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# Managed grassland alters soil N dynamics and $N_2O$ emissions in temperate steppe

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ABSTRACT

The reclamation of degraded grasslands as managed grasslands or even croplands has been increasingly accelerated in recent years in China. It is well known that land use change affects soil nitrogen (N) dynamics and nitrous oxide ($N_2O$) emissions. However, it remains unclear how large-scale grassland reclamation will impact the grassland ecosystem as a whole. Here, we invested the effects of the conversion from native to managed grasslands on soil N dynamics and $N_2O$ emissions by field experiments in Hulunber in northern China. Soil (0–10 cm), nitrate ($NO_3^-$), ammonium ($NH_4^+$), and microbial N were measured in plots in a temperate steppe (*Leymus chinensis* grassland) and two managed grasslands (*Medicago sativa* and *Bromus inermis* grasslands) in 2011 and 2012. The results showed that conversion of *L. chinensis* grassland to *M. sativa* or *B. inermis* grasslands decreased concentrations of $NO_3^-$–N, but did not change $NH_4^+$–N. Soil microbial biomass N was slightly decreased by the conversion of *L. chinensis* grassland to *M. sativa*, but substantially increased by the conversion to *B. inermis*. The conversion of *L. chinensis* grassland to *M. sativa* (i.e., a legume grass) increased $N_2O$ emissions by 26.2%, while the conversion to the *B. inermis* (i.e., a non-legume grass) reduced $N_2O$ emissions by 33.1%. The results also showed that conversion from native to managed grasslands caused large created variations in soil $NO_3^-$–N and $NH_4^+$–N concentrations. Net N mineralization rates did not change significantly in association with changes of growing season and vegetation type, while variations in net nitrification rate were significantly associated with changes in growing season. These results produce fresh, quantitative evidence on how grassland reclamation may impact the grassland ecosystem in terms of N dynamics and $N_2O$ emissions, in persuasion of grassland protection and restoration.

## Introduction

Nitrogen (N) is an important limiting nutrient for net primary productivity in most terrestrial ecosystems (LeBauer and Treseder, 2008; Xu, 2003). Various diverse chemical forms of soil N can be utilized by plants and microbes (Harrison et al., 2008; Kaštovská and Šantrůčková, 2011). For example, common forms of inorganic N, such as ammonium ($NH_4^+$) and nitrate

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($NO_3^-$), are important N sources for plants, although organic N in the form of free intact amino acids also can be taken up by plants (Jones and Kielland, 2002). Soil inorganic N is mainly derived from mineralization of organic matter by microbial activities in natural ecosystems (Wang et al., 2005). Moreover, nitrous oxide ($N_2O$) is released during N transformation, primarily *via* processes of denitrification and nitrification (Skiba and Smith, 2000; Lan et al., 2014). $N_2O$ is reported a potent greenhouse gas with a global warming potential 298 times that of carbon dioxide (IPCC, 2013) and the largest ozone depleter in the atmosphere (Reeves and Wang, 2015). Since 1750, the atmospheric $N_2O$ concentration has increased by about 120%, from around 270 ppb, to 324 ppb in 2011 (Wang et al., 2014).

The absorption and conversion of nitrogen are dependent on different soil nitrogen conversion processes. In nitrification, $NH_4^+$ is converted to nitrite ($NO_2^-$) and then to nitrate ($NO_3^-$), while in denitrification, $NO_3^-$ is gradually reduced to $NO_2^-$ and further to nitrogen gases (NO, $N_2O$, and $N_2$) (Wu et al., 2013). In soil, both nitrification and denitrification are performed by microorganisms (Liang et al., 2014). Land use change primarily affects plant production, species diversity and the quality of organic or nutrient inputs into the soil due to changes in plant cover (Raiesi and Beheshti, 2014; Zhao et al., 2007). For example, mowing of grassland was reported to increase species richness and community stability of herbaceous plants (Collins et al., 1998; Yang et al., 2012), and decrease the amount of available N in soil.

