

Nitrogen acquisition strategies during the winter-spring transitional period are divergent at the species level yet convergent at the ecosystem level in temperate grasslands

Linna Ma^a, Guofang Liu^a, Xiaofeng Xu^{c,**}, Xiaoping Xin^d, Wenming Bai^a, Lihua Zhang^a, Shiping Chen^a, Renzhong Wang^{a,b,*}

^a State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, The Chinese Academy of Sciences, Beijing, 100093, China

^b University of Chinese Academy of Sciences, Beijing, China

^c Biology Department, San Diego State University, San Diego, CA, 92182, USA

^d Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agriculture Sciences, Beijing, 100081, China

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ABSTRACT

Nitrogen (N) is a major limiting element for productivity in temperate grasslands, particularly during early spring when soil N availability is low and the vegetative demand for it is high. Therefore, knowing whether and how plant species adopt different N acquisition strategies during the winter-spring transitional period is essential for understanding ecosystem functioning in temperate grasslands. In this study, parallel experiments with ¹⁵N tracer were conducted to examine plant N acquisition strategies during winter-spring transition in a meadow and a typical steppe in northern China. We found that soil microbes immobilized ~20% of the ¹⁵N tracer during the spring thawing period at both sites, and then released half of it back to the soil before late spring, confirming that soil microbes competed effectively with the plant roots for mineral N in early spring. Perennial bunch grasses adopted an active N acquisition strategy at the beginning of the spring thawing period. In contrast, perennial forbs and rhizome grasses began to take up N in the middle of the spring thawing period, and they acquired more N than the bunch grasses. However, sagebrushes and legumes accounted for little ¹⁵N recovery, indicating their dependence on internal N accumulation or N fixation. At the ecosystem level, no significant difference in the magnitude of plant ¹⁵N uptake was observed between the meadow steppe and typical steppe, although the plant biomass N in the meadow steppe was twice that of the typical steppe during the thawing period. This was attributed to the higher soil inorganic N and faster net N mineralization rate in the meadow steppe than in the typical steppe. Our results suggest that temporal niche differentiation in N acquisition during early spring may facilitate species coexistence in temperate grasslands despite strong plant-microbe or plant-plant competition for N. The divergent N acquisition strategies at the species level and convergent N acquisition strategies at the ecosystem level should be considered for model development to better simulate vegetation growth particularly under spring N stress.

1. Introduction

It is well known that nitrogen (N) limitation is prevalent in temperate grasslands, which cover 25% of the global land area and approximately 40% of Chinese terrestrial ecosystems (Hooper and Johnson, 1999; Kang et al., 2007). During the growing season, soil N mineralization is insufficient for the N demand for plant growth in temperate grasslands (Harpole et al., 2007; Niu et al., 2008; Ma et al., 2014); therefore, the N uptake ability of plants during the non-growing season such as the winter-spring transitional period is essential for plant

growth. Many studies have found that soil microbial N immobilization during winter plays a critical role in early spring plant growth in diverse ecosystems, i.e., alpine meadow, subarctic heath, and arctic tundra ecosystems (Lipson et al., 1999; Bilbrough et al., 2000; Schmidt and Lipson, 2004; Tye et al., 2005; Edwards et al., 2006; Larsen et al., 2007; Kaiser et al., 2011). Compared with alpine and high-latitude ecosystems, temperate regions have a relatively shorter winter season and are more sensitive to temperature variation over late winter and early spring due to longer soil exposure to freeze-thaw cycling during transitional seasons (Wang et al., 2010). However, whether and how plant

* Corresponding author. State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, The Chinese Academy of Sciences, Beijing, 100093, China.

** Corresponding author. Biology Department, San Diego State University, San Diego, CA, 92182, USA.

E-mail addresses: xxu@mail.sdsu.edu (X. Xu), wangrz@ibcas.ac.cn (R. Wang).

species in temperate grasslands adopt different N acquisition strategies during early spring remains elusive.

Previous studies have demonstrated that soil microbes remain active in frozen or snow-covered soils (Panikov and Sizova, 2007; Wang et al., 2010; Zhang et al., 2011). Winter is a period for microbial biomass buildup, and immobilization of N into microbial cells (Schmidt and Lipson, 2004; Schmidt et al., 2007; Edwards and Jefferies, 2010; Kaiser et al., 2011). Microbial N immobilization during winter was found to account for almost one-third of the annual plant N demand in a beech temperate forest (Kaiser et al., 2011). During the winter-spring transition, the freeze-thaw cycle ruptures microbial cells (mostly cold-adapted psychrophiles), and the microbial N is released to the soil as plant-available N (Grogan et al., 2004). Some of the mineralized N is retained by the plant community and other microbial communities (e.g., endophytic fungi) or is lost via leaching (Schmidt et al., 2007).

Different plant species or functional types have various N uptake abilities as a result of their inherent differences in physiology and root morphologies. For example, N uptake during snowmelt constituted over 12% of season-long uptake for a graminoid species and averaged 7.4% for perennial forbs in the alpine tundra (Bilbrough et al., 2000). Tye et al. (2005) investigated the release of simulated snow N deposition with ^{15}N tracer following snowmelt and found that lichens and bryophytes rapidly immobilized a considerable proportion of the applied N, and approximately 40% of the tracer was apparently lost due to leaching in the high arctic tundra. Several studies have focused on divergences in timing of N uptake among species and/or plant functional groups during non-growing seasons, and substantial inconsistencies were found across arctic and alpine wet meadows (Jaeger et al., 1999; Edwards and Jefferies, 2010), high-arctic tundra (Tye et al., 2005), subarctic heath (Larsen et al., 2012), and a birch forest (Grogan and Jonasson, 2003). For instance, plant N uptake was high during early spring snowmelt in arctic and alpine wet meadows (Jaeger et al., 1999; Bilbrough et al., 2000), but very low at the same time in an arctic tundra ecosystem (Bilbrough et al., 2000). Larsen et al. (2012) reported a temporal differentiation among plant functional groups in the post-winter resumption of N uptake with the highest potential for early N uptake in evergreen dwarf shrubs, followed by deciduous dwarf shrubs and graminoids in a subarctic heath ecosystem.

To understand the N acquisition strategies adopted by different plant species, and the ability of N retention in two temperate grassland ecosystems during the winter-spring transitional period, we conducted a field experiment by injecting ammonium nitrate- $^{15}\text{N}_2$ ($^{15}\text{NH}_4^{15}\text{NO}_3$) into the soil before winter freezing and quantified ^{15}N tracer uptake by representative plant species (perennial bunch grasses, rhizome grasses, sagebrushes, forbs, and legumes) at consecutive harvests in the following winter freezing, spring thawing (early spring), mid-spring, and late spring in a meadow steppe and a typical steppe. We hypothesized that (1) the N acquisition strategy is divergent among plant species due to differences in the temporal patterns of photosynthetic capacities and N demands and in morphological traits (Yang et al., 2011). Given the competitive and photosynthetic capacity of dominant perennial bunch and rhizome grasses (e.g., *Stipa baicalensis* Roshev., *Stipa krylovii* Roshev. and *Leymus chinensis* (Trin.) Tzvel.), we expected that these species would have higher N acquisition potential than other species during the winter-spring transitional period (Zhang et al., 2008); (2) at an ecosystem level, the N acquisition strategies of plants during the winter-spring transitional period would differ between the two temperate grasslands due to their different edaphic conditions and community structures (Table 1).

