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Effect of different forage species on the nitrogen uptake in Hulunbeir

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Abstract: Knowledge of determining factors for nitrogen uptake preferences and how they are modified in changing environments are critical to understand ecosystem nitrogen cycling and to predict plant responses to future environmental changes. However, it remains unclear in this aspect for the main managed grassland (Medicago sativa, Bromus inermis, Leymus chinensis) and crop (Brassica campestris) under field condition in Hulunbeir area of Inner Mongolia of China. Two 15N tracer experiments utilizing a unique differential labelled nitrogen source were employed in both managed grassland (M. sativa, B. inermis and L. chinensis) and crop (B. campestris) in Hulunbeir area of Inner Mongolia of China. Tracing both labels in the above- and below ground plant biomass, soil NH_4^+ -N or NO_3^- -N and NH_4^+ -N or NO_3^- -N uptake by plants. There were differences between soil NO_3^-N concentration and NH4+-N concentration, and NO_3^-N concentration was higher than NH_4^+-N concentration. NO_3^-N concentration was led by different grass species. The NH_4^+-N concentration in August were higher than in July on the whole, the highest value for B. campestris and the lowest for B. inermis. The plant N concentration in B. inermis, L. chinensis and B. campestris showed decreasing trend, its mean value decreased by 20.1, 47.9 and 26.7%, respectively, and M. sativa increased by 13.7%. Among the four species, the individuals exhibited a preference for $^{15}NO_3^-$, indicated by higher ^{15}N signatures in $^{15}NO_3$-treatment than in $^{15}NH_4^+$ treatment.

1.Introduction

Nitrogen (N) is an important limiting nutrient for net primary productivity in most terrestrial ecosystems (LeBauer & Treseder, 2008). Nitrogen is made available to plants through decomposition

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of soil organic matter (SOM), but microbes also require N for growth. Plantemicrobe competition for N can be intense when both the demand for N by plants and microbes to grow is high (Dunn et al., 2006; Månsson et al., 2009; Inselsbacher et al.,2010;Xu et al.,2011) affecting plant community composition, ecosystem N retention and carbon (C) sequestration (Zak et al.,1990; van der Heijden et al., 2008; Bloor et al., 2009; Averill et al., 2014). Ammonium (NH_4^+) and nitrate (NO_3^-) being the two major inorganic N forms available for plants (Mahmood & Kaiser 2003; Wang et al, 2011). Recent studies reinforce the idea that plants clearly prefer inorganic N forms over organic forms in most systems (Harrison, Bol & Bardgett 2007; Ashton et al. 2008), and the optimization of the plant preference of different forms of inorganic N presumably enhances the survivorship and the fitness of plants (Wang & Macko, 2011). Recent years, some scholars studied the N uptake characterizes mainly including forest species (Zhang et al., 2014), crops (Sauer et al. 2015; Cicek, 2015; Chakwizira, 2015), and alpine ecosystems (Xu et al., 2006; Miller & Bowman, 2002) and so on. Whereas there are only a few reports on grasses (Ramirez-Garcia1 et al., 2015; Schmitt et al., 2013), especially perennial grasses (*Medicago sativa*, *Bromus inermis*, *Leymus chinensis*) (Zebarth et al., 2015; Lv et al., 2013; Mitra & Mandal, 2012; Chen et al., 2003).

Grasslands, one of the most dominant vegetation types over the world, occupy about 40% of the land area in China (Chen, 2000). In recent years, the natural grasslands in China are experiencing degradation in different degrees. A sharp decline in the grassland productivity often occurs during the degradation, and further affects the animal husbandry. To chieve high quality forage supply, managed grasslands with cultivation have been developed in the severely degraded grasslands in China. Cultivation has merged to collectively affect the productivity of the Chinese grassland ecosystems. Till to 2013, the total area of managed grasslands with cultivation was increasing to 1.2×10^7 hm^2 (National animal husbandry, 2014). It was more and more important to select the suitable grasses species.

Inner Mongolia possesses a large area of temperate grasslands and some of them have been intensive managed. Hulunbeir located in northeast Inner Mongolia, China. The natural grassland in Hulunbeir City, with 88000 km^2 in area which covers 33.5% of the total area. Hulunbeir was a typical area, natural grassland degradation and managed grassland planted. At present, *Medicago sativa*, *Bromus inermis*, *Leymus chinensis* was mainly planted in these areas. The planted areas are enlarging year by year.