Not only management practices, but also soil physical, chemical and biological conditions regulate N transformation (Knoepp and Swank, 2002). Soil pH, moisture content and texture are among the most important factors regulating $N_2O$ emissions (Pihlatie et al., 2004; Mørkved et al., 2007; Wang et al., 2013). $N_2O$ emissions increased with increasing water filled pore space (WFPS), the maximal $N_2O$ emissions were measured between 80% and 95% WFPS. (Schindlbacher et al., 2004; Qi et al., 2014).

Grasslands occupy 40% of the total land mass of China (Chen et al., 2014). Natural grasslands in China are experiencing degradation of different degrees (Li, 1997; Ye and Van Ranst, 2009). The productivity of managed grassland is several times higher than that of natural grassland. As such, substantial amount of degraded grasslands have been converted into managed grasslands and even croplands in recent years (Liu et al., 2009) in order to achieve a more stable supply of higher quality forages. The area of managed grasslands expanded from $9.6 \times 10^4$ km$^2$ in 2001 to $12.8 \times 10^4$ km$^2$ in 2014, a 33% jump in a period slightly longer than 10 years (National Animal Husbandry, 2013). Numerous studies have been conducted to investigate how land use change could affect the N cycle in temperate grasslands (Pan et al., 2016). Conversion of native to managed grasslands or croplands accelerates soil N mineralization (Li and Chen, 1998; Booth et al., 2005), resulting in a 8.8% reduction of total soil N in the top 1 m layer of soil (Li et al., 2005). Whether $N_2O$ emissions decrease nor increase after conversion is a fundamental question being hotly debated (Wen et al., 2005). What is clearer to date is that N losses from grasslands are closely related to N availability and microbial activity and thereby inherently affected by management practices such as fertilization, harvesting, and tillage (Geng et al., 2001; Ye et al., 2008).

In the Hulunber region, managed grasslands of legumes (*Medicago sativa*) or non-legumes (*Bromus inermis*) are common land use types to substitute the native temperate grasslands (*Leymus chinensis*). Both substitute grassland types are high productive of high quality forages. However, efforts to quantitatively investigate the impacts of grassland conversion on soil N dynamics and $N_2O$ emissions are still lacking. Therefore, we conducted field experiments to assess the influences of grassland conversion on soil N dynamics and $N_2O$ emissions in temperate grassland ecosystems in Inner Mongolia, China. We aimed to test the following hypotheses: (Attard et al., 2016) land use change from native to managed grasslands promotes net N mineralization; (Bernard et al., 2007) conversion to the legume *M. sativa* increases $N_2O$ emissions because biological N fixation provides more $NH_4^+$; and (Bedard-Haughn et al., 2006) soil moisture has stronger impacts on $N_2O$ emissions than temperature in this semiarid region.

## 1. Materials and methods

### 1.1. Research site

This study was conducted at the Hulunber Grassland Ecosystem Observation and Research Station (49°19′35″N, 119°56′52″) in north-eastern Inner Mongolia in China. The frost-free period is 110 days, and the average annual precipitation was totaled at 320 mm during the year of 2000–2010, 80% of which fell between July and September. Monthly mean temperature varied between the maximum of 36.2°C in July and the minimum of −48.5°C in January. Chestnut soil is the main soil type (Table 1).

Experiments were conducted on two managed grasslands (*M. sativa* and *B. inermis*) and a native grassland (*L. chinensis*). The plots were arranged in a split-plot design with land use as the main treatment. Each 10 × 10 m plot was consisted of

**Table 1 – Site characteristics and soil properties for the different grassland sites evaluated during the growing season.**

| Grassland type | *M. sativa* | *B. inermis* | *L. chinensis* |
|---|---|---|---|
| pH value | 6.96 ± 0.68a | 6.88 ± 1.05a | 6.72 ± 0.98a |
| Alkali hydrolyzable nitrogen (mg/kg) | 135.57 ± 5.79a | 124.37 ± 8.36b | 125.42 ± 5.55b |
| Available potassium (mg/kg) | 307.88 ± 21.87a | 205.81 ± 67.97c | 235.49 ± 34.45b |
| Total nitrogen (g/kg) | 2.40 ± 0.67a | 2.29 ± 0.24a | 2.31 ± 0.15a |
| Soil organic carbon (g/kg) | 16.17 ± 3.01a | 15.83 ± 2.65b | 16.02 ± 2.76b |
| Texture | Sandy soil | Sandy soil | Sandy soil |
| Soil depth (cm) | 10 | 10 | 10 |
| Average aboveground biomass (kg/ha) | 5843.36 ± 250.17a | 6044.91 ± 309.05a | 3044.85 ± 167.16b |