2. Materials and methods

2.1. Study site

Parallel experiments were conducted at two different temperate continental grasslands: a meadow steppe and a typical steppe. The

meadow steppe was in the Hulunber Grassland Ecosystem Observation and Research Station of the Chinese Academy of Agriculture Sciences, which is located at the east part of the Eurasian Steppe, Inner Mongolia, northeastern China (49° 33' N, 120° 06' E, 620 m a.s.l.). The typical steppe was in the Duolun Restoration Ecology Station of the Institute of Botany, Chinese Academy of Sciences, approximately 30 km from Duolun County of Inner Mongolia, northern China (42° 02' N, 116° 17' E, 1324 m a.s.l.) (Table 1). Both sites experience long and cold winter for 6–7 months, and mild growing seasons with apparent seasonality in temperature and precipitation. For the 1960–2000 period, the mean annual temperature at the meadow steppe was $-3\text{ }^\circ\text{C}$, while the mean annual temperature at the typical steppe was $2.1\text{ }^\circ\text{C}$ (<http://www.worldclim.com>). The typical steppe usually has a shallow winter snow cover ranging from 15 to 20 cm at late winter, and it is snow-free after mid-April. In contrast, the winter snow depth of the meadow steppe is typically 20–25 cm, and the snow completely melts by late April. The soils at both sites are classified as chestnut soil according to Chinese soil classification. The period from late March to late April (approximately 30 d) in the meadow steppe and from mid-March to mid-April (approximately 30 d) in the typical steppe are characterized as the spring thawing period (early spring), during which snow completely melt and frozen soils completely thaw (Table 1).

For the plant life forms, the local vegetation of both sites primarily includes five functional types (Yang et al., 2011). Perennial bunch grasses, rhizome grasses, forbs, and sagebrushes dominate the meadow steppe, while legumes occupy less territory. The representative species of each functional type in the meadow steppe are *S. baicalensis*, *L. chinensis*, *Artemisia tanacetifolia* Linn., *Artemisia frigida* Willd., and *Vicia amoena* Fisch., respectively. The typical steppe is dominated by perennial bunch grasses, sagebrushes, and rhizome grasses, followed by forbs, while legume species are present at lower densities. The representative species of each functional type in the typical steppe are *S. krylovii*, *A. frigida*, *L. chinensis*, *Potentilla bifurca* Linn., and *Melissitus ruthenicus* (L.) Peschkoua, respectively (Table 1).

2.2. Experimental design

Eighteen $1\text{ m} \times 1\text{ m}$ plots, with a 1 m buffer between any two adjacent plots, were established in late September 2014 at each site, covering a total area of $30\text{ m} \times 20\text{ m}$. Within each site, the experimental design of the ^{15}N treatment ($^{15}\text{NH}_4^{15}\text{NO}_3$), control treatment (injected with water instead of $^{15}\text{NH}_4^{15}\text{NO}_3$), and ambient (un-manipulated control) treatment was carried out using a randomized block design (3 treatments $\times$ 6 replicates). Five separated $20\text{ cm} \times 20\text{ cm} \times 15\text{ cm}$ (length $\times$ width $\times$ height) subplots were excavated within each plot and wrapped with nylon nets, and were subsequently placed back into the soil prior to the onset of winter freezing.

We applied $^{15}\text{NH}_4^{15}\text{NO}_3$ (98%, Shanghai Research Institute of Chemical Industry, China) as the tracer. A solution equivalent to $30\text{ mg }^{15}\text{N L}^{-1}$ of $^{15}\text{NH}_4^{15}\text{NO}_3$ was injected with a syringe guided by a grid frame with 100 holes and with each hole receiving 2 mL of label solution, thus adding a total of 200 mL per subplot, equivalent to $150\text{ mg }^{15}\text{N m}^{-2}$. The total amount of ^{15}N injected into each subplot contributed approximately 10–15% to the soil inorganic N, which is within the natural range of inorganic N levels for these systems; therefore, we do not expect any substantial detrimental effect on plant and microbial communities, ensuring the focus of the experiments on N allocation among the different components. The syringe needle was inserted down to the bottom (approximately 15 cm) of the soil and then ejected gradually to the top so as to dispense the ^{15}N solution evenly. Control treatments (injected with water instead of $^{15}\text{NH}_4^{15}\text{NO}_3$) did not differ from ^{15}N the treatments in terms of plant biomass N and microbial biomass N (MBN) (data not shown), suggesting that the injection of $^{15}\text{NH}_4^{15}\text{NO}_3$ did not produce a fertilization effect and that our results were robust for N allocation analysis.

Table 1Site information, soil, and plant community properties (mean $\pm$ standard error [SE]) in the meadow and typical steppes.