However, few studies have been carried out to investigate N uptake characteristics of soil and plants in different grass species and crop. Species differ in their ability to take up different forms of N, such partitioning may enable plants to efficiently utilize available soil N (Chapin et al., 1993; Leadley et al., 1997; Näsholm et al., 1998; Lipson et al., 2001). This paper reports how we met the gap through a field experiment in Inner Mongolia, China. Specifically, we assessed managed grassland & crop nitrogen uptake using stable nitrogen isotope in Hulunbeir Inner Mongolia, China. Experiments focusing on N dynamics was conducted at selected grasslands in Inner Mongolia in 2014. The hypotheses backing the experiments were: (1) plants prefer NO_3^- in the four species because it is a dominant inorganic N form in the temperate grasslands; (2) The preference for N form is affected by the soil NH_4^+/NO_3^- ratios.

2.Materials and methods

2.1. Research site

This study was conducted at Hulunbeir Grassland Ecosystem Observation and Research Station (N 49°19'35", E 119°56'52"), in the north-eastern Region of Inner Mongolia, China. The frost-free period is approximately 110 days in this area. The average precipitation is ranged from 350 mm to 400 mm from 2000 to 2010, about 80% of which falls between July and September. The annual mean air temperature ranged from -5 to -2 ℃, with the maximum monthly mean of 36.2 ℃ in July and the minimum of -48.5 ℃ in January. The soil is chestnut soil or chernozem (Table 1). In this study, we selected three managed grasslands (Medicago sativa, Bromus inermis and Leymus chinensis) and one type of crop (Brassica campestris) as a reference. The plots were arranged in a split-plot design as the main treatment. Each plot consisted of four subplots (3×5m). The seedling amounts of the M. sativa, B. inermis, L. chinensis grasslands and B. campestris were 15.0, 22.5, 37.5,75.0 kg hm^{-2}, respectively. The managed grasslands were perennial grasses, and planted in 2010, B. campestris was annual crop and planted in 2014. No fertilizer was applied for all the experimental grasslands.

2.2.Experimental design

The experiment was done July and August in 2014, respectively. Each selected experimental samples was carefully injected as a water suspension into the soil of four plots at a depth of 5 cm using the method described by Schimel and Chapin (1996) for injecting amino acid solution. Four plots were used as control, by injecting the same amount of water. $NaNO_3$ (10%) and $(NH_4)_2SO_4$ (10%) inputs corresponded to 1.82 and 1.41g m^{-2}, firstly, the drug was dissolved in the distilled water, 45 ml once, and sprayed to soil, and another 45 ml distilled water was used to clean and then sprayed to soil again. The beginning time was calculated and 16 h later, the samples were taken back to laboratory, including the aboveground, root and soil samples.

Roots were carefully separated from soil cores. Plant material was separated from the aboveground and the root, and heated 90 s by microwave oven, and then was dried at 75℃ for 48 h, weighed for total dry biomass and ground for measurement of total N content, $^{15}N/^{14}N$ ratios. Soil samples were sieved to 2 mm to remove coarse fragments for measuring pH, $^{15}NH_4^+$ and $^{15}NO_3^-$.Total nitrogen (N) content was measured by dry combustion on a LECO CNS-1000 elemental analyzer (LECO Corporation, St.Joseph, MI) (Du & Gao, 2006).

2.3.Calculations and statistics

Plant ^{15}N uptake was calculated as the following:

$$\text{Plant } ^{15}\text{N uptake } [\mu g\ N\ g^{-1}\ root] = N\% \times Biomass \div 100 \times APE \div 100 \times 1000000 \quad (1)$$

$$\text{Plant uptake } NO_3^- [\mu g\ N\ g^{-1} root] = \text{Plant } ^{15}\text{N uptake} \div [NO_3^-]soil \times {}^{15}\text{Nadded} \quad (2)$$

$$\text{Plant uptake } NH_4^+ [\mu g\ N\ g^{-1}\ root] = \text{Plant } ^{15}\text{N uptake} \div [NH_4^+]soil \times {}^{15}\text{Nadded} \quad (3)$$

$$\text{Plant } ^{15}\text{N uptake rate}[\mu g\ N\ g^{-1} root\ h^{-1}] = NO_3^- \text{ was absorbed by plants} \div Biomass(BG) \div t \quad (4)$$

And t means time from the beginning time of 15Nadded to the end of the samples taken away.