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three subplots of 15 $m^2$. The two managed grasslands were established by converting the original temperate steppe (*L. chinensis*) to *M. sativa* or *B. inermis* through re-seeding in 2009. Samples were collected from May to September on the third and fourth year after planting. The seeding amounts of the *M. sativa* and *B. inermis* grasslands were 15.0 and 22.5 kg/ha, respectively. The plowing depth was 2 cm in both grasslands. Before sowing, 2,4-D butyl ester was applied as subsurface treatment to clear weeds. No fertilizer was applied.

### 1.2. Meteorological data

Meteorological data was collected by an automatic meteo-station, located 100 m from the experimental plots.

### 1.3. Net N mineralization

Many researches revealed that for most of the arid and semiarid grasslands, $N_2O$ was predominantly produced by microbial nitrification (Cookson et al., 2006; Xu et al., 2008; Zhong et al., 2014). Heterotrophic nitrification reported to be the dominant process of $N_2O$ production in Inner Mongolia steppe, while the contribution of denitrification seemed not significant (Du et al., 2001; Du, 2006). Soil N mineralization and nitrification rates were measured using *in situ* incubations in May 2011. At the start of each incubation period, soil cores were taken in pairs using PVC tubes of 10 cm in height and 5 cm in diameter from three randomly chosen positions in each plot. The litter layer was removed before sample was taken. One of each pair of cores (initial sample) was removed and sent to the laboratory in an icebox to determine the initial soil ammonium ($NH_4^+$–N) and nitrate ($NO_3^-$–N) concentrations. The second core (incubation sample) was wrapped with low-density polyethene on the top and with gauze under the bottom allowing gas movement but preventing leaching. The core was then returned to its original position. During the incubation period, the samples were collected every 30 days from June to September in 2011 and 2012. The amount of mineral N accumulated in the sample was determined. Within each core, changes in inorganic N content during the incubation period represent the net N mineralized from the organic sources.

All collected soil cores were kept cool before reaching the laboratory and stored in a refrigerator at 4°C in laboratory until analyzed, usually within 36 hr. In the laboratory, the PVC tubes were removed and each soil core was well mixed by hand, excluding roots and stones. A 5 g sub-sample was extracted in 20 mL of 2 mol/L KCl for 1 hr, filtered through Whatman 42 filter paper and centrifugal oscillated for 30 min. The $NH_4^+$–N and $NO_3^-$–N contents were then analyzed with an ultraviolet spectrophotometer (752PC, Shanghai, China).

The inorganic N concentrations were expressed on a dry weight basis. To obtain oven-dry weight, a 20 g sub-sample was placed in a 105°C oven for >12 hr.

Rates of net N mineralization and nitrification were calculated using the following equations:

$$N_{min} = \left([NH_4^+] + [NO_3^-]\right)_j - \left([NH_4^+] + [NO_3^-]\right)_i / t_{(j-i)}$$

where $N_{min}$ is the net N mineralization rate(µg N dw (dry weight)/(g·day)), $[NH_4^+]$ is the $NH_4^+$ concentration (µg N dw/g), $[NO_3^-]$ is the $NO_3^-$ concentration (µg N dw/g), *j* is the soil incubation *j* days, *i* is the soil incubation *j* days, and $t_{(j-i)}$ is the soil incubation from *i*th to *j*th day. The net N nitrification rate ($N_{nit}$, µg N dw/(g·day)) is given by:

$$N_{nit} = \left([NO_3^-]_j - [NO_3^-]_i\right) / t_{(j-i)}.$$

### 1.4. Microbial biomass N (MBN)

Fresh soil samples were collected every month during growing season from June to September in 2011 and 2012 using an earth-boring auger. Three points were randomly selected in each plot, and three soil samples were taken at 0–10 cm depth. Soil samples were put into plastic bags, and immediately brought to the laboratory for measurements of MBN and soil moisture content.

