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ORIGINAL ARTICLE

Soil inorganic nitrogen composition and plant functional type determine forage crops nitrogen uptake preference in the temperate cultivated grassland, Inner Mongolia

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ABSTRACT

Plant nitrogen (N)-acquisition strategy affects soil N availability, community structure, and vegetation productivity. Cultivated grasslands are widely established to improve degraded pastures, but little information is available to evaluate the link between N uptake preference and forage crop biomass. Here an *in-situ* ¹⁵N labeling experiment was conducted in the four cultivated grasslands of Inner Mongolia, including two dicots (*Medicago sativa* and *Brassica campestris*) and two monocots (*Bromus inermis* and *Leymus chinensis*). Plant N uptake rate, shoot- and root biomass, and concentrations of soil inorganic-N and microbial biomass-N were measured. The results showed that the root/shoot ratios of the dicots were 2.6 to 16.4 fold those of the monocots. The shoot N concentrations of the dicots or legumes were 40.6% to 165% higher than those of the monocots or non-legumes. The four forage crops in the cultivated grassland preferred to uptake more NO₃⁻-N than NH₄⁺-N regardless of growth stages, and the NH₄⁺/NO₃⁻ uptake ratios were significantly lower in the non-legumes than in the legumes ($p < 0.05$). Significant differences in the NH₄⁺-N rather than NO₃⁻-N uptake rate were observed among the four forages, related to plant functional types and growth stages. The NH₄⁺ uptake rate in the perennial forages exponentially decreased with the increases in shoot-, root biomass, and root/shoot ratio. Also, the plant NH₄⁺/NO₃⁻ uptake ratio was positively correlated with soil NH₄⁺/NO₃⁻ ratio. Our results suggest that the major forage crops prefer to absorb soil NO₃⁻-N, depending on soil inorganic N composition and belowground C allocation. The preferential uptake of NO₃⁻-N by forages indicates that nitrate-N fertilizer could have a higher promotion on productivity than ammonium-N fertilizer in the semi-arid cultivated grassland.

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¹⁵N labeling; plant
functional type; soil
N availability; temperate
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1. Introduction

Nitrogen (N) is an essential element for plant growth and a limiting factor in the productivity of terrestrial ecosystems (LeBauer and Treseder 2008). Plants can directly take up NH₄⁺-N, NO₃⁻-N, and low molecular weight organic N from soils (Jones et al. 2005), and then they are assimilated and synthesized into proteins for the construction of plant tissues and organs. In order to take up enough N to meet the needs of its growth and development, plant develops various N-acquisition strategies (e.g., N uptake preference) (Silvertown 2004). Plant N-acquisition strategy directly and indirectly affects soil N availability, species coexistence and diversity, community structure, and vegetation productivity (Houlton et al. 2007). Therefore, understanding plant N-acquisition strategy is critical to promote productivity and stability in terrestrial ecosystems (Van Der Heijden, Bardgett, and Van Straalen 2008).

Ammonium (NH₄⁺) and nitrate (NO₃⁻) in soil solution diffuse and migrate to the root-soil interface before they are absorbed by plants. Ammonium ions are absorbed by the plant via ammonia (NH₃) transporters. Both absorbed and synthesized NH₃ is incorporated into amino acids via the glutamine synthetase-glutamate

synthase (GS-GOGAT) pathway (Masclaux-Daubresse et al. 2006). While nearly all the NH₃ in the root is usually incorporated into amino acids at the root itself (Kiyomiya et al. 2001), plants may transport significant amounts of NH₄⁺ in the xylem to be fixed in the shoots (Schjoerring et al. 2002). In contrast, NO₃⁻ is taken up by several NO₃⁻ transporters that use a proton gradient to power the transport (Sorgona et al. 2011). Nitrate is first reduced to nitrite (NO₂⁻) in the cytosol by NO₃⁻ reductase using NADH or NADPH, and NO₂⁻ is then reduced to NH₃ in the chloroplasts (plastids in roots) by a ferredoxin dependent nitrite reductase (Tischer 2000). Most of the NO₃⁻ reduction is carried out in the shoots while the roots reduce only a small fraction of the absorbed NO₃⁻ to NH₃ (Scheurwater et al. 2002). Thus, most plants exhibit higher affinity for absorption of NH₄⁺ than NO₃⁻ due to low energy consumption (5 ATP mol⁻¹ NH₄⁺ vs 20 ATP mol⁻¹ NO₃⁻), but the accumulation of NH₄⁺ in the cells is not conducive to plant growth due to ammonium toxicity (Boudsocq et al. 2012). It is still controversial that plants will preferentially absorb which N ion.

The ability of a plant to capture NH₄⁺-N or NO₃⁻-N from the soil depends on soil type, environment, and species, etc. Soil water content influences the rate of oxygen supply and several N transformation processes within the soils, while soil

properties especially hydraulic conductivity affects soil N loss pathways. In aerobic and arid soils where nitrification can occur, NO_3^- -N is usually the predominant form of available N that is absorbed (Xu et al. 2011); however, NH_4^+ -N can predominate in forests and in flooded, anaerobic soils like rice paddies (Houlton et al. 2007; Ishii et al. 2011). Plant N uptake preference is closely related to soil inorganic N composition ($\text{NO}_3^-/\text{NH}_4^+$ ratio, Kahmen, Wanek, and Buchmann 2008). Soil pH affects the plant N uptake preference through choosing the high- or low-affinity transport systems of NH_4^+ -N and NO_3^- -N (Van Beusichem, Kirkby, and Baas 1988). The rate of NO_3^- -N uptake is usually high and is favored under high-pH conditions, while NH_4^+ -N uptake is best under neutral condition (Hawkins and Robbins 2010). Additionally, the plant species with low nitrate activity (e.g., blueberries) are almost completely dependent on NH_4^+ -N as the N source, whereas the monocots particularly grasses seem to be willing to use NO_3^- -N (Maathuis 2009). Monocots generally have an excess of anion uptake over cation uptake which results in an increased pH in the rhizosphere. So monocots may prefer NH_4^+ -N in acid soil conditions, but NO_3^- -N under alkaline conditions (Marschner et al., 1991). Also, NO_3^- -N are presumably related to the cation exchange capacity of the root tissue, and the dicots are higher than the monocots (Haynes 1980).

