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REGULAR ARTICLE

Contrasting grass nitrogen strategies reflect interspecific trade-offs between nitrogen acquisition and use in a semi-arid temperate grassland

Nianxun Xi · Bi-Ru Zhu · Da-Yong Zhang

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Abstract
Background and aims Nitrogen (N) niche differentiations play an important role in community structure and biogeochemical cycling in terrestrial ecosystems. However, very few studies have examined how plant N strategies specialize via trade-offs between N acquisition and use abilities under natural field conditions.
Methods A field experiment was conducted to investigate N strategies by coexisting grass species using the *in-situ* stable isotope labeling technique. We injected ^{15}N–labeled nitrate, ^{15}N–labeled ammonium, and ^{13}C-^{15}N-labeled glycine solutions in early and late vegetative growing seasons. Shoot δ^{15}N and N concentration were measured to determine the N uptake and use abilities of 4 common species.
Results All plant species preferred to take up nitrate (the dominant N form) over ammonium and glycine, and N-acquisition capacity varied with temporal variation of soil N. However, the dominant species was more N-conservative than less-dominant species and had lower overall N uptake rates (shoot ^{15}N excess 48 hours after injection) and higher N use efficiencies (aboveground biomass : N ratio).
Conclusions The different N strategies may reflect mixed effects of environmental filtering and interspecific competition and have significant implications for species coexistence as well as for ecosystem functions such as nutrient cycling.

Keywords Niche differentiation · Nitrogen form · Plant phenology · Species coexistence · Stable isotope

Introduction

Nitrogen (N) is the key limiting nutrient for plant growth and maintenance in temperate grasslands (Vitousek and Howarth 1991). However, an increasing number of studies suggest that plant species may develop diverse N acquisition and use strategies to meet N demand depending on environmental conditions such as N availability (Reich 2014; Wooliver et al. 2016). Plant N uptake, use, and loss are key controls over the cycling of N in grassland ecosystems (Schimel and Bennett 2004), and divergent plant N strategies may promote

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Statement of Data Availability
All data analysed during this study are included in this published article and its supplementary information files.

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N. Xi · B.-R. Zhu · D.-Y. Zhang (✉)
State Key Laboratory of Earth Surface Processes and Resource Ecology and MOE Key Laboratory for Biodiversity Science and Ecological Engineering, College of Life Sciences, Beijing Normal University, Beijing 100875, China
e-mail: zhangdy@bnu.edu.cn

N. Xi
Centre for Integrative Conservation, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Yunnan 666303, China

species coexistence within local communities by avoiding competitive exclusion (Chesson 2000). A better understanding of plant N strategies is critical for predicting mechanisms underlying species coexistence and managing ecosystem nutrient cycling.

At the global scale, plants can be spread along a continuum with exploitative and conservative strategies as end points based on trade-offs between morphological traits related to plant N acquisition and use (Díaz et al. 2004; Wright et al. 2004; Reich 2014). N-exploitative species are characterized by traits linked to fast growth ability, high N capture, and low N use efficiency (high leaf N content and specific leaf area but low leaf dry matter content), whereas N-conservative species have the opposite trait suites (Maire et al. 2009; Wooliver et al. 2016). The relative abundance of N-exploitative versus conservative plants may vary primarily with soil nutrient availability because of environmental filtering; N-exploitative species dominate N-rich environments, and N-conservative species dominate N-poor environments (Maire et al. 2012; Lhotsky et al. 2016). In recent years, an increasing number of studies have been conducted on morphological and physiological traits associated with N strategies of individual species under variable environments (Chen et al. 2005; Bermúdez and Retuerto 2014). However, to the best of our knowledge, research simultaneously investigating plant N acquisition and use abilities under natural field conditions is still lacking.

Within a given habitat, coexisting plant species may partition soil N on the basis of chemical N form because soil N exists in a wide variety of chemical forms (Jones et al. 2005) and plants can take up intact molecules of inorganic N and dissolved organic N (Näsholm et al. 2009). Pot experiments with temperate grassland species have revealed interspecific differences in preferential use of chemical N forms, with slow-growing species capturing more organic N than fast-growing species (Weigelt et al. 2005). Nonetheless, recent advances indicate that both fast- and slow-growing grassland species may take up the most N in the inorganic form (i.e., nitrate and ammonium) under natural field conditions, driven by high N mineralization and strong plant competition for inorganic N (Schimel and Bennett 2004). Indeed, most studies do not explicitly distinguish nitrate from ammonium (Bardgett et al. 2003; Harrison et al. 2007), and a limited number of experiments have indicated inconsistent patterns of plant preference for nitrate versus ammonium depending on grassland productivity or N availability (Kahmen et al. 2008; Wilkinson et al. 2015). As a result of this uncertainty, further work needs to test in situ how coexisting plant species differ in the preferential capture of nitrate, ammonium and organic N.