The MBN was determined by the chloroform fumigation direct extraction method. Paired samples of moist soil of 10 g in weight were placed in 120 mL glass cups. One sample of the pair was extracted with 50 mL of 0.5 mol/L $K_2SO_4$ by shaking for 1 hr, and the resulting suspension was filtered. The second sample was fumigated with ethanol-free chloroform for 24 hr in the dark at 22°C. The chloroform was then removed and the sample was extracted with 0.5 mol/L $K_2SO_4$ as described above. The MBN was calculated as the difference in dissolved organic N between the fumigated and non-fumigated extracts using the following equation:

$$MBN = \left(N_{fumigated} - N_{non\text{-}fumigated}\right) / 0.45$$

where $N_{fumigated}$ is the total dissolved organic N extracted after fumigated with chloroform for 24 hr, and $N_{non\text{-}fumigated}$ is the total dissolved organic N directly extracted without fumigation.

### 1.5. Soil properties

Bulk density was calculated as the quotient between the dry weight and the core volume. Soil pH was measured using a pH probe (SG2, Beijing, China) in 1:2 soil water solution of 10 g dry soil in 20 mL water. Total N content was measured by dry combustion on a LECO CNS-1000 elemental analyzer (LECO Corporation, St. Joseph, MI, USA) (Du and Gao, 2006).

### 1.6. Measurement of $N_2O$ emissions

Nitrous oxide emissions were measured using the dark static closed chamber method from June to September in 2011 and 2012, respectively. The chamber volume was 0.2 $m^3$ (0.5 m × 0.5 m × 0.8 m). The chamber collars were installed into the soil in June 2011 to a soil depth of 10 cm. The $N_2O$ emissions were typically measured at local time from 9:00 to 10:00 (demonstrated greenhouse gas fluxes measured during this time period are representative of the daily mean flux) on each sampling day and 1–2 times per week during the growing season (*i.e.*, June through September) (Xu et al., 2015). Gas samples were taken at 0, 10, 20, 30 and 40 min after chamber installation using 50 mL polypropylene syringes equipped with three-way stopcocks. After sampling, $N_2O$ flux was analyzed

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using a gas chromatograph (Chemical Engineering Department Dalian bright chemical industry research Institute, Dalian, China) equipped with a $^{63}N_i$ electron capture detector (ECD) operated at 300°C. The fluxes were calculated based on the assumption of linear concentration increase inside of a chamber to minimize the negative effect of chamber closure on $N_2O$ production (Wang et al., 2014). Prior report showed that the increase in $N_2O$ concentrations remained linear for up to 2 hr following chamber closure, the coefficients of determination ($R^2$) of the linear regression were greater than 0.98 ($p < 0.001$) (Zhang and Wang, 2008). Q15

The $N_2O$ emissions were calculated as the slope of the linear regression of the $N_2O$ concentration change over time.

The $N_2O$ emissions inside the chambers were estimated using the following equation:

$$F = [M \times V \times (C2 \times 273 \times P2)/(P0 \times T2) - C1 \times 273 \times P1/(P0 \times T1)]/A \times (T2 - T1) \times 22.4$$

where, $F$ (μg $N_2O$–N/(m$^2$·day)) is the $N_2O$ flux; $M$ (g/mol) is the molecular weight of $N_2O$–N; $V$ (0.2 m$^3$) is the volume of the chamber; $A$ (0.25 m$^2$) is the area from which $N_2O$ was emitted into the chamber; $T1$ and $T2$ (day) are the sampling and end times, respectively; $C1$ and $C2$ are the concentrations of $N_2O$ (cm$^3$/m$^3$) at the beginning and end of gas collection, respectively; $T1$ and $T2$ are the soil absolute temperatures (K) at the beginning and end of gas collection, respectively; $P1$ and $P2$ are the atmospheric pressures inside the chamber (Pa) at the beginning and end of gas collection, respectively; and $P0$ is the standard atmospheric pressure at 273 K (Pa). We assumed that $P2 = P1 = P0$.