	Term	Meadow steppe	Typical steppe
Site information	Location	49° 33' N, 120° 06' E	42° 02' N, 116° 17' E
	MAT (°C)	- 3	2.1
	MAP (mm)	420	383
	Elevation (m)	620	1324
	Winter time	From early October to late March (c. 170 d)	From early October to mid-March (c. 160 d)
Soil property (0–15 cm)	Winter-spring transitional time	From late March to late April (c. 30 d)	From mid-March to mid-April (c. 30 d)
	SOM (g kg ⁻¹)	53.90 $\pm$ 1.72 ^{NS} (summer)	48.18 $\pm$ 1.94 (summer)
		50.10 $\pm$ 2.73 ^{NS} (winter)	45.87 $\pm$ 2.22 (winter)
	DON (g m ⁻²)	0.76 $\pm$ 0.06 ^{NS} (summer)	0.62 $\pm$ 0.04 (summer)
		1.21 $\pm$ 0.10 ^{NS} (winter)	1.01 $\pm$ 0.10 (winter)
	IN (g m ⁻²)	1.55 $\pm$ 0.09 ^{**} (summer)	0.95 $\pm$ 0.04 (summer)
		1.51 $\pm$ 0.06 ^{**} (winter)	1.04 $\pm$ 0.05 (winter)
	20–2000 μ m (%)	63.32 $\pm$ 1.53 ^{NS}	58.24 $\pm$ 1.96
	2–20 μ m (%)	27.58 $\pm$ 0.65 ^{**}	35.22 $\pm$ 0.77
	< 2 μ m (%)	9.61 $\pm$ 0.23 ^{**}	6.10 $\pm$ 0.16
	pH	7.70 $\pm$ 0.06 ^{NS}	7.60 $\pm$ 0.07
	BD (g cm ⁻³)	1.37 $\pm$ 0.13 ^{NS}	1.25 $\pm$ 0.11
	Biomass (g m ⁻²)	877.28 $\pm$ 50.88 ^{**}	422.16 $\pm$ 32.62
	Cover (%)	67.50 $\pm$ 6.94 ^{**}	41.50 $\pm$ 4.22
	PBG	<i>Stipa baicalensis</i>	<i>Stipa krylovii</i>
Plant community property	PRG	<i>Leymus chinensis</i>	<i>Leymus chinensis</i>
	SB	<i>Artemisia frigida</i>	<i>Artemisia frigida</i>
	PF	<i>Artemisia tanacetifolia</i>	<i>Potentilla bifurca</i>
	LE	<i>Vicia amoena</i>	<i>Melissitus ruthenicus</i>
	PBG cover (%)	10.30 $\pm$ 0.65 ^{**}	5.15 $\pm$ 0.45
	PRG cover (%)	9.80 $\pm$ 1.04 ^{**}	5.30 $\pm$ 0.54
	SB cover (%)	4.20 $\pm$ 0.37 ^{NS}	4.35 $\pm$ 0.55
	PF cover (%)	5.05 $\pm$ 1.26 ^{NS}	2.15 $\pm$ 0.38
	LE cover (%)	0.95 $\pm$ 0.15 ^{NS}	1.05 $\pm$ 0.18

Significant differences between sites are reported from one-way ANOVA as non-significant [NS] ($P > 0.05$), * ($P < 0.05$), ** ($P < 0.01$); MAT, mean annual temperature; MAP, mean annual precipitation; SOM, soil organic matter; DON, dissolved organic N; IN, inorganic N; BD, bulk density; PBG, perennial bunch grass; PRG, perennial rhizome grass; SB, sagebrush; PF, perennial forb; LE, legume.

2.3. Sampling and processing

Soil temperatures were measured with a Digital thermometer (DS1921G, Maxim, USA), which automatically recorded soil temperature at the depth of 3 cm with 3 h intervals in each plot throughout the study period. Total N in the plant tissues and soil was analyzed (2 g for plant tissue, 0.8 g for soil) with an elemental analyzer (Elementar analyzer Vario Max CN, Germany). The in situ soil net N mineralization rate was measured using a polyvinyl chloride plastic (PVC) cores method (Raison et al., 1987). A pair of PVC cores (5 cm in diameter $\times$ 18 cm in length) was inserted vertically into a depth of 15 cm in the soil layer to incubate a volume of soil in the absence of plant uptake in each plot. One soil core from each pair was taken as the uninoculated sample to measure initial NH_4^+ -N and NO_3^- -N concentrations by a flow injection autoanalyzer (Scalar SAN^{plus} segmented flow analyzer, Netherlands). The other core was incubated in situ lasting an average of 14 d in capped cores to prevent leaching. Soil NH_4^+ -N and NO_3^- -N concentrations in the incubated samples were also analyzed following incubation. Net N mineralization rates were calculated as the changes in NH_4^+ -N and NO_3^- -N concentrations in the initial and incubated samples (Wang et al., 2006).

One labeled subplot was randomly sampled in each plot during each of the five field campaigns (meadow steppe: 1 December 2014, 1 April 2015, 14 April 2015, 1 May 2015, 27 May 2015; typical steppe: 3 December 2014, 27 March 2015, 10 April 2015, 29 April 2015, 24 May 2015). Within 4 h after collection, three soil cores (5 cm inner diameter) were randomly collected from the excavated subplot. The three replicates were combined into one composite sample, and then each composite sample was sieved using a 2 mm sieve. Two subsamples of each sample were obtained; one was air-dried for routine soil analyses and the other was stored at -20°C for microbial biomass analysis. The plants were gently removed from the soil cores and then rinsed in distilled water to remove the ^{15}N from the root surfaces. The plants

were dissected into shoots, coarse roots, fine roots, and rhizomes, and were then oven-dried at 65°C for 48 h.

Soil microbial biomass carbon (C) and N were measured using the fumigation-extraction method (Vance et al., 1987). Briefly, the fresh soil samples were incubated in the dark for one week at 25°C after adjusting to 60% of water holding capacity. The moist samples (15 g dry weight equivalent) were then fumigated for 24 h with chloroform. Soil extracts from the fumigated and unfumigated samples were obtained by shaking the soil samples with a solution of 60 mL 0.5 M potassium sulfate (K_2SO_4) for 30 min. The extracts were filtered through 0.45 μm filters and their extractable organic C and N contents were analyzed by the CN elemental analyzer. Microbial biomass C and N were calculated as the difference in extractable organic C and N contents between the fumigated and the unfumigated samples using conversion factors (k_{ec} and k_{en}) of 0.38 and 0.45, respectively (Lovell et al., 1995).

Phospholipid fatty acids (PLFAs) were extracted from 8.0 g (dry weight equivalent) soils (Bossio and Scow, 1998). The separation and identification of extracted PLFAs were carried out according to the standard protocol of the Sherlock Microbial Identification System V4.5 (MIDI) with a Gas Chromatograph (Agilent 6850, USA). The fatty acids a13: 0, i14: 0, i15: 0, i16: 0, i17: 0, a17: 0, 16: 1 ω 7c, 17: 1 ω 8c, 18: 1 ω 5c, 18: 1 ω 9t, 17: 0cy and 19: 0cy were chosen to represent the bacterial group, and three fatty acids (16: 1 ω 5c, 18: 2 ω 6, 9c and 18: 1 ω 9c) were chosen to represent the fungal group (Frostegård and Bååth, 1996).

2.4. Isotopic analyses

The ^{15}N in the plant tissues and soil were analyzed (2 g for plant tissue, 0.8 g for soil) with an elemental analyzer linked to an isotope-ratio mass spectrometer (IRMS, Thermo Finnigan MAT DELTA^{plus} XP, USA). For ^{15}N analysis of the total dissolved N (TDN), a 0.5 M K_2SO_4

soil extract was oxidized by the alkaline persulfate method (Zhou et al., 2003).