In addition to differing in the uptake of chemical N forms, plants may display temporal variability in their N-uptake capacity (Fargione and Tilman 2005; Gao et al. 2014). The capacity of plants to take up soil N differs depending on the phenological stages of plant growth (Bilbrough and Caldwell 1997). Greater plant N capture is expected to occur in early vegetative growth stage when plant growth rate and N demand are higher than in late stage (James and Richards 2005). This temporal pattern of plant N uptake may be reinforced by seasonal variations of soil N availability, given that large soil nutrient pulses commonly occur in early spring because of rising soil microbial activities (James and Richards 2005; Schimel et al. 2007). In theory, coexisting plant species with different N strategies may occupy different temporal N niches if they display asynchronous phenologies (McKane et al. 2002; Fargione and Tilman 2005; Kahmen et al. 2006). In practice, however, it is largely unknown whether coexisting native species have different temporal N patterns under natural field conditions.

In this study, we investigated temporal patterns of nitrogen acquisition and use by coexisting plant species in a temperate grassland using an in-situ stable isotope labelling technique. The temperate grassland was strongly N-limited with inorganic N dominating the soil N pool, and grass species accounted for more than 90% of the total aboveground biomass (Zhu 2011). Previous studies in this site have suggested that the dominant species *Stipa grandis* showed more resource-conservative strategies - such as lower growth rate, lower specific leaf area, higher leaf dry matter content and lower leaf N content - than less-dominant grass species (Zhu 2011). We injected ^{15}N–labeled nitrate and ammonium, and ^{13}C-^{15} N-labeled glycine solutions in early and late vegetative growing seasons. Glycine was used to measure amino acid N uptake because it is one of the most abundant amino acids in the soils of temperate grasslands (Streeter et al. 2000; Jones et al. 2005) and previous studies have also suggested that grassland species show a greater uptake capacity for glycine compared with the more complex amino acids (Weigelt et al. 2005; Harrison et al. 2007). We tested the following hypotheses: (1) the dominant species has lower total N uptake, but higher N use efficiency, than less-dominant species; (2) coexisting plant species are differentiated by preferentially taking up different forms of N; (3) temporal peaks of N uptake vary among coexisting plant species.

Materials and methods

Study site

The study was conducted in a low-productivity grassland located at the Inner Mongolia Grassland Ecosystem Research Station of the Chinese Academy of Sciences, Inner Mongolia, China (43°32′ N, 116°40′ E, 1220 m a.s.l.). The site is temperate with a mean annual precipitation of 346 mm and a mean annual temperature of 0.3 °C. The vegetation cover and soil conditions are spatially uniform (Xu et al. 2010). The plant community is dominated by the C_3 grass *Stipa grandis*, which typically makes up more than 65% of the total cover, with coexisting C_3 grasses such as *Leymus chinensis*, *Agropyron cristatum*, and *Achnatherum sibiricum* each making up 5% - 20% of the total cover. The four species have similar phenology, and the growing season begins in late April and persists until late September. The soil type is Calcic Chemozems (28.2% C, 2.6% N, a pH_{H2O} of 8.4) according to IUSS Working Group WRB (2015). The soil texture was analyzed by Schneider et al. (2008), with 48% - 50% sands, 32% - 36% silt and 16% - 17% clay. In early April 2009 we enclosed a 100 × 100 m land with fence in the natural grassland and mowed it once every year. The area was divided into five blocks (24 × 20 m), with each block consisting of 30 plots (4 × 4 m).