The cumulative $N_2O$ fluxes were calculated by interpolating the $N_2O$ fluxes that were measured during the sampling periods (Dong et al., 2000; Peng et al., 2011). Q16 Q17

### 1.7. Statistical analyses

Correlation analysis was used to evaluate the relationships between $N_2O$ emissions and temperature, soil moisture content, $NO_3^-$–N, $NH_4^+$–N, MBN, N mineralization rate and nitrification rate. Models were fit after data were tested for normality. Analyses were conducted using SAS V9.2 (SAS Institute Inc., Cary, NC, USA). For multiple linear regression analysis, stepwise variable selection was used in most cases. Principal component analysis (PCA) was used to determine the factors influencing $N_2O$ emissions, and analysis of variance was used to analyze the differences in $N_2O$ emissions between species and months of the year.

## 2. Results

### 2.1. Meteorological conditions

The average temperature of experimental site of 2011 and 2012 was −1.60 and −1.86°C, respectively, lower than the average annual temperature of 0.25°C between 2000 and 2010. Precipitation in 2011 was 317.5 mm and lower than the average precipitation of 323 mm during, while precipitation in 2012 (*i.e.*, 320 mm) was similar to the average level. Fig. 1 shows the average daily temperature and precipitation in 2011 and 2012. During the field observation period from June to September in each year, the daily average temperatures were between 20 and

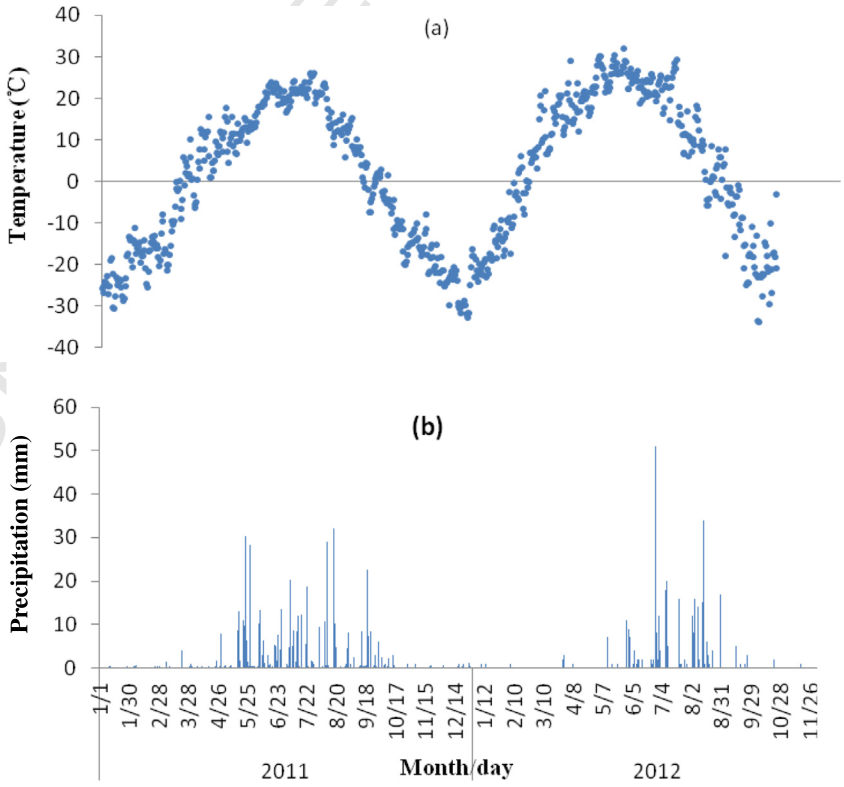

**Fig. 1 – Seasonal changes of air temperature and precipitation in the three different types of grassland.**

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30°C and the total precipitation accounted for about 70% of the total annual precipitation.