BG means belowground

The significance of differences in ^{15}N uptake as well as N contents between plant species was examined using one-way analysis of variance (ANOVA). Critical LSD values for 5% error probability were calculated. The standard errors of means are presented on the figures as a variability parameter. One-way ANOVA was used to test the difference in mean biomass, N content, ^{15}N uptake by plants between the treatments. Significance of all tests was accepted at $P < 0.05$.

3.Results

3.1.Biomass

Among the four species, plant biomass (aboveground + belowground) at the time of sampling was remarkably higher in *B. inermis* compared to the three other species, while plant biomass of *L. chinensis* secondary and *M. sativa* was lowest in July, and there was significant among four species (Fig. 1). *L. chinensis* is highest in August, but there is not significantly (P>0.05) (Fig.1). There were significant different in aboveground or belowground among four species both July and August (P<0.05). The R:S ratio(ratios of root to sheet) showed the similar trend, that is decreased after July on the whole. *B. inermis* (3.74 and 0.97) was higher than any other species. *L. chinensis* (1.57 and 0.52) secondary, *B. campestris* is the lowest, only 0.21(in July) and 0.07(in August).

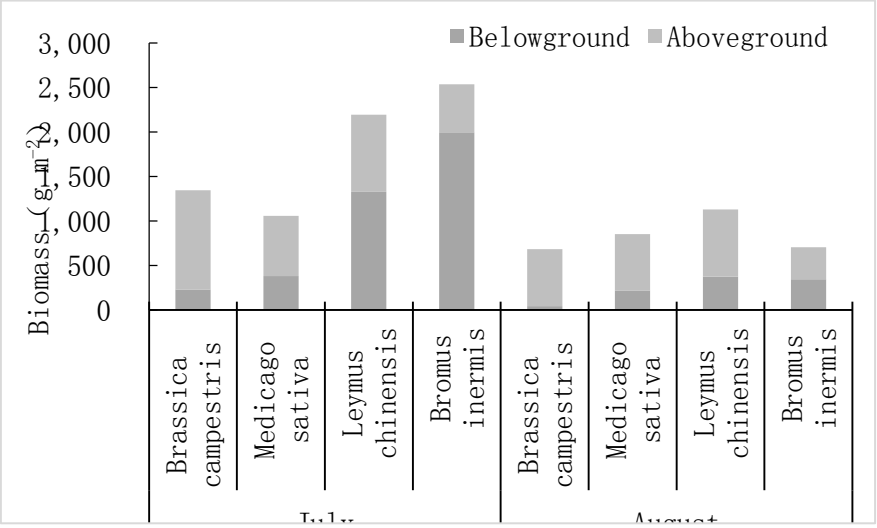


Figure1. The Biomass change in different treatments. The figure indicated the biomass change in July and August, biomass including aboveground and belowground. ANOVA method was used to analyze the difference of aboveground and belowground among four species, Values are means (±SE) of four replicates, corrected by those in the control plots. Letters indicate significant differences at 0.05 error probability level, small letters stand for the differences of grass species in same months, and capitals stands for the differences of months as to the same species.

3.2.Change of soil N concentration

There were differences between soil NO_3^--N concentration and NH_4^+-N concentration, and NO_3^--N concentration was higher than NH_4^+-N concentration. NO_3^--N concentration was led by different grass species. In July, NO_3^--N concentration in *M. sativa* was higher than any other grass species, which were 4.4, 1.8 and 2.5 times larger than in *B. inermis*, *L. chinensis* and *B. campestris*, respectively, and there was significant positive difference among four grass species (P<0.05) (Fig.2-a). In August, NO_3^--N concentration in *L. chinensis* was the highest, compared to in July, *B. inermis* and *B. campestris* have the tendency of increase, and *M. sativa* decreased, and significant among three species (P<0.05). The same specie was not significant between July and August (P>0.05).