China's natural grassland area is approximately 400 million ha, accounting for 40% of the country's land area (Li et al. 2017). It is estimated that 31.8% of grassland in China (1.05×10^8 ha) has been undergoing different degrees of degradation due to climate change and overgrazing (Wang et al. 2017). Cultivated grassland has higher productivity than natural pasture, which can to some extent relieve the pressure of natural pasture and increase the number of livestock. In 2013, the area of cultivated grassland in China has reached 1.2×10^7 ha (Xu et al. 2018), becoming a necessary business model of modern grassland farming system. However, the conversion of natural grassland to cultivated grassland will raise several ecological problems, including decline in soil water and nutrients (Connell et al. 2011) and decrease in ecosystem stability (Xu et al. 2018). Species-specific differences in direct uptake of different N forms in the temperate grassland ecosystems have been regarded as an important mechanism influencing species coexistence (Weigelt, Bol, and Bardgett 2005). Exploring the N-acquisition strategies of representative forage crops as well as its driving mechanisms is critical to construct reasonable cropping pattern and fertilizer regime. Unfortunately, previous studies mainly focused on natural grassland ecosystems, and little information is available about forage crops N-acquisition strategies in cultivated grassland.

In the Hulunber region of Inner Mongolia, managed grasslands of legumes (*Medicago sativa*), non-legumes (*Bromus inermis*), and oil crop (*Brassica campestris*) are common land-use types to substitute the native temperate grasslands (*Leymus chinensis*). To date, the characteristics and controlling factors of the N preferential use in the four forage crops are still unclear. We hypothesized that plant N content was closely related to soil mineral N content. The four test species could prefer to absorb more NO_3^- -N than NH_4^+ -N because NO_3^- -N dominates inorganic N in the aerobic and managed grassland

soil (Xu et al. 2018). Also, we hypothesized that the N uptake preference could depend on plant adaptation to soil conditions, which is closely related to the plant functional type (dicots vs monocots), above- and belowground biomass, and soil N composition. In order to test the two hypotheses, we conducted an *in situ* ^{15}N labeling experiment in Hulunber. The main objectives were to: (1) investigate the effects of different forage crop cultivations on general soil properties, above- and belowground biomass, plant and microbial biomass N concentration, and soil NH_4^+ -N and NO_3^- -N concentration; (2) compare the rates of NH_4^+ -N and NO_3^- -N uptake of the four species at the different growth stages; and (3) clarify the relationships between forage N uptake rates and key driving factors.

2. Materials and methods

2.1. Site description and herbage cultivation

This study was conducted at the Hulunber Grassland Ecosystem Observation and Research Station (N 49°19'35", E 119°56'52", 650 m a. s. l.), located in the northeast of Inner Mongolia, China. The region has a characteristic of mid-temperate semi-arid continental climate. Average monthly temperature varies from -48.5°C in January to 36.2°C in July, with a mean annual temperature of -1.5°C . Accumulated temperature above 10°C is between 1700°C and 2300°C , and the frostless season is about 110 days. The mean annual precipitation ranges from 350 mm to 400 mm between 2000 and 2010, of which approximately 80% falls in June to September (Xu et al. 2018). The soil in this region is classified as a chernozem (IUSS WG WRB 2015). The type of land use is farmland, which has been continuously planting oilseed rape (*Brassica campestris*) for more than two decades. In 2010, some oilseed rape cropland was converted and grow leymus, alfalfa, and brome grass, respectively. Here, we use oilseed rape farmland as a control.

Two monocots (*Bromus inermis* and *Leymus chinensis*) and two dicots (*Medicago sativa* and *Brassica campestris*) were selected to compare the differences in N uptake. Both *B. inermis* and *L. chinensis* are perennial grasses, *M. sativa* is a perennial legume forage, while *B. campestris* is an annual oil crop. The four species are widely cultivated in the Hulunber region. The multiple-species cultivation experiment was established using a randomized block design in 2010. Each treatment had four replicated plots, with an area of $12\text{ m} \times 20\text{ m}$ for each plot. The seeding rates for *M. sativa*, *B. inermis*, *L. chinensis*, and *B. campestris* were 15.0, 22.5, 37.5, and 75.0 kg ha^{-1} , respectively. Three perennial forages were cultivated in 2010, while *B. campestris* was cultivated for four years since 2010. The aboveground biomasses were removed and the roots were retained in the soil at the end of September. No fertilizer was applied for any experimental treatments. The general soil properties in the surface layer (0–20 cm) in different experimental treatments are shown in Table 1.

2.2. ^{15}N labeling experiment

In 2014, the mean annual temperature and mean annual precipitation in Hulunber area were -1.6°C and 365 mm, respectively, which was close to the multi-year average of air

Table 1. General soil properties in the 0–20 cm soil depth under different forage crop cultivations after four years of forage crop cultivation.