Soil microbial ^{15}N was determined by alkaline persulfate oxidation (Zhou et al., 2003) followed by a modified diffusion technique for recovering ^{15}N (Stark and Hart, 1996). A fraction of the K_2SO_4 extract was oxidized with 3% potassium persulfate in 0.15 M sodium hydroxide (NaOH), and then the reagent was poured into a 250 mL glass jar. Then, we added 250 mg magnesium oxide, 250 mg Devarda's alloy, and a diffusion package that consisted of a glass fiber filter (15 mm length, 5 mm width; Millipore APEFD02500) containing 10 μL 2.5 M potassium bisulfate to each jar. The jars were sealed and diffused at 25 °C for 7 d, and then an additional diffusion package was sealed in the bottle again to recover as much ^{15}N as possible. The two diffusion packages were subsequently freeze-dried in a 10 mL glass vial, and these were analyzed for isotopic composition by mass spectrometry. The microbial ^{15}N content was calculated as the difference between the ^{15}N content of the fumigated and non-fumigated samples. Soil-immobilized ^{15}N content was calculated as the ^{15}N content in the dried soil samples minus the ^{15}N content of MBN and TDN.

2.5. Calculations and statistics

As the clonal *L. chinensis* has a rhizome, unlike the other species, the ^{15}N tracer recovery and biomass of the rhizome in this plant were ascribed to that of the roots. For shoot and root biomass, the ^{15}N tracer recovery (% of added ^{15}N) was calculated as: $(^{15}\text{N}_i - ^{15}\text{N}_a) \times \text{Biomass} \times 100 / ^{15}\text{N}_t$. $^{15}\text{N}_i$ and $^{15}\text{N}_a$ are the ^{15}N concentrations ($\text{g } ^{15}\text{N g}^{-1}$ sample) in the labeled samples and ambient samples, respectively. Biomass represents the harvested biomass at each sampling time (g m^{-2}), and $^{15}\text{N}_t$ is the total added amount of ^{15}N tracer (g m^{-2}). For MBN, TDN, and soil immobilized N, the ^{15}N tracer recovery (% of added ^{15}N) was calculated as: $(^{15}\text{N}_i - ^{15}\text{N}_a) \times \text{BD} \times \text{V} \times 100 / ^{15}\text{N}_t$. BD represents the bulk density (g cm^{-3}) and V is the soil volume for the 0–15 cm soil profile ($\text{cm}^3 \text{ m}^{-2}$).

Soil mineral N, microbial biomass C and N, plant biomass C and N, and ^{15}N pool data were tested for the effects of sampling time for each individual fraction by one-way analysis of variance (ANOVA) followed by Duncan's tests at a significance level of $P < 0.05$. Dependent variables were not log-transformed prior to analysis. Non-parametric Mann-Whitney U tests were used to test whether the $\delta^{15}\text{N}$ in the labeled samples was significantly higher than the ambient samples. All statistical analyses were performed using SPSS 21.0 software (SPSS, Chicago, IL, USA).

3. Results

3.1. Background environmental conditions

The mean annual temperature and annual precipitation were similar for the two sites. The temperature of the top soil layer (0–3 cm) was similar, but the edaphic conditions, including organic matter, dissolved organic N, inorganic N content and texture were significantly different between the two sites during the winter-spring transitional period ($P < 0.05$) (Table 1; Fig. 1A and B). The lowest temperatures of the top soil layer over the winter freezing period were -20°C and -17°C in the meadow steppe and typical steppe, respectively. The daily minimum soil temperature was close to 0°C on 26 September in the meadow steppe and on 10 October in the typical steppe. From 24 March to 22 April in the meadow steppe and 15 March to 13 April in the typical steppe (spring thawing period), the daily mean soil temperature gradually increased from -5 to 5°C , and most of the snow disappeared and soil thawing was completed by the end of the period (Fig. 1A and B).

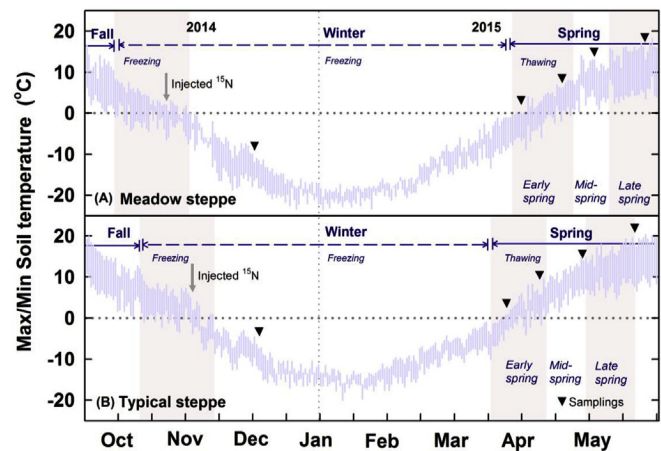


Fig. 1. Soil temperatures over fall, winter, and spring in a meadow and a typical steppe of northern China. Vertical lines are daily ranges in soil temperature measured at 3 cm depth. The period from 15 September (late fall) to 30 May (late spring) is indicated as well as the times of addition of $^{15}\text{N}_2$ -labeled NH_4NO_3 and sampling at both sites.

3.2. Soil properties and microbial biomass under the treatments

Soil NH_4^+ -N dominated the inorganic N pool in the soils, and accounted for approximately 60–65% of the inorganic N pool during the winter-spring transitional period in the meadow and typical steppes (Fig. 2A and B). Soil NH_4^+ -N slightly accumulated over winter and reached the highest level during the spring thawing period at both sites, whereas NO_3^- -N did not change significantly over the study period. From the winter freezing period to late spring, soil net N mineralization rates gradually increased at both sites, which the faster net N mineralization rate in the meadow steppe than in the typical steppe was observed during the spring thawing period (Fig. 2C and D).

Soil microbial biomass C increased by about 53% and 49% in the meadow and typical steppe, respectively, from the winter freezing to spring thawing period and then decreased to its original level at late spring (Fig. 2E and F). In contrast to the seasonal patterns of microbial biomass C, microbial biomass N remained unchanged over the study period (Fig. 2G and H).

Soil fungal biomass (PLFAs) reached the highest value over winter, and then gradually dropped at both sites (Fig. 2I and J). In contrast to fungal biomass, soil bacterial biomass did not change significantly over the study period in the meadow steppe, while it gradually increased from the spring thawing period to late spring in the typical steppe.

3.3. Plant species N concentration and biomass

The shoot N concentration of the sagebrushes increased significantly from the spring thawing period to late spring at both sites ($P < 0.05$; Fig. 3A and B). In contrast, the root N concentration of sagebrush dropped rapidly and then slightly increased in the meadow steppe, whereas root N concentration of it gradually decreased in the typical steppe over the study period ($P < 0.05$; Fig. 3A and B). Only the root N concentration of the forb species increased significantly in the meadow steppe, and the root N concentration of the rhizome grass decreased gradually in the typical steppe over the period ($P < 0.05$; Fig. 3A and B). However, the shoot and root N concentrations of bunch grasses and legumes did not change over the study period at both sites (Fig. 3A and B).