Experimental design

Three chemical N form treatments were established both at the end of May and of July (corresponding to the early and late vegetative growth stages at the site, respectively). The three chemical N form treatments were: (1) labeled potassium nitrate and unlabeled ammonium chloride and glycine ($^{15}KNO_3$, NH_4Cl and $C_2H_5NO_2$); (2) labeled ammonium chloride and unlabeled potassium nitrate and glycine ($^{15}NH_4Cl$, KNO_3 and $C_2H_5NO_2$); and (3) dual-labeled glycine, unlabeled potassium nitrate and ammonium chloride ($^{13}C_2H_5{}^{15}NO_2$, KNO_3 and NH_4Cl). Labeled potassium nitrate, ammonium chloride and glycine (from Cambridge Isotope Laboratories, Inc. Andover, USA) were ≥98% enriched in ^{15}N or ^{13}C. Additionally, an unlabeled control treatment (KNO_3, NH_4Cl and $C_2H_5NO_2$) was established to assess ^{15}N and ^{13}C natural abundances in plant tissue. Each of the eight treatment combinations was replicated five times, resulting in a total of 40 quadrats of 35 cm × 35 cm. In each block we randomly chose eight plots and assigned them to experimental treatments. In each plot one quadrat including the four species was selected as one experimental replicate. Each quadrat was at least 2 m apart from other quadrats.

N application was in liquid form. Each solution contained equal contents of individual N forms and the total N addition was equal for all quadrats (i.e., 0.22 g m^{-2}). N solutions (100 ml per quadrat) were uniformly injected into the top 15 cm of the soil of the quadrats at 25 injection points along a grid (Streeter et al. 2000). For each injection point, a syringe with a 15 cm-long and side-port needle was inserted into the soil and solutions were injected as the needles were slowly withdrawn. All quadrats for May or July injections were labelled at the same day. Plant communities were harvested after 48 h. The incubation of 48 h was used in accordance with previous findings of maximal label uptake at this time (Streeter et al. 2000; Weigelt et al. 2005).

Samplings and analyses

At each harvest, shoots of all quadrats were cut and sorted by species. Shoot biomass was dried (70 °C, 48 h) prior to weighing to determine dry mass. For the four common species (*Stipa grandis*, *Leymus chinensis*, *Agropyron cristatum*, and *Achnatherum sibiricum*), the dried shoot material of each species and each quadrat was ground and analyzed for $\delta^{15}N$ and N concentration (for all quadrats) and $\delta^{13}C$ and C concentration (for labeled-glycine and control quadrats) by a continuous flow isotope ratio mass spectrometry coupled to an elemental analyzer system (Flash EA1112HT, Thermo Finnigan, USA). We were aware that estimation of plant N uptake may be not accurate without root ^{15}N measurements. However, root systems cannot be clearly sorted out to individual species in field conditions, and there are high risks of losing a large amount of absorptive roots. Values of $\delta^{15}N$ ($\delta^{13}C$) were converted to ^{15}N (^{13}C) atom-percentages using the following equations:

$$R_{\mathrm{sample}} = \left(\frac{\delta^{15}N}{1000}+1\right)\times R_{std}$$

$$\frac{^{15}N}{N} = \frac{R_{\mathrm{sample}}}{1+R_{\mathrm{sample}}}$$

where R_{sample} is the ratio of ^{15}N to ^{14}N and R_{std} is the natural abundance standard for N. Standards for ^{15}N: ^{14}N and ^{13}C: ^{12}C ratios were atmosphere N_2 ($R_{std} = 0.003676$) and Pee Dee belemnite ($R_{std} = 0.011237$), respectively. Shoot ^{15}N concentrations (μmol g^{-1} dry mass) were calculated as follows:

$$^{15}N_{\mathrm{content}} = N_{\mathrm{content}} \times \frac{^{15}N}{N}$$

where $N_{content}$ refers to total N concentration in shoots (μmol g^{-1} dry mass). We did similar calculations for shoot ^{13}C concentrations (μmol g^{-1} dry mass). To assess plant uptake for labeled N forms, shoot ^{15}N and ^{13}C excesses (μmol g^{-1} dry mass) were calculated using mean values of ^{15}N and ^{13}C concentrations of the unlabeled control plants as references (Näsholm et al. 1998). Total N concentrations in shoots were used to assess the ratio of aboveground biomass to N content (g dry mass g^{-1} N). Biomass: N ratios can be used as an estimate of nitrogen use efficiency (Fargione and Tilman 2006), although the mean residence time of N in plants may also affect biomass production per unit N (Berendse and Aerts 1987).