Soil properties [†]	Forage crops [‡]			
	<i>M. sativa</i>	<i>B. campestris</i>	<i>B. inermis</i>	<i>L. chinensis</i>
TN (g kg ⁻¹)	2.40 ± 0.67a	2.02 ± 0.11a	2.29 ± 0.24a	2.31 ± 0.15a
SOC (g kg ⁻¹)	16.17 ± 3.01a	14.07 ± 0.98c	15.83 ± 2.65b	16.02 ± 2.76a
AK (mg kg ⁻¹)	307.88 ± 21.87a	201.45 ± 11.00b	205.81 ± 67.97b	235.49 ± 34.45b
pH	6.82 ± 0.01a	7.01 ± 0.03a	6.80 ± 0.02a	6.96 ± 0.01a
BD (g m ⁻³)	1.08 ± 0.06b	1.10 ± 0.07b	1.13 ± 0.05b	1.36 ± 0.03a
MS (%)	16.38 ± 1.98a	17.51 ± 3.05a	19.12 ± 0.81a	12.37 ± 0.34b

[†]TN, SOC, AK, BD, and MS are total nitrogen, soil organic carbon, available potassium, bulk density, and soil moisture, respectively.

[‡]Data are mean ± standard error ($n = 4$). Different lowercase letters indicate significant differences among the four species cultivations.

temperature (−2~−1°C) and precipitation (300–500 mm). The forage crops were growing well because there was no obvious drought. ¹⁵N labeling was performed for each experimental treatment during the flowering phase (in July) and maturation phase (in August), respectively. At each plot, three micro-plots (30 cm × 30 cm) were randomly established to label N, including ¹⁵N-labeled (NH₄)₂SO₄ (¹⁵NH₄⁺), ¹⁵N-labeled KNO₃ (¹⁵NO₃⁻), and a zero N control. The ¹⁵N labeling experiment was conducted at 10:00 a.m. on a sunny day. At each micro-plot, an equal amount of 23.2 mg ¹⁵NO₃⁻-N (K¹⁵NO₃, 10 atom% ¹⁵N) or ¹⁵NH₄⁺-N ((¹⁵NH₄)₂SO₄, 10 atom% ¹⁵N) was dissolved in 20 mL deionized water. A previous study by in the same region showed that temperate plants acquired 67% of their N from the 0–5 cm soil layer and 33% from the 5–15 cm layer (Liu et al. 2016). Therefore, we focused on the uptake of targeted plants from the upper 5 cm soil and injected N at 2.5 cm depth. In order to avoid N fertilization effect, we used N at a low rate of 2.58 kg ha⁻¹, equal to <5.0 µg g⁻¹ soil. To ensure the even distribution of the ¹⁵N, a total of 4 mL of labeled solution was injected into the soil around the plant using a triangular fashion (i.e., with the plant generally growing in the center of a triangle). The solution was injected at a distance of about 0.5 cm from the center to avoid injecting the roots. The control micro-plots only received the same amount of deionized water.

2.3. Crop harvest and measurement

After labeling for 24 h, shoot (leaf plus stem) and root samples in each micro-plot of 30 cm × 30 cm were collected. The shoot was cut off, and then the soil core in the 0–30 cm depth was excavated. All the roots were carefully separated from the soil. The shoot and root samples were dried at 75°C for 48 h to measure their biomass using the oven-drying method.

A part of dried shoot and root subsamples were ground to a fine powder in a planetary mill (FSD-100A, China). ¹⁵N/¹⁴N ratio and N concentration of samples were determined simultaneously on an isotope ratio mass spectrometer (Finnigan MAT-253, Thermo Electron) coupled to an automatic, online elemental analyzer (Flash EA1112, Thermofinnigan) (Fang et al. 2011).

2.4. Soil sampling and measurement

The soil core in the 0–30 cm depth at each micro-plot was excavated after plant collection. The soil samples were sieved (2 mm mesh) to remove coarse plant residues and gravels, and

stored at 4°C for the measurements of soil NH₄⁺-N, NO₃⁻-N, and microbial biomass N (MBN).

Soil samples were extracted using 2 M KCl solution (soil:water = 1:10) and shaken for 1 h. Then, soil suspensions were filtered using Whatman #1 filters, and the extracts were determined for NH₄⁺-N and NO₃⁻-N concentrations on a continuous-flow auto-analyzer (AA3, SEAL company, Germany) (Fang et al. 2014). Soil MBN was determined on a 15-g oven-dry equivalent field-moist soil sample using the chloroform fumigation–extraction method (Brookes et al. 1985). In brief, organic N from the fumigated (24 h) and non-fumigated (control) soil were quantified by a CN analyzer (Model TOC-V/CPH-TN; Shimadzu, Kyoto, Japan). The non-fumigated control values were subtracted from the fumigated values. The MBN was calculated using a kEC factor of 0.54 (Jenkinson 1988). The MBN content was determined once a month during the growing season (from May to September). Also, another soil subsamples were ground to a fine powder to measure ¹⁵N/¹⁴N ratio and N concentration on an isotope ratio mass spectrometer (Finnigan MAT-253, Thermo Electron). Except soil total N (TN), other general soil properties including soil organic carbon (SOC), available potassium (AK), soil moisture (MS), bulk density (BD), and pH value were measured by the potassium dichromate volumetric method, ammonium acetate extraction method, oven-drying method, ring knife method, and pH meter, respectively (Lu 1999).

2.5. Data calculation and statistical analysis

¹⁵N uptake of by plants (¹⁵N_{uptake}, mg ¹⁵N m⁻²) was calculated using equation (1) (McKane et al. 2002):

$${}^{15}\text{N}_{\text{uptake}} = [N]_{\text{plant}} \times APE \times \text{Biomass} \quad (1)$$

where $[N]$ is plant N concentration (%), APE is atom percent excess (atom%_{labeled} – atom%_{control}), $Biomass$ is the sum of shoot and root biomass (g m⁻²).