The NH_4^+-N concentration in August were higher than in July on the whole, the highest value for *B. campestris* and the lowest for *B. inermis* in July, and the highest value for *B. campestris* were 1.6, 1.4 and 1.3 times larger than *M. sativa*, *B. inermis* and *L. chinensis*, and there was not significant

difference between *B. campestris* and three other species, and there was no significant difference among these three species (*M. sativa*, *B. inermis*, and *L. chinensis*) (Fig. 2-b).

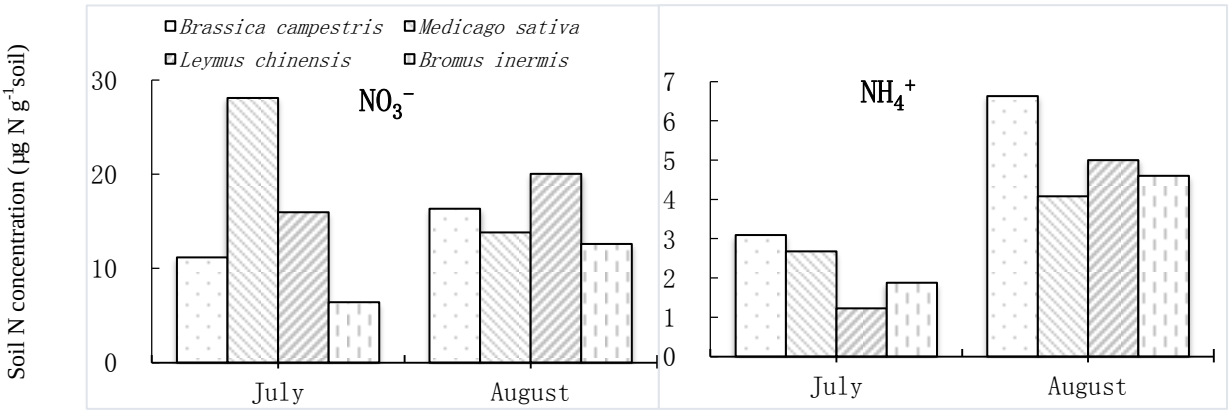


Figure2. Soil N concentration of different species in different months, including NO_3^- -N concentration and NH_4^+ -N concentration. Values are means (±SE) of four replicates, corrected by those in the control plots. Letters indicate significant differences at 0.05 error probability level, small letters stand for the differences of grass species in same months, and capitals stands for the differences of months as to the same species.

3.3.Change of plant N concentration

The plant N concentration in *B. inermis*, *L. chinensis* and *B. campestris* showed decreasing trend, its mean value decreased by 20.1, 47.9 and 26.7%, respectively, and *M. sativa* showed different trend compared to three other species, the plant N concentration showed increasing trend from July to August, its value increased by 13.7%. Among the four species, the plant N concentration was higher in *M. sativa* compared to the three other species, while the plant N concentration of *B. inermis* and *L. chinensis* was similar, and the lowest. *B. campestris* was secondary. There was significant different among four species in July and August ($P<0.05$). *M. sativa* and *B. inermis* were not different in different months, but *L. chinensis* and *B. campestris* not.

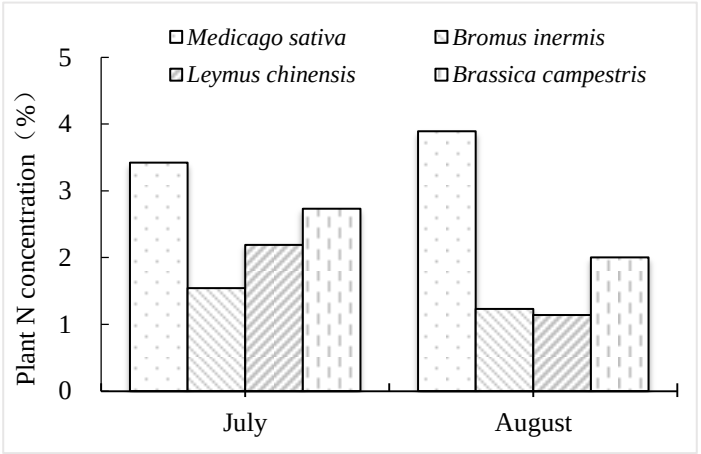


Figure3. Change of plant N concentration. Values are means (±SE) of four replicates, corrected by those in the control plots. Letters indicate significant differences at 0.05 error probability level, small letters stand for the differences of grass species in same months, and capitals stands for the differences of months as to the same species.