Soil samplings for measurements of inorganic N and free amino acids were carried out at roughly one-month intervals from the end of April until the start of August. For each soil sampling, six soil cores (5 cm diameter, 15 cm deep) were randomly taken outside experimental quadrats and returned to the laboratory. All soil samples were sieved at 5 mm and maintained at 5 °C prior to analysis. Soil inorganic N (nitrate and ammonium) was extracted by shaking 5 g of freshly sieved soil with 25 ml of 2 M KCl on a reciprocating shaker for 1 h. The KCl extracts were filtered through Whatman No 42 papers and analyzed by colorimetric measurements and auto-analyzer procedures (Bran & Luebbe AutoAnalyser 3, Germany). Soil free amino acids (glycine and other 19 amino acids, see Table S1) were measured by shaking 5 g subsamples of the sieved soil in 37.5 ml double-deionized water on a reciprocating shaker for 10 mins, followed by centrifugation (8000 rpm) for 4 min. The water extracts were filtered through Whatman GF/A papers and analyzed using liquid chromatography-mass spectrometry (LC-MS/MS) procedures (UltiMate 3000, UltiMate, USA; 3200 Q TRAP, AB, USA). An additional 5 g of sieved soil samples were oven-dried (105 °C, 24 h) to determine the soil water content.

Statistical analysis

To test the temporal patterns of soil N, data (nitrate, ammonium, glycine and total free amino acid) were analyzed using one-way analysis of variance (ANOVA) with the sampling date as the factor. Plant species collected from the same quadrat were not truly independent. To avoid pseudoreplication, we analyzed species-level aboveground biomass and biomass: N ratios using a mixed model procedure for partly nested two-way ANOVA; season was the fixed whole-plot factor, species was the fixed sub-plot factor and quadrat was the random factor (Quinn and Keough 2002). Shoot ^{15}N excess data were analyzed using a mixed model procedure for partly nested three-way ANOVA with the chemical N form as an additional whole-plot factor. Differences between treatments were determined using Tukey's honest significant difference post hoc tests. Regression analyses for ^{15}N and ^{13}C excess in shoot biomass treated by dual-labeled glycine were conducted to assess the uptake of intact glycine for each season, and the relationships between ^{15}N and ^{13}C excess were used to calculate the fractions of intact amino acid uptake (Näsholm et al. 1998). All analyses were performed using R and mixed model procedures were conducted using the nlme package in R (R Development Core Team 2013). Data were log- or square-root-transformed to meet assumptions of variance homogeneity and residual normality if necessary.

Results

Temporal patterns of soil N

Soil nitrate concentration showed a pronounced temporal variation; the lowest value of 0.74 ± 0.30 (standard deviation, SD) μg N g^{-1} dry soil was recorded at the end of May (corresponding to the first isotope labeling) and the highest value of 10.72 ± 0.86 (SD) μg N g^{-1} dry soil was recorded at the end of April ($F_{3,20} = 104.90$, $P < 0.001$; Fig. 1). Soil ammonium concentration was

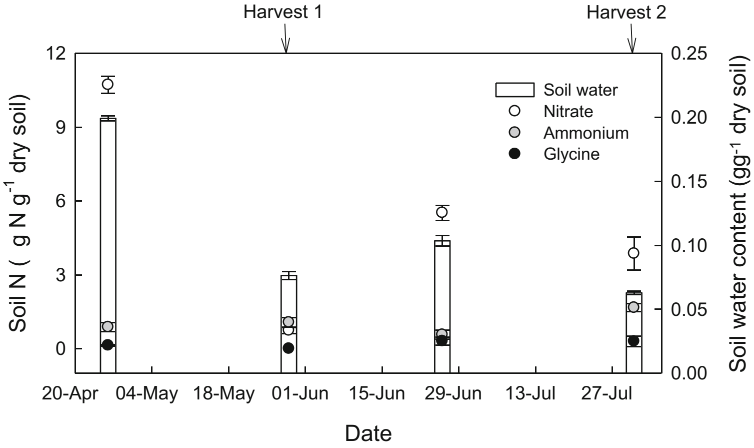

Fig. 1 Temporal patterns of soil nitrate, ammonium and glycine concentrations, and soil water content. Values are the means ± SE ($n = 6$)