The uptake rates of NO₃⁻-N or NH₄⁺-N by plants ($MN_{\text{uptake rate}}$, mg N kg⁻¹ root h⁻¹) were calculated using the equations (2) and (3) (Xu et al. 2011):

$$MN_{\text{uptake}} = {}^{15}\text{N}_{\text{uptake}} / {}^{15}\text{N}_{\text{added}} \times [MN] \quad (2)$$

$$MN_{\text{uptake rate}} = MN_{\text{uptake}} / (\text{Root biomass} \times T) \quad (3)$$

where MN_{uptake} is the rate of NO₃⁻-N or NH₄⁺-N uptake by plants, ${}^{15}\text{N}_{\text{added}}$ is the total amount of added ¹⁵NO₃⁻ or ¹⁵NH₄⁺ (mg kg⁻¹), $[MN]$ is the concentrations of soil NO₃⁻-N or NH₄⁺-N in the control plot (mg kg⁻¹), Root biomass is equal to belowground biomass (g m⁻²), T is the duration of ¹⁵N labeling experiment (24 h).

One-way analysis of variance (ANOVA) combined with Tukey's honestly significant difference (HSD) test was used to evaluate the effects of the four forage crop cultivations on general soil properties, shoot and root biomass, N concentrations of shoot and microbial biomass, soil inorganic N concentration, and mineral N uptake rates in plants at different growth stages. Paired *t*-test was used to examine the difference in each response variable between the flowering and maturation phases. Also, the relationships between mineral N uptake rates in plants and shoot-, root biomasses, and root/shoot ratios, as well as the relationship between plant $\text{NH}_4^+/\text{NO}_3^-$ uptake ratios and soil $\text{NH}_4^+/\text{NO}_3^-$ ratios were evaluated using the stepwise regression analyses. All statistics analyses were performed using the SPSS software package (version 16.0) and the graphs were drawn using the Sigmaplot software (version 12.5). Statistical significant differences were set with *p* values <0.05 unless stated otherwise.

3. Results

3.1. Soil general properties

After four years of forage crop cultivation, there was no significant difference in the TN concentration and pH value in the 0–20 cm depth among the four species cultivation treatments (Table 1). However, the SOC concentration was the highest in the cultivated *M. sativa* and *L. chinensis* plots, followed by the *B. inermis* plots, and the *B. campestris* plots was the lowest (Table 1). Similar to the pattern of SOC, soil available potassium concentration followed the order: *M. sativa* > *L. chinensis* > *B. inermis* > *B. campestris* (Table 1). *L. chinensis* cultivation tended to increase soil bulk density and decrease soil moisture content relative to the other three species cultivations (Table 1).

3.2. Shoot and root biomass

During the flowering phase, the shoot biomass of *B. campestris* was significantly higher than those of the other species;

moreover, the shoot biomass of *L. chinensis* was significantly higher than that of *B. inermis* (Figure 1(a-d)). However, no significant difference in the shoot biomass among the four species cultivations was observed during the maturation phase (Figure 1(a-d)). Also, the shoot biomass of *B. inermis* and *B. campestris* was higher during the flowering phase than during the maturation phase (Figure 1(a-d)).

For the root biomass of flowering period, the two monocots, *B. inermis* and *L. chinensis*, were higher than the two dicots (i.e., *M. sativa* and *B. campestris*); the former was 3.5 to 8.8 fold those of the latter (Figure 1(e-h)). Similar pattern was observed during the maturation phase, and the root biomass of *L. chinensis* was significantly higher than those of *M. sativa* and *B. campestris* (Figure 1(e-h)). Except the cultivated *M. sativa* treatment, the root biomass was higher during the flowering phase than during the maturation phase (Figure 1(e-h)). At the different growth stages, the root/shoot ratios in the two monocots, *B. inermis* and *L. chinensis*, averaged 2.13 and 1.05, respectively, which was greater than those of the two dicots (0.41 for *M. sativa* and 0.13 for *B. campestris*) (Figure 1).

3.3. Nitrogen concentrations in shoot, soil, and microbial biomass

The N concentration of *B. inermis* shoot was the lowest among the four species, while that of *M. sativa* was the highest, attributed to the nature of atmospheric N_2 fixation (Figure 2). During the flowering phase or the maturation phase, the N concentrations of dicotyledonous *M. sativa* and *B. campestris* shoots were higher than those of monocotyledonous *B. inermis* and *L. chinensis* ($p < 0.05$, Figure 2). Significant difference in the shoot N concentration between the flowering phase and the maturation phase was observed ($p < 0.01$, Figure 2). From the flowering to maturity, the shoot N concentrations of *B. inermis*, *L. chinensis*, and *B. campestris* significantly decreased by 17.1% to 47.8%, whereas the shoot N concentration of *M. sativa* increased by 10.9% ($p < 0.01$, Figure 2).