Plant biomass N in the meadow steppe was twice that in the typical steppe during the early spring (Fig. 3C and D). Shoot biomass N of the bunch grass, rhizome grass, sagebrush, forb and legume species increased by 0.08, 0.07, 0.05, 0.11, and 0.05 g N m^{-2} ($P < 0.05$) in the meadow steppe, and the shoot biomass N of these species increased by

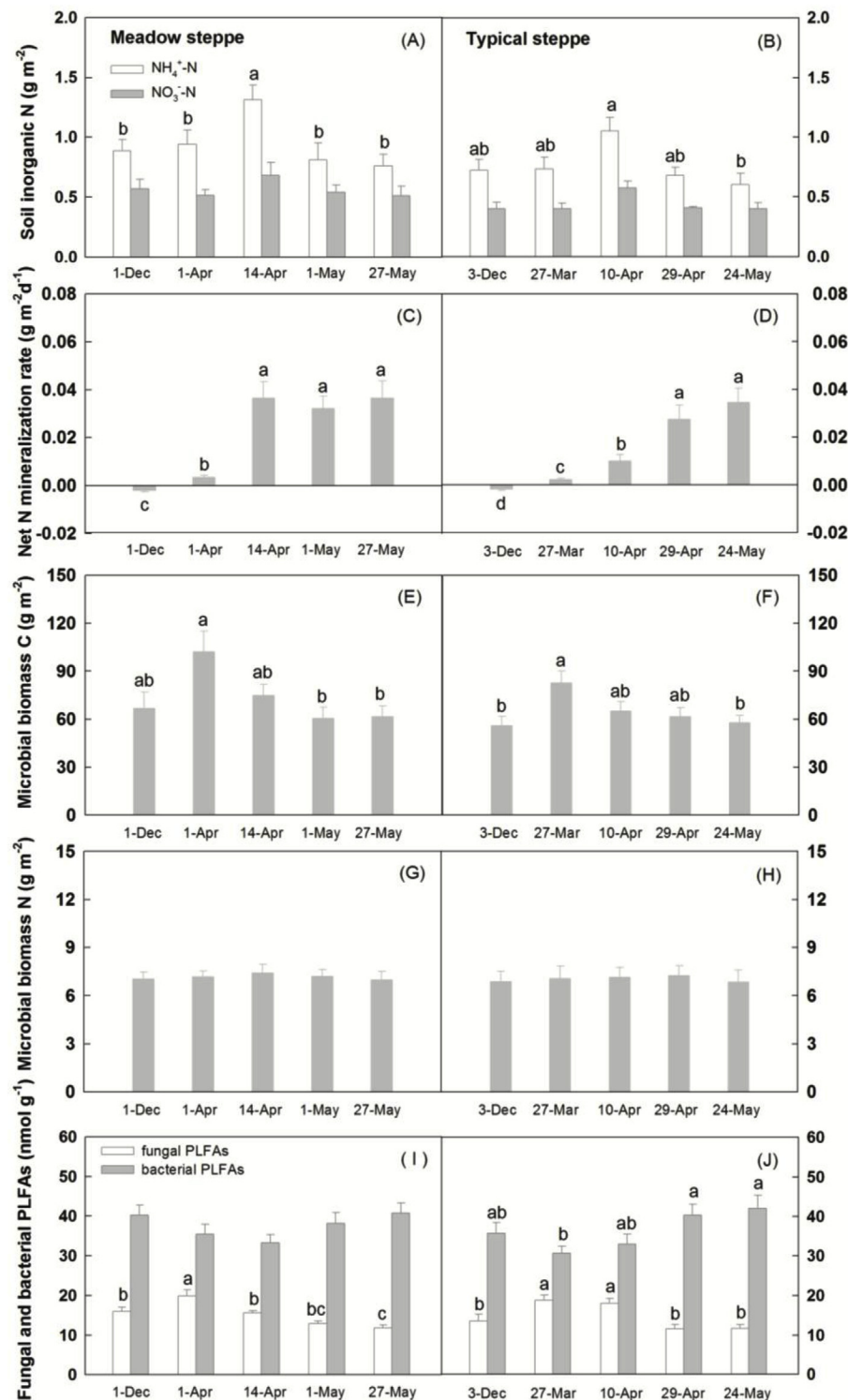


Fig. 2. Soil inorganic N (NH_4^+ and NO_3^-), net N mineralization rate, microbial biomass C and N, and soil fungal and bacterial PLFAs from December 2014 to May 2015 in a meadow and a typical steppe of northern China. Vertical bars indicate SE of the means ($n = 6$). Different lowercase letters indicate statistically significant differences ($P < 0.05$) in pools at the different sampling periods.

0.05, 0.08, 0.04, 0.06, and 0.03 g N m^{-2} ($P < 0.05$) in the typical steppe at the end of study period. In contrast, the attached root biomass N of the bunch grass, rhizome grass, sagebrush, forb and legume species increased by 0.10, 0.20, 0.08, 0.16, and 0.06 g N m^{-2} ($P < 0.05$) in the meadow steppe, and the attached root biomass N of these species increased by 0.12, 0.18, 0.09, 0.21, and 0.04 g N m^{-2} ($P < 0.05$) in the typical steppe, respectively (Fig. 3C and D).

3.4. Microbial N immobilization and plant N acquisition

Over the entire study period, the total recovery of the injected ^{15}N tracer was 65% and 61% in the meadow and typical steppes, respectively. The ^{15}N tracer recovery was highest in the soil immobilized N fractions (41–45%) and microbial biomass (10.5–12.3%), with small amounts of ^{15}N tracer in the roots (6.0–6.7%), shoots (2.0–2.3%), and