lower compared to soil nitrate over the experimental period (Fig. 1). Unlike nitrate, we observed higher soil ammonium concentration at the end of July (corresponding to the date of second labeling) than at the previous three sampling dates ($F_{3,20} = 6.55$, $P = 0.003$; Fig. 1). Soil glycine concentration was very low during the experimental period (0.18 ± 0.34 (SD) μg N g^{-1} dry soil) and did not vary with time ($F_{3,20} = 1.15$, $P = 0.352$; Fig. 1). However, total free amino acids accounted for a large proportion of the soil N pool (3.03 ± 3.49 (SD) μg N g^{-1} dry soil; Table S1), and there was no temporal pattern during the experimental period ($F_{3,20} = 1.65$, $P = 0.211$).

Aboveground biomass and biomass: N ratio

Aboveground biomass had greater values in July than in May regardless of plant species (Table 1; Fig. 2a). Plant species varied significantly in their aboveground biomass (Table 1). In general, *Stipa grandis* dominated the plant communities across the seasons and contributed 53.2% of the total biomass, followed by *Leymus chinensis*, which accounted for 21.5% of the total biomass (Fig. 2a). The least dominant species were *Agropyron cristatum* and *Achnatherum sibiricum*, accounting for 8.8% and 6.5% of the total biomass, respectively (Fig. 2a). Plant biomass showed no significant season × species interaction (Table 1; Fig. 1a).

As with the aboveground biomass, the biomass: N ratio varied with both season and species (Table 1). In general, the biomass: N ratio was greater for the second harvest compared with the first harvest, and *Stipa grandis* had a greater biomass: N ratio than the less-dominant species (Fig. 2b). However, interspecific differences varied across season (significant season × species interaction; Table 1). Plant species showed similar values of biomass: N ratio in May, whereas in July the increase in biomass: N ratio was more pronounced in *Stipa grandis* than in the other species (Fig. 2b).

Uptake of chemical N forms by plants

All plant species tested in this study were capable of taking up nitrate, ammonium and glycine-derived N, as proved by the shoot ^{15}N excess of all plant species 48 h after ^{15}N label injection (Fig. 3). In general, plant species showed significant interspecific differences in N uptake (Table 2), with greater shoot ^{15}N excess in less

Table 1 Effects of season and species on aboveground biomass and biomass: N ratio

Effect	*df*	Aboveground biomass		Aboveground biomass: N ratio	
		F	*P*	*F*	*P*
Season	1, 38	35.80	<0.001	121.49	<0.001
Species	3, 114	62.67	<0.001	24.08	<0.001
Season × Species	3, 114	2.22	0.090	4.15	0.008

F- and *P*-values derived from ANOVA are shown with *df*

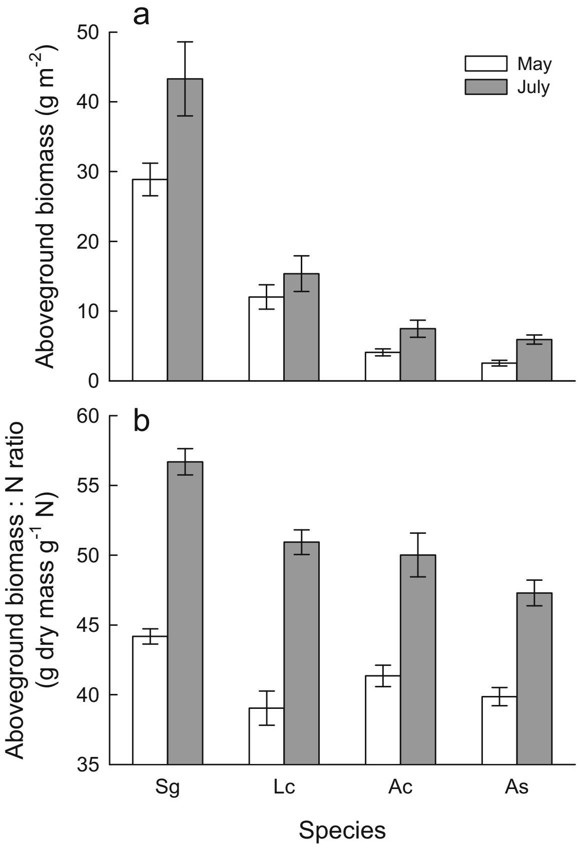

Fig. 2 Seasonal patterns of **a** species-level aboveground biomass and **b** biomass: N ratio. Values are the means ± SE ($n = 20$). *Sg: Stipa grandis*, *Lc: Leymus chinensis*, *Ac: Agropyron cristatum*, *As: Achnatherum sibiricum*