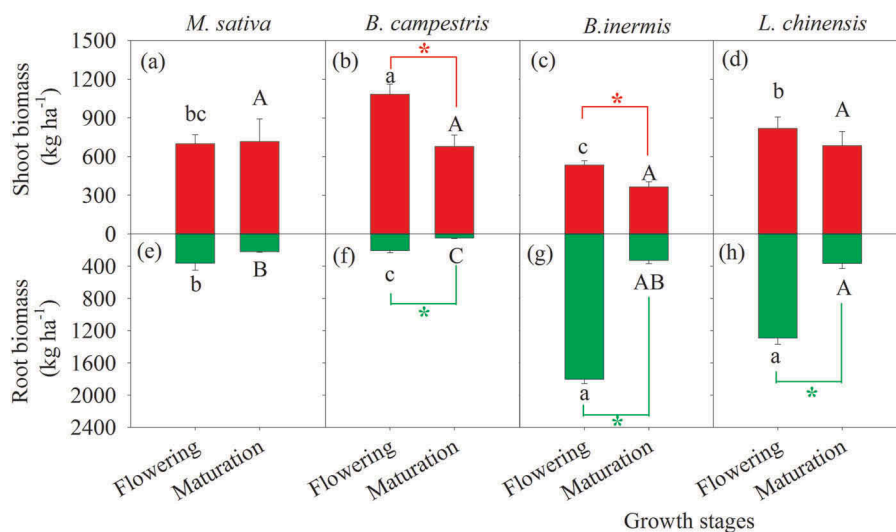


Figure 1. Shoot- and root biomass in the four forage crop cultivations during the flowering and maturation phases. Values are means \pm standard error ($n = 4$). Different lowercase and uppercase letters mean significant difference in a given variable during the flowering and maturation phases, respectively. Asterisk (*) means a significant difference between the two growth stages at the level of 0.05 based on *t*-test.

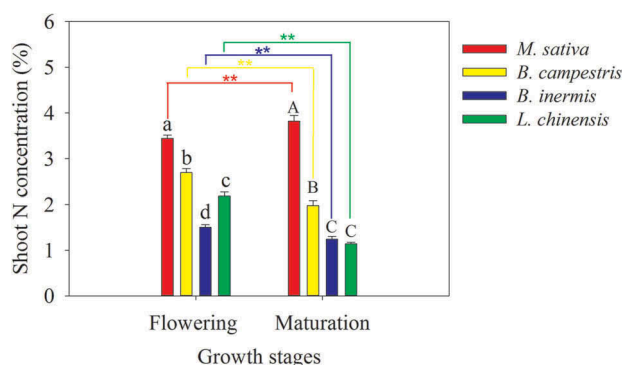


Figure 2. Shoot N concentrations in the four forage crops during the flowering and maturation phases. Values are means \pm standard error ($n = 4$). Different lowercase and uppercase letters mean significant difference in shoot N concentration during the flowering and maturation phases, respectively. Two Asterisks (**) mean a significant difference between the two growth stages at the level of 0.01 based on t -test.

In the temperate cultivated grassland, soil inorganic N is dominated by NO_3^- -N, and soil NO_3^- -N concentrations were 1.4 to 14.9 fold those of soil NH_4^+ -N at the different growth stages and species cultivations (Figure 3). During the flowering phase, soil NO_3^- -N concentrations in the dicotyledonous *M. sativa* and *B. campestris* cultivations were significantly higher than those of monocotyledonous *B. inermis* and *L. chinensis* cultivations ($p < 0.01$, Figure 3). Similarly, during the maturation phase, soil NO_3^- -N concentration was the highest in the *M. sativa* cultivation, while the lowest soil NO_3^- -N concentration was observed in the *B. inermis* cultivation plots (Figure 3). Significant difference in soil NO_3^- -N concentration was found in the dicots plots between the two growth stages (Figure 3). In contrast, there was no significant difference in soil NH_4^+ -N concentration among the four species cultivations during the flowering phase, but the soil NH_4^+ -N concentration was significantly lower in the *B. inermis* cultivation than in the

B. campestris cultivation during the maturation phase (Figure 3). Furthermore, the soil NH_4^+ -N concentration was lower during the flowering phase than during the maturation phase regardless of plant functional types (Figure 3).

There was significant monthly variation in the soil MBN content, with the peak occurring in July to September for the different species cultivations ($P < 0.05$, Figure 4(a)). Compared with the other non-legumes, *M. sativa* cultivation exhibited a higher MBN content throughout the growing season. However, the lowest soil MBN content was observed in the *B. campestris* cultivation treatment (Figure 4(a)). The average soil MBN content in the *M. sativa* cultivation was 27.9% higher than that of *B. campestris* cultivation (Figure 4(b)).

3.4. Inorganic N uptake by plants

Forage crop species and growth stages influenced the preferential uptake of inorganic N in soils. In the artificial grasslands of Hulunbeier, forage crops absorbed more NO_3^- -N than NH_4^+ -N regardless of forage crop species and growth stages (Figure 5). The average ratio of plant $\text{NH}_4^+/\text{NO}_3^-$ uptake ranged from 0.021 to 0.24 during the flowering phase, which was lower than those of the maturation phase ranging from 0.21 to 0.48; moreover, the average ratio of plant $\text{NH}_4^+/\text{NO}_3^-$ uptake followed the order: *B. campestris* < *L. chinensis* < *B. inermis* < *M. sativa* ($p < 0.05$, Figure 5). Also, there was no significant difference in plant NO_3^- -N uptake rate during the flowering and maturation phases among the four species, but the plant NH_4^+ -N uptake rate was significantly different among the four species regardless of the growth stages (Figure 5). The NH_4^+ -N uptake rate of leguminous *M. sativa* was significantly higher than those of the other non-legumes during the flowering phase, but was significantly lower than that of *B. inermis* during the maturation phase ($p < 0.05$, Figure 5). In addition, the difference in the NO_3^- -N uptake

