 $\pm$ 0.07 ^A	0.16 $\pm$ 0.06 ^A	0.54 $\pm$ 0.03 ^{Ba}	0.56 $\pm$ 0.09 ^{Ba}	0.34 $\pm$ 0.05 ^A	0.13 $\pm$ 0.02 ^a	0.69 $\pm$ 0.04 ^{Ba}	0.86 $\pm$ 0.11
NH_4^+ – ^{15}N	0.14 $\pm$ 0.05 ^{Aa}	0.12 $\pm$ 0.04 ^A	1.20 $\pm$ 0.06 ^{Bb}	1.25 $\pm$ 0.22 ^{Bb}	0.23 $\pm$ 0.11 ^A	0.44 $\pm$ 0.03 ^{Ab}	1.58 $\pm$ 0.14 ^{Bb}	1.06 $\pm$ 0.24 ^B
NO_3^- – ^{15}N	0.45 $\pm$ 0.09 ^b	0.35 $\pm$ 0.15	0.91 $\pm$ 0.27	0.82 $\pm$ 0.13	0.37 $\pm$ 0.18	0.22 $\pm$ 0.03 ^{Aa}	0.98 $\pm$ 0.22	0.70 $\pm$ 0.16 ^B
Total	0.85 $\pm$ 0.12	0.63 $\pm$ 0.17	2.65 $\pm$ 0.29	2.63 $\pm$ 0.27	0.94 $\pm$ 0.02	0.79 $\pm$ 0.07	3.25 $\pm$ 0.26	2.62 $\pm$ 0.37

Different capital letters indicate significant differences ($P < 0.05$) for each N form contrast in the same community and soil depth between two months, different lowercase letters show significant differences ($P < 0.05$) for three N forms contrasts in the same months and soil depth

the uppermost soil and that upper roots competed more effectively than deeper roots (Schenk 2006). Therefore, both *L. chinensis* and *S. grandis* could absorb much more N in topsoil than subsoil. On the other hand, although the thickness of subsoil (5–15 cm) was two times that of topsoil (0–5 cm), the N uptake rate by plant at topsoil was still higher than that of subsoil and varied significantly between two soil depths (Table 2, Fig. 4), which indicated an important pool serving as plant nutrients at topsoil would be found.

Comparing the two communities, plant N uptake by *L. chinensis* and *S. grandis* differed greatly (Table 2). Overall, total N uptake rates in *S. grandis* were higher than that in *L. chinensis* (Fig. 4). Plant uptake rates of inorganic N were higher than that of N-derived from glycine, and plant uptake rates of inorganic N between NH_4^+ -N and NO_3^- -N varied significantly across seasons and communities (Figs. 3 and 5). There are several possible reasons for this N absorption pattern:

Firstly, our results confirmed that the inorganic N forms were still the dominant N sources and were considered to be the preferred N forms taken up by higher plants compared with N-derived from glycine when supplied with equal concentrations (Harrison et al. 2007; Kahmen et al. 2009; Gioseffi et al. 2011).

Secondly, considering the differences in these life forms, *L. chinensis* and *S. grandis*, possess distinct architectural characteristics of their root systems. *L. chinensis* is a type of rhizomatous clonal grass with a guerrilla strategy, which enables *L. chinensis* to spread quickly in horizontal space and lets *L. chinensis* more readily escape from stressful microsites to more favorable ones (Doust 1981, 1987; Ye et al. 2006; Li et al. 2007). Therefore, the N acquisition strategy of *L. chinensis* was more flexible and more dependent upon the relative availability of different N forms in the soil. *S. grandis* is a type of tussock grass, which may absorb N more concentratedly, possessing a relatively stable N acquisition strategy (Fig. 5).

In addition to the root architectures, the plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi could also play important roles in facilitating plant nutrient acquisition by enhancing the nutrient availability in close proximity of the soil-root interface and regulating the iron uptake and translocation (Pii et al. 2015). Both *L. chinensis* and *S. grandis* can form arbuscular mycorrhizal symbiosis, having the potential to reduce the drought stress on the host plants, and improved plant growth by enhancing plant nutrient uptake and storage, such as promoting N-capture from complex organic compound (Hodge et al. 2001; Al-Karaki et al. 2004). The differences in N uptake by plants between two monodominant communities might be partly caused by activities of PGPR and rate of mycorrhizal colonization across various seasons and soil depths, though the biochemical mechanisms underlying N acquisition process in plant still remains unclear.

Additionally, from July to August, the inorganic soil N pool in two communities seemed to increase (Table 1), especially for NO_3^- . Both communities produced more biomass in August, this could result in more C released as rhizodeposition and provide more labile C to nitrifying bacteria and enhance nitrification. Young plants have a greater nutrient concentration and probably absorb more ions through mass-flow at the early growing stage of their life (Barber 1962). The early blooming species of alpine wetland often takes up N more efficiently from soil in early growing season (Gao et al. 2014). *L. chinensis* often blooms in early-July and matures in early-August (Zhong et al. 1998), while *S. grandis* often heads in mid-July (Sun et al. 2015), blooms in early-August and matures in mid-August. Soil concentration of inorganic N in *L. chinensis* community was lower in July (Table 1) than in August, plants have to acquire more organic N to meet their growing requirements (Fig. 5). By comparison, the phenological phase of *L. chinensis* occurs earlier than *S. grandis*, low investment to roots by *L. chinensis* could decrease uptake of NO_3^- -N in mid-August, since the transfer of NO_3^- -N is more energy-consuming than NH_4^+ -N (Engels and Marschner 1995) (Figs. 3 and 4).

Moreover, uptake of NH_4^+ and NO_3^- is mediated by several transporters which formed two uptake systems: high-affinity transport system (HATS) and low-affinity transport system (LATS) (Miller and Cramer 2004). Two distinct gene families, NRT1 and NRT2, have been identified for NO_3^- (Crawford and Glass 1998), and two distinct groups within AMT family have been denoted as AMT1 and AMT2 for NH_4^+ (Shelden et al. 2001). As for amino acids, the transporters were known to be active in root uptake including lysine histidine transporter (LHT1), amino acid permease 1 (AAP1), and amino acid permease 5 (AAP5) (Näsholm et al. 2009). Thus, the variations in uptake patterns of N forms by plant species could be partly due to the different presence of specific roots operating transporters under various concentrations of N available in the soil over seasons and soil depths.

Previous studies have shown that plants take up different N forms depending upon the effects of interactions among species over seasons and soil depths (Gao et al. 2014; Schleuss et al. 2015). In this study, by comparing two typical monodominant plant communities, we conclude that two plant species with different life forms vary in their N acquisition strategies and show different seasonal-temporal patterns. The physical and chemical properties of soil and biological factors such as rhizosphere microbes, enzymes, and transporters which determine that still needs further investigation. Overall, the observed N acquisition strategies have important implications for understanding how plants acquire N to meet their N requirement in N-limited environments and for the further assessment of the contributions of different N forms to plant N nutrition.

In addition, in this study we only explored the issues of N acquisition strategies by plants in monodominant communities (dominated by *L. chinensis* or *S. grandis*), and excluded the competition influences from other interspecific plant species on N acquisition strategies. In the future, a further series of gradient studies from monodominant (dominated by *L. chinensis* or dominated by *S. grandis*) to co-dominant (dominated by *L. chinensis* and *S. grandis*) communities should be conducted to illustrate how N uptake by different plant species and how the N acquisition strategies affected by interspecific competition.

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