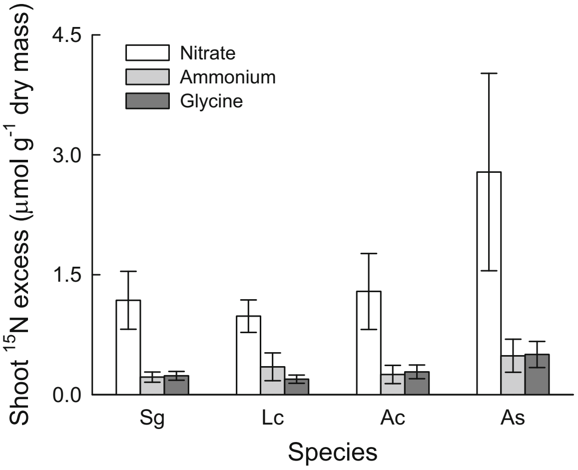

Fig. 3 Species-level shoot ^{15}N excess in response to chemical N form. Values are mean ± SE ($n = 10$). For abbreviations, see Fig. 2

Table 2 Effects of season, chemical N form and species on shoot ^{15}N excess

Effect	*Df*	*F*	*P*
Season	1, 24	209.78	<0.001
N form	2, 24	59.21	<0.001
Species	3, 71	11.80	<0.001
Season × N form	2, 24	3.50	0.046
Season × Species	3, 71	8.82	<0.001
N form × Species	6, 71	0.56	0.762
Season × N form x Species	6, 71	1.86	0.099

F- and *P*-values derived from ANOVA are shown with *df*

dominant species than in more dominant species (Figs. 3 and 4). However, the interspecific differences varied across seasons (significant season × species interaction; Table 2). *Achnatherum sibiricum* had a significantly greater shoot ^{15}N excess than other common species (*Stipa grandis*, *Leymus chinensis* and *Agropyron cristatum*) at the end of May, whereas no significant differences in shoot ^{15}N excess among plant species were found at the end of July (Fig. 4). Shoot ^{15}N excess responded significantly to chemical N form (Table 2). In general, all plant species preferred to capture nitrate N rather than ammonium and glycine N, and the ratio between the three N fractions was approximately 5: 1: 1 (Fig. 3). Shoot ^{15}N excess showed no response to a significant N form × species or season × N form × species interaction (Table 2). Moreover, significant relationships between shoot ^{15}N and ^{13}C excess in dual-labelled glycine quadrats were not detected for any species and season combination (Fig. S1).

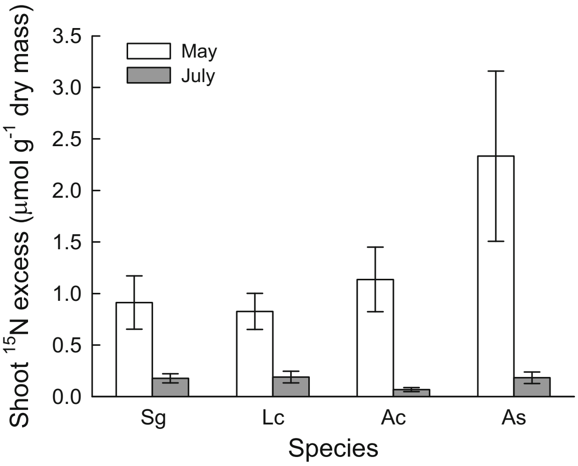

Fig. 4 Species-level shoot ^{15}N excess in response to season. Values are the means ± SE ($n = 15$). For abbreviations, see Fig. 2

Discussion

Despite the growing recognition that N strategy differentiations play an important role in plant species coexistence and nutrient cycling in terrestrial ecosystems (Schimel and Bennett 2004; Kahmen et al. 2006), very few studies have examined how plant N strategies specialize by trade-offs between N total acquisition and use abilities (Soussana et al. 2005; Maire et al. 2009), or between different methods of N acquisition (e.g., temporal or chemical N partitioning; Näsholm et al. 1998; McKane et al. 2002). The present study provides valuable information to fill the knowledge gap by examining trade-offs at these two dimensions in natural grassland ecosystems.