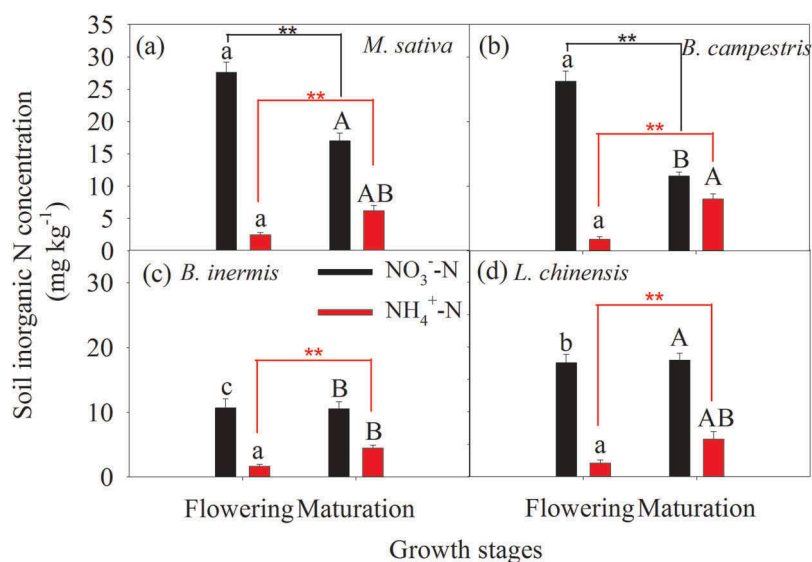


Figure 3. Soil NO_3^- -N and NH_4^+ -N concentrations in the four species cultivations during the flowering and maturation phases. Different lowercase and uppercase letters mean significant difference in soil NO_3^- -N or NH_4^+ -N concentration during the flowering and maturation phases, respectively. Two asterisks (**) mean a significant difference between the two growth stages at the level of 0.01 based on t -test.

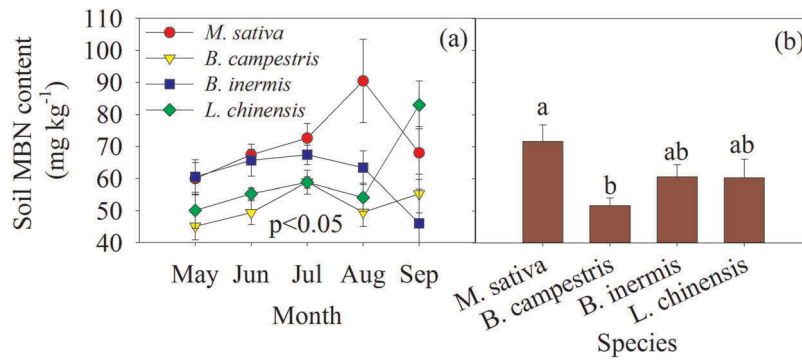


Figure 4. Monthly variation and mean of soil microbial biomass N (MBN) concentration in the different species cultivation plots. Different lowercase letters indicate significant difference among the four species cultivations.

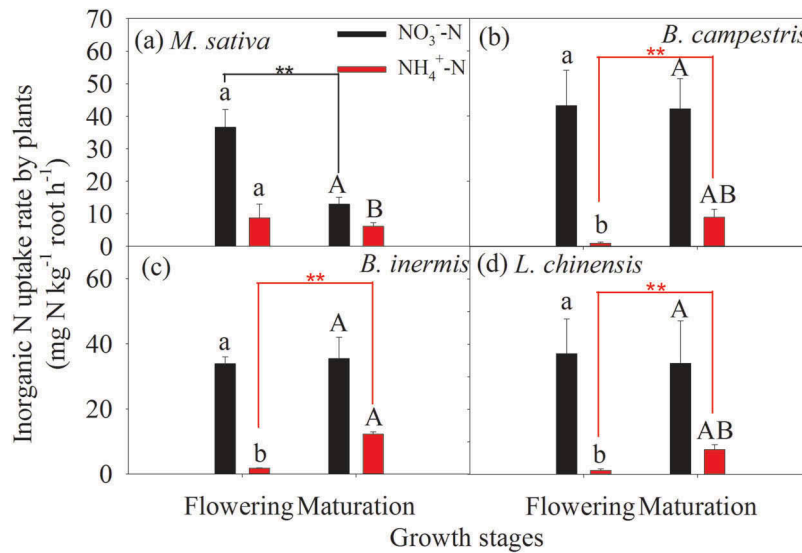


Figure 5. Plant inorganic N uptake rates in the four species cultivations during the flowering and maturation phases. Different lowercase and uppercase letters mean significant difference in plant NO_3^- -N or NH_4^+ -N uptake rates during the flowering and maturation periods, respectively. Two asterisks (**) mean a significant difference between the two growth stages at the level of 0.01 based on *t*-test.

rate by *M. sativa* between the two growth stages as well as the differences in the NH_4^+ -N uptake rate by the three non-legumes between the two growth stages were significant ($p < 0.01$, Figure 5).

3.5. Relationships between plant N uptake and driving factors

Except the *M. sativa* cultivation treatment, the plant NO_3^- uptake rates did not change obviously with the increase in shoot- and root biomass, and root/shoot ratios (Figure 6(a-c)). Except the annual *B. campestris*, the NH_4^+ uptake rates in the perennial forages significantly decreased with the increases in shoot- and root biomass, and root/shoot ratios, and the relationships could be well fitted by linear or exponential decay equations (Figure 6(d-f)). Also, the plant $\text{NH}_4^+/\text{NO}_3^-$ uptake ratios were positively correlated with soil $\text{NH}_4^+/\text{NO}_3^-$ ratios, and the latter could explain 47% of the former's variation (Figure 7).