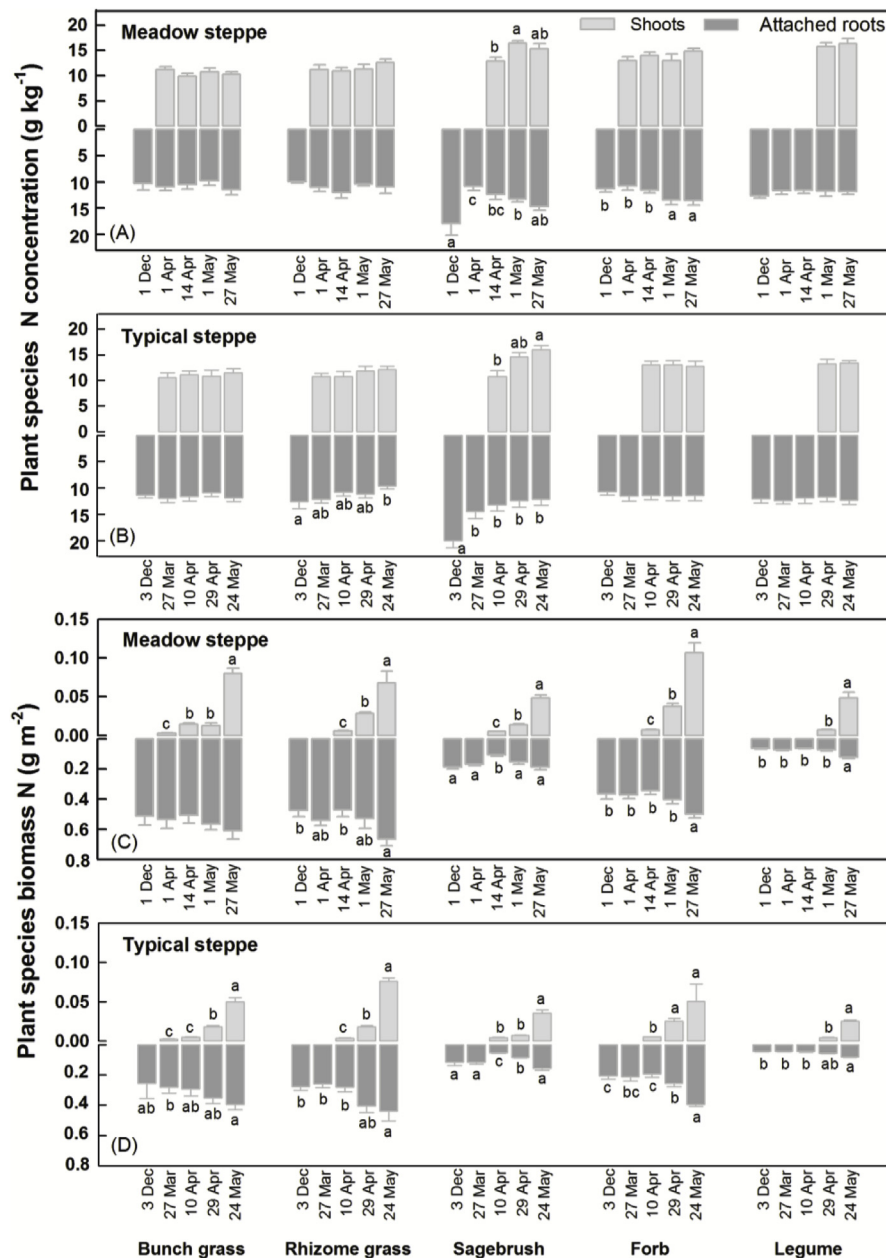


Fig. 3. Plant N concentration and biomass N of selected species from December 2014 to May 2015 in a meadow and a typical steppe. Vertical bars indicate SE of the means ($n = 6$). Different lowercase letters indicate statistically significant differences ($P < 0.05$) in pools at the different sampling periods.

total dissolved N (1.1–1.2%); the higher figures always being found in the meadow steppe (Fig. 4A, B, C, D).

At both sites, the ¹⁵N tracer immobilized by the soil microbes declined from 20% during spring thawing to 10–12% in late spring (Fig. 4A, C). As the microbial biomass N pool did not fluctuate significantly during the study period (Fig. 2E and F), the pattern of microbial ¹⁵N tracer recovery reflects uptake/release processes.

The ¹⁵N tracer recovery in the shoots and attached roots of all plant species increased significantly from spring thawing to late spring in both steppes (Fig. 4B, D). There was no significant difference in added ¹⁵N recovery in total vegetation biomass between both sites during the experimental trial (Fig. 4B, D), whereas the plant species showed significant divergences in magnitude and timing of added ¹⁵N uptake (Fig. 5A and B).

The fine roots of the perennial bunch grasses (*i.e.* *S. baicalensis* and *S. krylovii*) first acquired ¹⁵N at the beginning of the spring thawing period. The total ¹⁵N tracer recovery of bunch grass reached 1.64% and

1.53% in the meadow and typical steppes in late spring, respectively (Fig. 5A and B). In contrast to bunch grasses, rhizome grass and forb species began to take up N in the middle of spring thawing at both sites (Fig. 5A and B). In the meadow steppe, ¹⁵N tracer recovery in the belowground biomass of the rhizome grass and forb gradually increased to 2.96% and 2.01%, especially in the rhizome (Fig. 5A and B). Shoot ¹⁵N recovery in the rhizome grass and forb only reached 0.41% and 1.18% at the end of study period, respectively (Fig. 5A and B). Similar to the meadow steppe, the added ¹⁵N recovery in the roots of rhizome grass and forb increased to 2.87% and 1.18%, and the ¹⁵N recovery in their shoots increased to 0.5% and 1.1% at the end of study period in the typical steppe, respectively (Fig. 5A and B). However, ¹⁵N recovery in the sagebrush and legume species was the smallest component of total plant ¹⁵N recovery, and was as low as 0.10% and 0.29% in the meadow steppe, and 0.05% and 0.14% in the typical steppe, respectively (Fig. 5A and B).

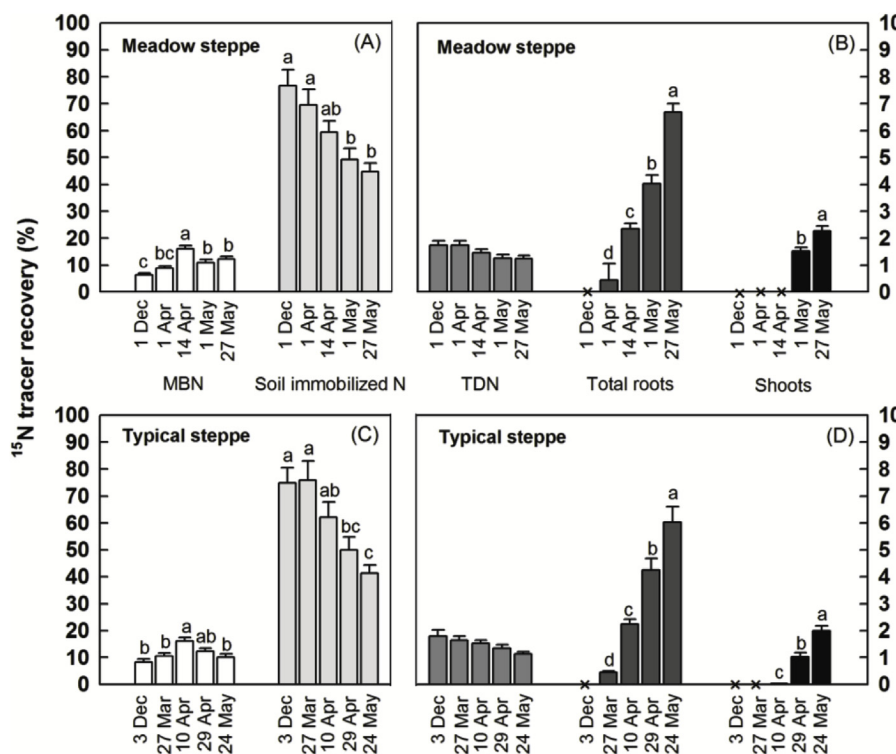


Fig. 4. Partitioning of added ^{15}N tracer shown as ^{15}N tracer recovery of MBN, soil immobilized N, TDN, total shoots and attached roots in % of addition from December 2014 to May 2015 in a meadow and a typical steppe of northern China. Soil immobilized ^{15}N is the added tracer recovered in the soil samples minus the ^{15}N tracer recovery of MBN and TDN. Vertical bars indicate SE of the means ($n = 6$). Different lowercase letters indicate statistically significant differences ($P < 0.05$) in the pools at the different sampling periods.