Our result provides some evidence for interspecific trade-offs between N acquisition and use under natural field conditions. Plant overall N uptake decreases with species relative abundance, whereas plant N use efficiency has the opposite tendency. This species N strategy-relative abundance pattern confirms previous findings that resource-poor habitats are dominated by conservative resource strategies that increase the ability of plants to tolerate and survive in such environments (Soussana et al. 2005; Maire et al. 2012; Reich 2014). The temperate grassland of the present study may be more strongly nutrient-limited than other temperate grasslands because we recorded low N availability and soil moisture over the plant growing season (Harrison et al. 2007; Maire et al. 2012).

Herbaceous plants are expected to change resource allocation between shoots and roots depending on the soil resource availability; this reflects a physiologically intrinsic trade-off between N acquisition and use (Tilman and Wedin 1991; Osone and Tateno 2005; Díaz et al. 2004). Plants with high N use efficiency are considered to be competitively superior under N-limited conditions that entail a high biomass production per unit of N captured by plants (Soussana et al. 2005; Fargione and Tilman 2006). However, the less-dominant species (e.g., *Achnatherum sibiricum*) may have a relative advantage in N acquisition over dominant species. The interspecific trade-offs between N acquisition and use likely promote species coexistence by avoiding the competitive exclusion of N-exploitative species in severe N-limited environments. Our results indicate that there may be more niche partitioning related to nitrogen use efficiency rather than N acquisition. Additional work is needed to determine whether a trade-off between two components of nitrogen use efficiency (nitrogen productivity and mean residual time of N, Berendse and Aerts 1987) can contribute to species coexisting in the field conditions. The dominance of N-conservative species could have strong implications for ecosystem nutrient cycling by concurrent changes in litter inputs and root exudates that modify carbon inputs to the soil and influence mineralization-immobilization dynamics (de Vries and Bardgett 2012).

Based on a growing awareness that differentiation in uptake of chemical N forms can explain plant species coexistence (Näsholm et al. 2009), we hypothesized that coexisting plant species are differentiated by preferentially taking up different forms of N. Our data did not support this prediction; soil nitrate was the most abundant N form over the plant growing season, and all plant species preferred to capture nitrate over ammonium and glycine. This contrasts with previous findings that N partitioning with respect to chemical N forms occurs among coexisting species in arctic tundra and alpine ecosystems where soil-dissolved organic N is the primary source of plant-available N (McKane et al. 2002; Miller and Bowman 2003). Nonetheless, our results are consistent with previous temperate grassland studies where plant species display a greater uptake of inorganic than organic N under natural conditions (Bardgett et al. 2003; Harrison et al. 2007).

Interestingly, most of the previous studies that had found N form complementarity among coexisting species (Miller and Bowman 2003; Weigelt et al. 2005; Kahmen et al. 2006) or ammonium preferences by plants (Bardgett et al. 2003; Wilkinson et al. 2015) were conducted in acidic soil. In alkaline soil such as that in our study, speedy transformations of amino acids to ammonium via mineralization and to nitrate subsequently via nitrification may increase the effluxes of the amino acids and ammonium pools, and the influx of the nitrate pool as a consequence, thereby resulting in the dominance of nitrate in soil N pool (Jones et al. 2005; Luo et al. 2013). Species that prefer to capture ammonium or organic N may not meet their N demands and thus are particularly prone to be outcompeted by plants with preference for soil nitrate, evidenced by the fact that this grassland is dominated by few common species and species richness is much lower compared with arctic tundra or alpine grasslands (McKane et al. 2002; Miller and Bowman 2003).