4. Discussion

According to the content of SOC in surface soil layer (Table 1), we found that *M. Sativa* and *L. chinensis* cultivations were more conducive to maintain SOC content compared with *B. campestris* cultivation. Although the shoot biomass in the *B. campestris* cultivation treatment was the highest, its root biomass was the lowest either during the flowering phase or during the maturation phase (Figure 1). Considering that most of the aboveground biomass was usually removed before winter, the root biomass plays a dominant role in SOC pool dynamics (Ma et al. 2008). Also, the root/shoot ratios of the two monocots, *B. inermis* and *L. chinensis*, were greater than those of the two dicots (*M. Sativa* and *B. campestris*), indicating that the former has a higher return of plant residues than the latter.

The shoot N concentrations in the dicots especially legumes were significantly higher than those of the monocots. Generally, the content of Rubisco enzyme in dicots is higher than that of monocots (Sage and Seemann 1993), and N is the

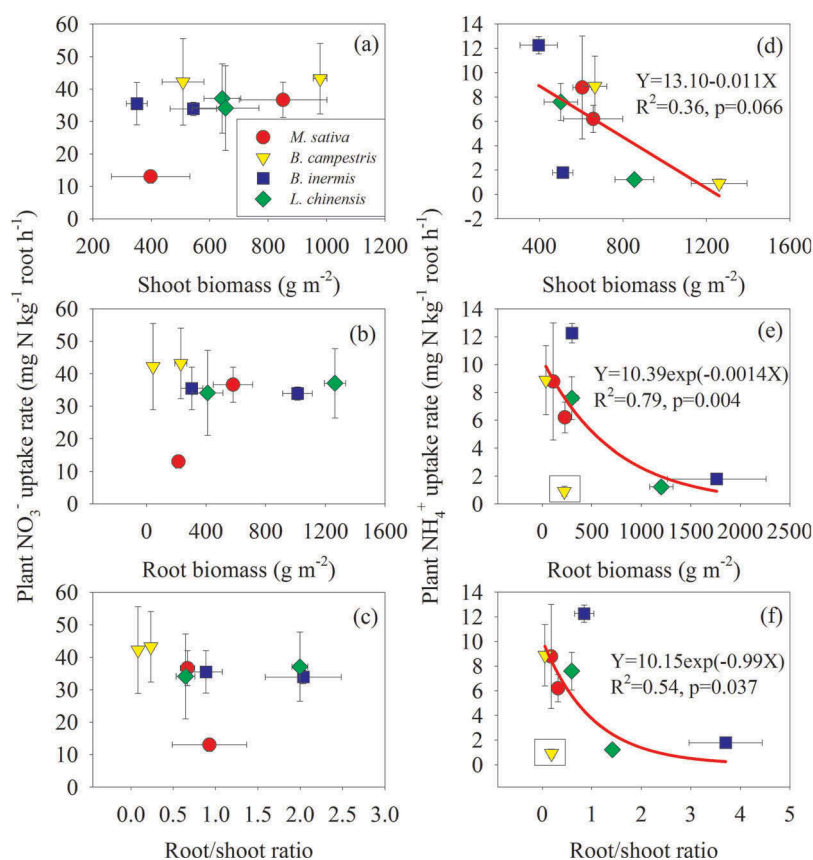


Figure 6. Relationships between plant inorganic N uptake rates and shoot-, root biomass, and root/shoot ratios. *B. campestris* is removed from the statistical analysis.

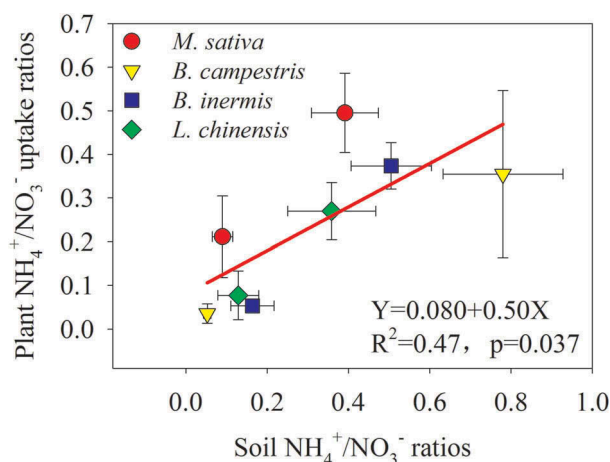


Figure 7. Relationships between plant $\text{NH}_4^+/\text{NO}_3^-$ uptake ratios and soil $\text{NH}_4^+/\text{NO}_3^-$ ratios.

major constituent element of this enzyme (Han et al. 2005). Also, *M. sativa*, a leguminous forage, requires less inorganic N from the soil because it can fix N_2 in the atmosphere through establishing a symbiosis with rhizobium and producing NH_3 (Cui et al. 2017). In this study, the contents of soil inorganic N in the dicot cultivations were significantly higher than those of monocot cultivations, attributed to higher N concentration of plant residues (Figure 3), and symbiotic N fixation (e.g., *M. sativa*), and higher soil microbial activity relative to the monocot cultivations. For instance, legumes

cultivation tends to promote soil nitrifying bacteria activity leading to an accumulation in soil NO_3^- -N (Ollivier et al. 2011). Based on the resource competition/ratio theory proposed by Tilman (1982), species with lower nutrient content are more suitable for growth in nutrient-limited areas in the long run. Plants with lower N and phosphorus (P) contents will become dominant species in N-limited or P-limited environments (Tilman 1997). Thus, the monocots with low N content are more competitive in growth and development relative to the dicots with high N content (Sewell 2004), which should be fully considered in plant-mixed farming. The N concentration in plant tissue, such as leaves and petioles, strongly depends on N availability (Subedi and Ma 2005). Overall, tissue N concentrations can be used to determine critical and optimal N concentrations in specific plant parts at defined periods of growth. Timing and rates of fertilizer N applications can be optimized based on critical N values to increase N use efficiency and prevent N losses.

