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

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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 ^{15}N 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 ^{15}N 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 ^{15}N recovery, indicating their dependence on internal N accumulation or N fixation. At the ecosystem level, no significant difference in the magnitude of plant ^{15}N 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

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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 inconsistences 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-$^{15}N_2$ ($^{15}NH_4{}^{15}NO_3$) 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 °C, while the mean annual temperature at the typical steppe was 2.1 °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 m × 1 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 m × 20 m. Within each site, the experimental design of the ^{15}N treatment ($^{15}NH_4{}^{15}NO_3$), control treatment (injected with water instead of $^{15}NH_4{}^{15}NO_3$), and ambient (un-manipulated control) treatment was carried out using a randomized block design (3 treatments × 6 replicates). Five separated 20 cm × 20 cm × 15 cm (length × width × 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 $^{15}NH_4{}^{15}NO_3$ (98%, Shanghai Research Institute of Chemical Industry, China) as the tracer. A solution equivalent to 30 mg $^{15}N\ L^{-1}$ of $^{15}NH_4{}^{15}NO_3$ 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 mg $^{15}N\ m^{-2}$. 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 $^{15}NH_4{}^{15}NO_3$) 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 $^{15}NH_4{}^{15}NO_3$ did not produce a fertilization effect and that our results were robust for N allocation analysis.