We observed an equivalent amount of ammonium and glycine-derived ^{15}N in the shoot biomass of each plant species, reflecting the same uptake rates of soil ammonium and glycine by plants. But we cannot rule out the possibility that some labelled glycine was mineralized to ammonium prior to plant uptake, because we failed to detect a significant relationships between shoot ^{15}N and ^{13}C excesses for all plant species. Lack of shoot ^{15}N and ^{13}C relationships may also have resulted from the mixed effects of variable plant background N concentrations and the small amount of dual-labelled glycine additions. We were fully aware of the necessity for root measurements if we want to accurately estimate plant capacities of N form uptake, despite the fact that even species-level root ^{15}N excess were not measured due to limitations of the stable isotope labelling technique (Näsholm et al. 2009). For now, we cannot determine how root-absorbed N is allocated between shoot and root components depending on chemical forms. Therefore, future work needs to develop a novel technique to accurately estimate ^{15}N in root biomass and background soil for a better understanding of plant N preferential use in field conditions. Our results highlight that species-specific preferential uptake of chemical N forms observed in pot experiments (Weigelt et al. 2005) may not be applicable under natural field conditions, especially for low species-richness communities. Coexisting plant species in natural grasslands are more likely to have evolved mechanisms of a better competition for the dominant soil N form (Harrison et al. 2007; Wilkinson et al. 2015).

Experimental and theoretical works have suggested that temporal niche differentiations are likely to occur in resource-limited ecosystems (Chesson 2000; Chesson et al. 2004), resulting in efficient use of limiting soil resources. Therefore, we tested whether coexisting plant species staggered their temporal peaks of N uptake. Our results did not support this hypothesis; all plant species showed greater total N uptake in the early growing season, although there was a tendency for the dominant species to have a larger proportion of N uptake in the late growing season. As with previous studies, the greater N uptake in the early growing season may reflect the mixed effects of a high plant growing rate, N demand and soil N availability at the end of the previous winter (Jaeger et al. 1999; James and Richards 2005). Interestingly, plant-available N decreased substantially in the early spring as compared to the end of winter, implying that plants may have strong effects on ecosystem nutrient cycling in soils via nutrient uptake and utilization (Boudsocq et al. 2012). Of course, however, the reduction in soil N can be a result of high activities of soil microbes in the early spring (Schimel et al. 2007).

All plant species were in tune with the temporal dynamics of soil N availability and had a greater capacity to exploit larger N pulses. The lack of species-specific temporal N patterns in this study contradicts the previous experimental results that coexisting species or functional groups (e.g. grasses, forbs and shrubs) can spatiotemporally partition the soil N pool in arctic tundra and alpine ecosystems (McKane et al. 2002; Miller and Bowman 2003; Gao et al. 2014). This finding is perhaps not surprising because all the grassland plants studied on this site are C_3 grass species with similar phenologies. Previous studies with grassland communities have suggested that plant species partition soil N pool by taking up N from different depths (von Felten et al. 2009, 2012). We cannot rule out spatial N differentiations as a possible mechanism underlying coexistence of the grass species under study. Partitioning soil organic N pool may contribute to species coexistence in the grassland ecosystem as well, given a large variety of amino acid forms were recorded in the soils. Future work is needed to test whether spatial N differentiation or partitioning organic N pool occurs in the Inner Mongolia grassland communities.

All in all, our results suggest that coexisting plant species follow different N strategies to meet their N demands. In general, the dominant species is more nutrient-conservative than less-dominant species in N-limited grasslands. All plant species prefer to take up the dominant N form, and N-acquisition capacity varies synchronously with temporal variation of soil N. The interspecific trade-offs between N acquisition and use could promote species coexistence by equalizing species' competitive ability (Chesson 2000). In contrast, the lack of significant chemical or temporal N differentiations implies that there is no stabilizing mechanism in this grassland community. It is the effects of environmental filtering that necessarily select functionally similar species within a local community (Grime 2006). We note that our results are consistent with the nearly neutral explanation of community organization (Zhou and Zhang 2008), in which rare species are slightly less competitive and are maintained in the community not by niche differences but by immigration from outside. In a similar vein, Zhang et al. (2016) also find, by analyzing a 30-year observed data set of a nearby plant community, that the commoner species tend to have a stronger competitive ability for soil nutrients than rarer species and the long-term dynamics of the rarer species is essentially driven by stochastic processes such as ecological drift. Of course, the claim for a nearly neutral explanation of species coexistence in the temperate grassland is still rather speculative, and further work is needed to rule out niche partitioning as a possible mechanism, such as spatial N niche differentiation or partitioning organic N pool. This study is focused on nitrogen strategies of plants because N is generally the most limiting resource in semi-arid grasslands. In the future, additional work should investigate how coexisting plant species specialize in other resources (e.g., water, light), given that temperate grassland species are also frequently limited by soil moisture or light.

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