Plant N uptake preference largely depends on the most abundant form of N in the soil, and soil $\text{NH}_4^+/\text{NO}_3^-$ ratio is a pivotal proxy (Cui et al. 2017). In NH_4^+ -enriched forest soil, plants preferentially absorb soil NH_4^+ -N, and foliar $\delta^{15}\text{N}$ is positively correlated with $\delta^{15}\text{N}$ - NH_4^+ in soil (Cheng et al. 2010). In contrast, the four forage crops, *M. sativa*, *B. inermis*, *L. chinensis*, and *B. campestris*, preferred to absorb more NO_3^- -N than NH_4^+ -N regardless of plant functional types and growth stages, which confirmed our first hypothesis. In the temperate-cultivated grassland, soil NO_3^- -N concentration accounted for 59% to 94% of total inorganic N concentration

in different species cultivation and growth stages (Figure 3). Semi-arid climate and artificial grassland management (e.g., tillage, irrigation, and fertilization, etc.) could be responsible for soil NO_3^- -N accumulation (Sapek, Estavillo, and Corre 2000). Similarly, in the alpine cultivated grassland where the soil $\text{NH}_4^+/\text{NO}_3^-$ ratio less than 1, Cui et al. (2017) documented that both legumes (alfalfa) and grasses preferred NO_3^- -N to NH_4^+ -N during their growth stages. Here we observed that the plant $\text{NH}_4^+/\text{NO}_3^-$ uptake ratios were positively correlated with soil $\text{NH}_4^+/\text{NO}_3^-$ ratios (Figure 7), which was partly supported our second hypothesis. Beside soil $\text{NH}_4^+/\text{NO}_3^-$ ratio, the preferential uptake of soil NO_3^- -N by the four temperate forage crops was attributed to other aspects. For instance, NO_3^- -N synergistically promotes the uptake of cations, such as K, Ca, and Mg, while NH_4^+ -N competes for the uptake with these cations (Sonneveld and Voogt 2009). Nitrates also limit the uptake of harmful elements, such as chloride, into large quantities (Cram 1973). Also, NO_3^- -N is the major anion taken up by most monocots in calcareous soil, and its uptake would contribute to antagonize Fe deficiency through elevating pH in the rhizosphere for the function of phytosiderophores (Abadía, et al. 2012). The conversion of NO_3^- and NH_4^+ into amino acids occurs in the leaves and roots, and they are fueled by solar energy and carbohydrates, respectively; the former is an energy-efficient process, whereas the latter are at the expense of other plant life processes, such as plant growth and fruit fill (Sonneveld and Voogt 2009). Forage preferential absorption of NO_3^- -N suggests that soil NO_3^- -N accumulation resulting from exogenous N inputs or accelerated soil nitrification could have a promotion on vegetation productivity in the cultivated grassland.

From the flowering phase to the maturation phase, the uptake rate of NO_3^- -N in the leguminous *M. sativa* decreased while the uptake rate of NH_4^+ -N in the other non-legumes significantly increased (Figure 5). This suggests that plant N uptake has a significant difference in different growth stages, and N in the plant tissues will be moved or reallocated during the senescence. The cumulative N uptake curve of annual crops from planting to maturity generally follows an S-shaped (sigmoid) curve (Martins et al. 2017). Little N is taken up during germination and seedling development. Nitrogen uptake is high and reaches a maximum during vegetative growth as the root system expands and leaves and stems develop. During the reproductive phase, the uptake pattern flattens off as N is moved or reallocated from the vegetative components to reproductive structures, such as seeds, fruits or tubers (Lemaire and Gastal 1997; Lemaire et al. 2008). Although the four forage crops in the temperate cultivated grassland prefer to absorb NO_3^- -N, the uptake rate of non-legumes did not variate obviously with the increase in shoot- and root biomass, indicating that soil NO_3^- -N content in the temperate cultivated grassland is sufficient and redundant for non-legumes growth. When N supply is adequate, crop N uptake mainly depends on crop growth rate and is influenced by nutrient and water availability (Gastal and Lemaire 2002). In contrast, the NH_4^+ -N uptake rate of perennial forages significantly decreased with the increases in shoot- and root biomass as well as root/shoot ratios (Figure 6(d-f)), suggesting the negative effect of plant NH_4^+ -N uptake on biomass

accumulation. The accumulation of NH_4^+ -N in plant tissue cells can cause toxicity, which is not conducive to plant growth (Britto and Kronzucker 2002). Both NH_4^+ and NH_3 easily diffuse through the cellular membranes, which leads to the protons are being transported from thylakoids to stroma; as a consequence, increased pH in thylakoids and decreased pH in stroma are toxic to plant photosynthesis (Werdan, Heldt, and Milovancev 1975). These results partly supported our second hypothesis that the N uptake preference could depend on plant adaptation to soil conditions.

5. Conclusions

Based on an *in situ* ^{15}N labeling experiment and auxiliary plant and soil measurements, we found that the shoot N concentration was higher in the dicots or legumes than in the monocots or non-legumes. The test forage crops in the cultivated grassland preferred NO_3^- -N to NH_4^+ -N regardless of plant functional type and growth stages. If the influences of other factors (e.g., genetic) are not considered, the rate of NH_4^+ -N uptake could largely determine the difference in the N concentration in various forage shoots. MBN is the highest in soils planted with leguminous alfalfa, suggesting that the potential N supply and retention capacity is the strongest. The plant N uptake preference largely depended on soil inorganic N composition as well as plant belowground C allocation. Our results reveal to a certain extent the mechanisms of N acquisition in the representative forages, which contributes to the management of fertilization and mix farming in the temperate artificial grassland.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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