### 2.2. Dynamics of $NH_4^+$–N, $NO_3^-$–N and MBN

The $NO_3^-$–N concentrations were higher and more varied between the three grassland types in 2011 than in 2012. In 2011, the $NO_3^-$–N concentrations in the managed grasslands were 19.0%–21.4% lower than those in the native grassland (31.04 ± 6.74 μg N dw/g) (Fig. 2a). The $NH_4^+$–N concentrations in the native grassland (22.63 ± 4.38 μg N dw/g) were 1.07 and 1.10 times higher than those in the *M. sativa* grassland and *B. inermis* grassland, respectively. In 2011, the $NO_3^-$–N concentrations in the native grassland (13.91 ± 4.8 μg N dw/g) were 0.90 and 1.76 times higher than those in the *M. sativa* grassland and *B. inermis* grassland, respectively during the growth season. In 2012, the $NH_4^+$–N concentrations in the native grassland (12.31 ± 2.86 μg N dw/g) were slightly lower than those in the managed grassland (Fig. 2b).

The ratios of $NH_4^+$–N to $NO_3^-$–N in the three grassland types were only higher than 1 in August and October, 2011. The $NH_4^+$–N/$NO_3^-$–N ratios ranged between 0.26 and 2.44 for the native *L. chinensis* grassland, between 0.12 and 3.24 for *M. sativa*, and between 0.34 and 16.61 for *B. inermis*. In June, July and September, $NO_3^-$ dominated the inorganic N and accounted for 64.4%–82.3% of total inorganic N. In August and October, $NH_4^+$–N dominated the inorganic N and accounted for 56.4%–79.2% of total inorganic N in 2011. From July to September in 2012, $NH_4^+$–N accounted for 56.1%–94.3% of the total inorganic N. There was a significant correlation between the $NO_3^-$–N concentrations and the vegetation type or the growing season ($p < 0.05$), but as for $NH_4^+$–N, the only significant correlation was found with the growing season ($p < 0.05$) (Table 2).

The MBN values greatly varied across the three grassland types during the whole growing season ($p < 0.05$) (Fig. 2c, Table 2). The MBN values were much higher in 2012 than 2011. The MBN values in the *M. sativa* grassland were 17.01%–41.57% higher than those in the native grassland (66.38 ± 1.01 and