4. Discussion

Temporal partitioning of N between plants and microbes is of great importance for plant growth in the following spring (Lipson et al., 2002; Schmidt et al., 2007). Extensive research has indicated that plant N uptake ceases and microbes switch from net N mineralization to N immobilization during the non-growing seasons in alpine and arctic systems (Lipson et al., 1999; Schadt et al., 2003; Andresen and Michelsen, 2005; Edwards et al., 2006; Christopher et al., 2008; Edwards and Jefferies, 2010). Unlike previous observations, however, soil microbial biomass C increased significantly over the winter freezing period, in this study, and soil microbial biomass N did not vary (Fig. 2E, F, G, H). A possible explanation is the significant increase in fungal biomass during winter at both sites (Fig. 2I and J), as fungi require relatively low N per unit of biomass and fungal C storage is 26 times greater than the corresponding bacterial C storage (Six et al., 2002). In addition, the low microbial N immobilization in temperate grasslands could have been due to much lower mid-winter soil temperatures (-16 to -20°C) in comparison with the arctic and alpine sites (-5 to -8°C). The shallow snow cover (< 25 cm) in temperate grasslands may not act as a thick insulating layer to maintain relative high soil temperature and biotic activities.

Approximately 20% of added ^{15}N was recovered in the microbes after the subsequent spring thawing period, and then declined to 10–12% in late spring at both sites. This finding confirms that the soil microbes competed effectively with the plant roots for mineral N in the short-term (Harrison et al., 2007; Larsen et al., 2007; Song et al., 2007; Zhu et al., 2013) due to their inherent uptake capacity (i.e., high surface-area to volume ratios) (Rosswall, 1982). However, the plants subsequently become more effective competitors for N in the long-term (Fig. 5), likely because microbial lysis releases N back to the plant-soil system (Hodge et al., 2000). From an evolutionary perspective, the competition between plants and soil microorganisms for early spring N resources prevents N leaching losses, and lends further support to the view that it is a strategy to maintain ecosystem stability (Kuzakov and Xu, 2013).

Plant species differ in terms of the magnitude and timing of N

acquisition during the winter-spring transitional period (Fig. 5). The significant increases in ^{15}N recovered in the fine roots of *S. baicalensis* and *S. krylovii* at both sites (Fig. 5) indicate that these perennial bunch grasses effectively acquired N at the beginning of the spring thawing period (when the maximum daily soil temperature reached 0°C). The ability of dominant plant species to take up N at or below freezing temperature has also been documented in subarctic, arctic, alpine, and temperate ecosystems (Billings et al., 1977; Onipchenko et al., 2009; Larsen et al., 2012). These findings demonstrated that the roots of some species are active under freezing conditions, and could obtain a certain proportion of added ^{15}N tracer under snow-covered soils in temperate grasslands. Plant activity at or below freezing may be a crucial contribution to season-long nutrient demand, and may facilitate rapid plant growth and development once the temperature increases (Bilbrough et al., 2000; Miller et al., 2009).

In both study sites, perennial rhizome grasses and forbs (*L. chinensis*, *A. tanacetifolia* and *P. bifurca*) began to take up ^{15}N in the middle of the spring thawing period (Fig. 5). In contrast to hypothesis 1, rhizome grasses and forbs acquired more applied ^{15}N than bunch grasses. Several possible factors have led to the inter-species differences in N acquisition strength. First, bunch and rhizome grasses have distinct root architectural traits. In temperate grasslands, *L. chinensis* depends mainly on clonal growth to maintain its role in the plant community (Dong, 1999). The clonal nature of *L. chinensis* enables it to escape from stressful environments and to compete for available nutrients successfully with a guerrilla strategy (Doust, 1981), whereas *S. grandis* possesses a more stable N acquisition strategy in comparison to *L. chinensis* in temperate grasslands (Wang et al., 2016). Second, from a phenological perspective, the flowering and maturation period of *L. chinensis* is earlier than that of *S. grandis* (Cui et al., 1996; Sun et al., 2015), and thus *L. chinensis* is likely to absorb more N to meet its growth requirements. In contrast, among the tested plant species, perennial forbs took up the highest ^{15}N tracer, which is consistent with its fast-growing characteristics (Zhang et al., 2008). These traits are associated with fast rates of ecosystem processes such as nutrient acquisition or decomposition, from a plant economic strategy perspective (Reich, 2014). The ability of perennial rhizome grasses and forbs to acquire N may play an