3.4.Change of plant N uptake rate

Among the four species, the individuals exhibited a preference for NO_3^-(Fig.3), indicated by higher ^{15}N signatures in $^{15}NO_3^-$treatment than in $^{15}NH_4^+$ treatment. On the whole, the plant N uptake rate to NO_3^- in July was higher than in August. And there was significantly different in *M. sativa* from July to August, decreased by 64.4% ($P<0.05$), but three other species uptake preferences did not obviously change ($P>0.05$).

Whereas four species had also a preference for NH_4^+, three species (*B. inermis*, *L. chinensis* and *B. campestris*) generally showed the same trends, which the plant N uptake rate to NH_4^+ in August was higher than in July, and there were significantly different in different months and species($P<0.05$), increased by 5.8, 5.2 and 8.8 times. But *M. sativa* showed different trend, decreased by 29.4%.

4.Discussion

4.1.Species preferred to NO_3^-

N mineralization and nitrification should be different for organic matter derived from different sources, i.e. soil, litter or added algae. Whatever the source material is, ammonium and nitrate produced are liberated into the soil solution from which they are taken up by the plants in an identical way (Xu et al.,2006). We studied the change of nitrogen content and soil ammonium and nitrate concentration of the four species, the results indicated that nitrogen content in *M. sativa* was higher than any others (Fig.3). M. sativa is legume, N fixed by nodule, which can improve available nitrogen content. From the flowering period to the fruit period, the nitrogen content of M. sativa is increasing.

Different plants showed different preferences for N (NH_4^+ and NO_3^-), a multitude of studies have shown that most plants prefer NO_3^-–N to NH_4^+–N (Britto and Kronzucker,2002; Marschner,1995). Azarmi and Esmaeilpour (2010) observed that cucumber grown in a 25:75 NH_4^+: NO_3^- solution had the highest total fruit yield. Strawberry has also been found to produce the highest vegetative growth and fruit yield even at 50:50 NH_4^+: NO_3^- (Tabatabaei et al., 2008). These studies imply that different plant species may have different optimal NH_4^+: NO_3^- ratios. We studied the change of nitrogen content of the four species, the results indicated that *M. sativa* was higher than any others. M. sativa is legume, N fixed by nodule, which can improve available nitrogen content.

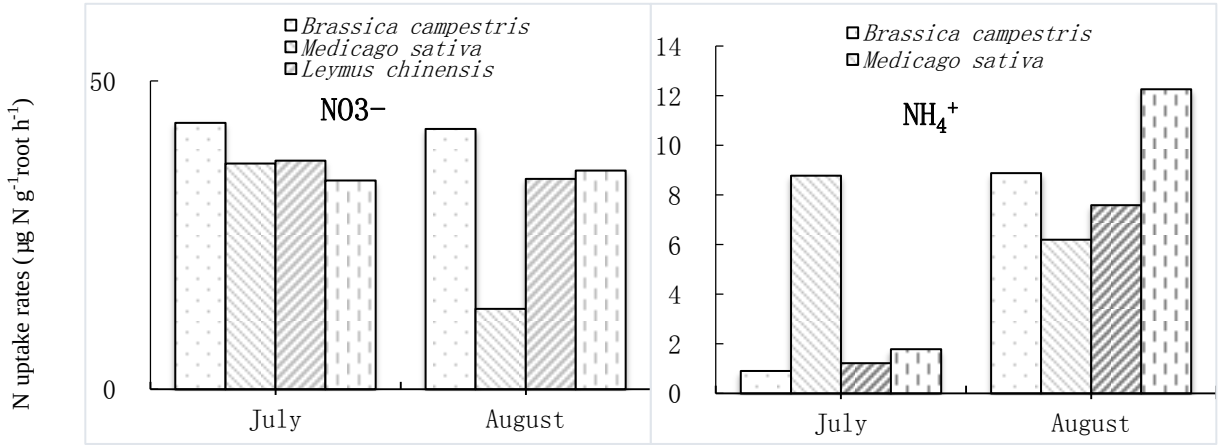


Figure4. Change of plant N uptake rate. Values are means (±SE) of four replicates, corrected by those in the control plots. Letters indicate significant differences at 0.05 error probability level, small letters stand for the differences of grass species in same months, and capitals stands for the differences of months as to the same species.

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