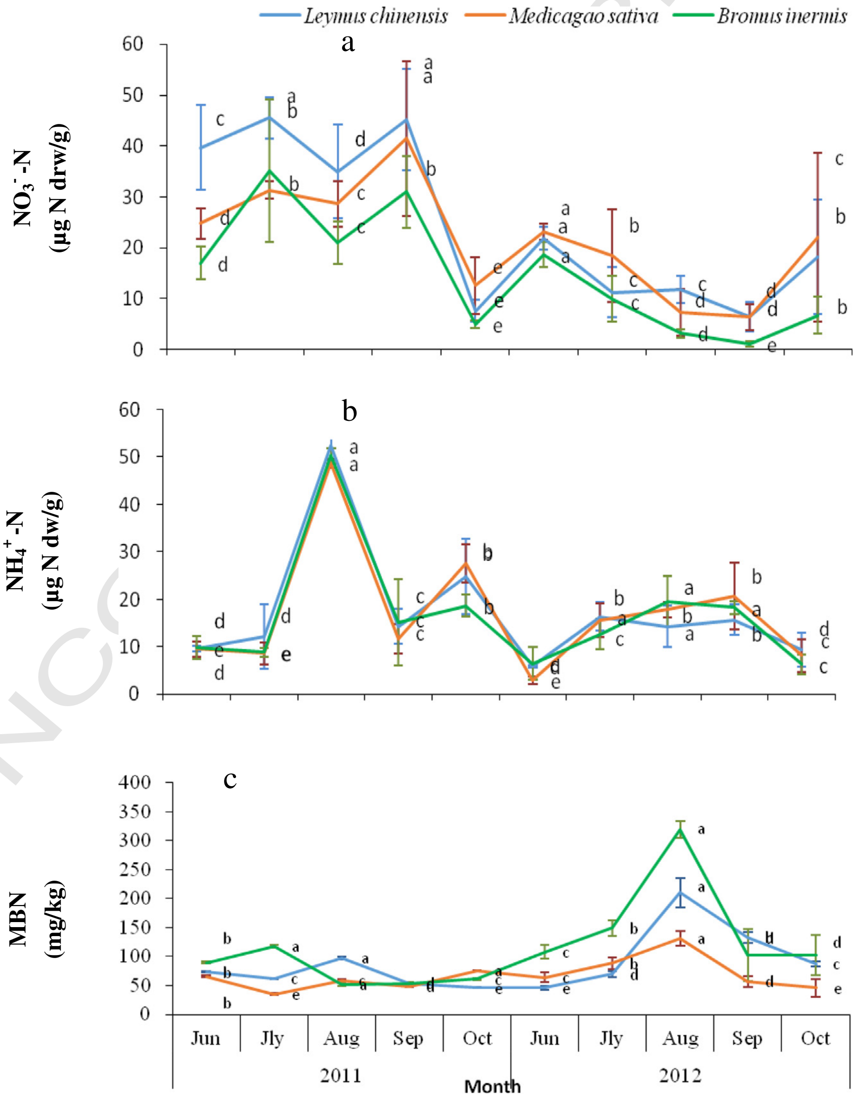

**Fig. 2 – Seasonal dynamics of (a) soil nitrate ($NO_3^-$–N), (b) soil ammonium ($NH_4^+$–N), and (c) soil microbial biomass nitrogen (MBN). The values indicate average ± standard error of three replicates. Different lowercase letters indicate significant differences between grassland types for each month ($p < 0.05$).**

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**Table 2 – Analysis of variance analysis between grassland types and growing season and their interaction.**

| Index | Source | DF | Mean square | *F* value | *p* |
|---|---|---|---|---|---|
| $NO_3^-$ | Growing season | 4 | 394.84 | 9.80 | **<0.0001** |
| | Type | 2 | 238.99 | 5.93 | **0.007** |
| | Growing season × Type | 8 | 28.94 | 0.72 | 0.674 |
| $NH_4^+$ | Growing season | 4 | 287.54 | 38.67 | **<0.0001** |
| | Type | 2 | 4.67 | 0.63 | 0.54 |
| | Growing season × Type | 8 | 14.76 | 1.98 | 0.08 |
| MBN | Growing season | 4 | 32,897.91 | 2982.75 | **<0.0001** |
| | Type | 2 | 23,702.63 | 2149.05 | **<0.0001** |
| | Growing season × Type | 8 | 4571.87 | 414.52 | **<0.0001** |
| $N_2O$ | Growing season | 3 | 0.00029 | 0.83 | 0.49 |
| | Type | 2 | 0.00075 | 2.14 | 0.14 |
| | Growing season × Type | 6 | 0.0007 | 1.99 | 0.11 |
| $N_{min}$ | Growing season | 4 | 0.05 | 0.80 | 0.53 |
| | Type | 2 | 0.09 | 1.38 | 0.27 |
| | Growing season × Type | 8 | 0.12 | 1.82 | 0.11 |
| $N_{nit}$ | Growing season | 4 | 1.79 | 7.24 | 0.09 |
| | Type | 2 | 0.95 | 3.85 | 0.14 |
| | Growing season × Type | 8 | 0.56 | 2.28 | **0.001** |

DF means degrees of freedom, microbial biomass nitrogen, and soil moisture.
Numbers in bold indicate significant difference at $p < 0.05$.
MBN: microbial biomass nitrogen.

109.15 ± 9.59 mg/g in 2011 and 2012, respectively). In contrast, the MBN values in the *B. inermis* grassland were 11.15%–30.09% lower than those in the native grassland (Fig. 2c).