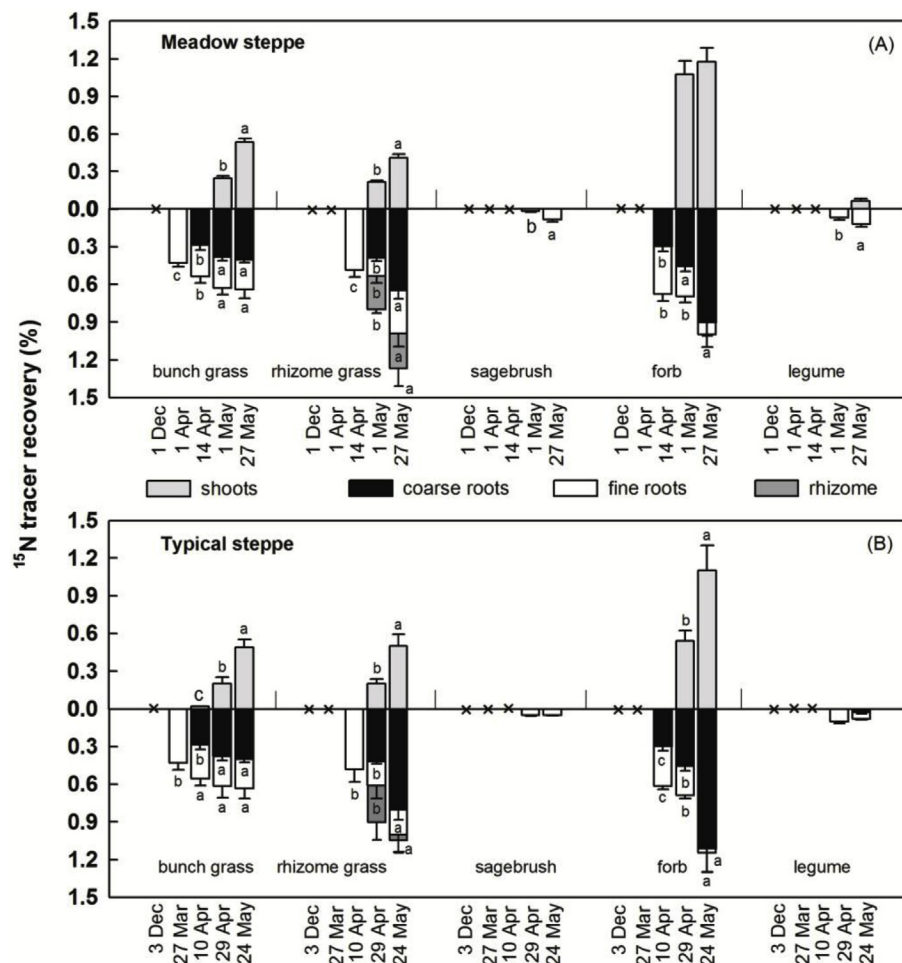


Fig. 5. Partitioning of added ^{15}N tracer among plant species shown as ^{15}N tracer recovery in % of addition from December 2014 to May 2015 in a meadow and a typical steppe of northern China. Root biomass (fine roots, coarse roots, and rhizomes) only includes roots that were still attached to the shoots after excavation. Vertical bars indicate SE of the means (n = 6). Different lowercase letters indicate statistically significant differences (P < 0.05) in the pools at the different sampling periods.

important role in maximizing ecosystem primary production, which contributes approximately 20–45% of annual gross photosynthesis at the study sites (Ma et al., 2012).

However, the legume species (*V. amoena* and *M. ruthenicus*) showed a low added ^{15}N recovery (0.14–0.29%), and even sagebrush species (*A. frigida*) almost did not take up added ^{15}N (< 0.1%) during the study period at both sites (Fig. 5). These results contrast with other studies in alpine and arctic tundra, subalpine grasslands, and a subarctic heath (Bilbrough et al., 2000; Tye et al., 2005; Larsen et al., 2012) that reported that all the plant species acquired applied ^{15}N during the snowmelt period. A possible explanation is that the N acquired by legume species in early spring may be attributable to symbiotic N fixation (Lonati et al., 2015). In contrast to legumes, we found that the N concentration in the sagebrush roots during winter was significantly higher than that in early spring at both sites (Fig. 4). This finding indicates that the initial growth of sagebrush probably relies on internal N resources, which were withdrawn during senescence the previous year.

We observed active ^{15}N acquisition by the roots, and transportation to the shoots, in perennial bunch grasses, rhizome grasses, and perennial forbs from the spring thawing period at both sites (Fig. 5). These trends are consistent with numerous studies in many cold ecosystems (Schimel and Clein, 1996; Jaeger et al., 1999; Bilbrough et al., 2000; Bardgett et al., 2002; Sorensen et al., 2008; Miller et al., 2009; Legay et al., 2013), but contrast with other studies in alpine or arctic meadows in which N absorbed during spring was not translocated to the shoots,

but remained in the roots and rhizomes (Jaeger and Monson, 1992; Edwards and Jefferies, 2010). In this study, perennial forbs translocated a much larger portion of the acquired ^{15}N to the shoots than perennial bunch grasses and rhizome grasses. This has also been attributed to the relatively rapid growth rate (Zhang et al., 2008), and thus a requirement for high photosynthetic rates in perennial forbs probably contributes to the rapid translocation to the shoots. The finding suggests that plant N acquisition in the winter-spring transitional period is not only used for root growth, but also for initial shoot growth in the temperate grasslands.

In contrast to hypothesis 2, at an ecosystem level, no significant difference in magnitude of plant ^{15}N uptake was observed between the two sites, although plant biomass N in the meadow steppe was twice that in the typical steppe during the early spring (Table 1; Figs. 2, 4 and 5). This is contrary to several studies conducted at high-latitude ecosystems (Bilbrough et al., 2000; Legay et al., 2013). Bilbrough et al. (2000) compared N acquisition by alpine and arctic tundra plants during snowmelt and observed that alpine plants acquired 100 times more added ^{15}N than arctic plants due to winter abiotic conditions. A possible explanation is that the initial plant N uptake partially relied on newly mineralized N; this is corroborated by the higher net N mineralization rate during early spring in the meadow steppe than that in the typical steppe (Fig. 2C and D). This finding suggests that soil N processes strongly affect plant N acquisition during the winter-spring transitional period in temperate grasslands.

In our study, a temporal niche differentiation in plant N acquisition may facilitate species coexistence in the N-limited temperate grasslands, despite the existence of strong competition for N in microbe-plant and plant-plant interactions. Given the findings of the present study, we anticipated three research efforts to better understand the N retention in temperate grasslands during winter-spring transitional periods. First, it is forecasted that temperate ecosystems would experience climate warming and snow fall regime changes over winter to the end of the 21st century (IPCC, 2013). An increase in the frequency of late winter warming events is very likely to contribute to early episodes of soil thawing, potentially reducing the ecosystem N retention in early spring. Further field experimental study and monitoring, in particular during fall and summer, are needed to understand the winter warming impacts on soil N cycling and plant N acquisition strategies. Second, it is still not clear how biological factors interact with the environmental factors controlling the plant strategies for taking up N; further experiments would contribute to our understanding of the biological and environmental controls on N acquisition strategies. Third, the present ecosystem models consider the plant-microbe competition for N in a very straightforward manner (Thornton et al., 2009), without taking into account the different strategies of plant species adapted to N-limited ecosystems. These models should be improved by including a more mechanistic representation of plant N acquisition strategies.

5. Conclusions

Perennial bunch grasses showed active N acquisition at the beginning of the spring thawing period, and perennial forbs and rhizome grasses subsequently began to take up N in the middle of the thawing period, whereas legumes and sagebrushes acquired little early spring N resources in the meadow and typical steppes. Therefore, the temporal niche differentiation of microbe-plant or plant-plant interactions may facilitate species coexistence and N retention in N-limited temperate grasslands. At the ecosystem level, no significant differences in magnitude of plant ^{15}N uptake were observed between the two grasslands, although vegetation biomass N in the meadow steppe was twice that in the typical steppe during the early spring. This finding is consistent with higher net N mineralization rate in the meadow steppe, suggesting a strong regulation of soil N processes and storage on plant N acquisition during the winter-spring transitional period.

Our discovery of different N acquisition strategies during early spring has important implications for understanding plant N uptake strategies. The different strategies adopted by various plant species help to explain multi-species coexistence under N stress. Given the important role of plant N uptake on carbon sequestration, the different plant N acquisition strategies should be included in model development to better simulating vegetation growth, particularly during N stress in spring.

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