### 2.3. Net mineralization and nitrification

Net mineralization rates varied over the growing season from June to October in each year, and the overall patterns of change were similar in both years (Fig. 3). During the period from June to August, net mineralization rates increased from −0.93 ± 0.04 to 0.99 ± 0.15 μg N dw/(g·day) in *L. chinensis* grassland, from −0.51 ± 0.07 to 0.74 ± 0.11 μg N dw/(g·day) in *M. sativa* grassland, and from −0.84 ± 0.1 to 0.91 ± 0.23 μg N dw/(g·day) in *B. inermis* grassland in 2011 (Fig. 3a). The majority of the positive and negative peaks occurred in August and September in 2011. There was no significant correlation between net mineralization rate, vegetation type and plant growing season ($p > 0.05$). The net mineralization rates did not change significantly with the growing season ($p > 0.05$) (Table 2).

Compared with the net mineralization rates, the soil net nitrification rates were much lower in magnitude and smoother in variation over time. The net nitrification rate in *L. chinensis* grassland was only positive in July and September, 2011, and in June and October, 2012. The maximum net nitrification rate was 0.72 ± 0.60 μg N dw/(g·day) in October 2011. The net nitrification rates in *M. sativa* grassland were positive except in August and October, 2011 and in July and August, 2012. The maximum net nitrification rate in *M. sativa* grassland was 0.53 ± 0.48 μg N dw/(g·day) in October 2012. The maximum net nitrification rates in *B. inermis* grassland were positive only in July and September, 2011 and June and October, 2012, with negative values for all other periods. The maximum net nitrification rate in *B. inermis* grassland was 0.43 ± 0.36 μg N dw/(g·day) in June 2011. The peak net nitrification rates in *L. chinensis* grassland occurred in July 2011 and October, 2012, and the lowest rates were measured in October 2011 and July, 2012. The highest net nitrification rate in *M. sativa* grassland occurred in September 2011 and October 2012, the lowest rates occurred in October 2011 and July 2012. The highest net nitrification rate in *B. inermis* grassland was measured in July 2011 and October 2012, and the lowest rates occurred in October 2011 and July 2012 (Fig. 3b). The positive values of net nitrification rate indicated that $NO_3^-$–N produced by soil nitrification remained as residual $NO_3$ in soil after immobilization by microbes. There was a significant correlation ($p < 0.05$) between net nitrification rate, vegetation type, and growing season (Table 2).

### 2.4. Nitrous oxide emissions

$N_2O$ emissions varied greatly across treatments. The average $N_2O$ emissions showed similar patterns across the three types of grasslands with the highest emissions observed during late June and July and lower values thereafter (Fig. 4). The $N_2O$ internal fluctuation was clear in the *L. chinensis* grassland. The seasonal pattern of $N_2O$ emissions in the *M. sativa* grassland was similar to that in the *L. chinensis* grassland, but with significantly lower ($p < 0.05$) emissions rates in 2011 (85.4 ± 13.12 to 1746.3 ± 35.47 μg $N_2O$–N/($m^2$·day)). The $N_2O$ emissions showed a net positive flux throughout the growing season (272.6 ± 39.46–3931.8 ± 101.37 μg $N_2O$–N/($m^2$·day)), exhibiting relatively large fluctuations in the early stage of the wet summer and autumn in 2012. Emissions from *B. inermis* grassland were relatively low in 2011, but fluctuated greatly in early growing season in 2012 (Fig. 4). The flux rates ranged between 129.7 ± 30.06 and 2847.4 ± 291.08 μg $N_2O$–N/($m^2$·day) during the whole season.

Overall, the average rates of $N_2O$ emissions were different between grassland types from 2011 to 2012, decreasing in the order *M. sativa* (1038.7 ± 89.19 μg $N_2O$–N/($m^2$·day)) > *L. chinensis* (823.3 ± 46.35 μg $N_2O$–N/($m^2$·day)) > *B. inermis* (695.0 ± 78.29 μg $N_2O$–N/($m^2$·day)). Compared with the *L. chinensis* grassland, the average $N_2O$ emissions of the *M. sativa* grassland were 26.2% higher, while emissions of the *B. inermis* grassland were

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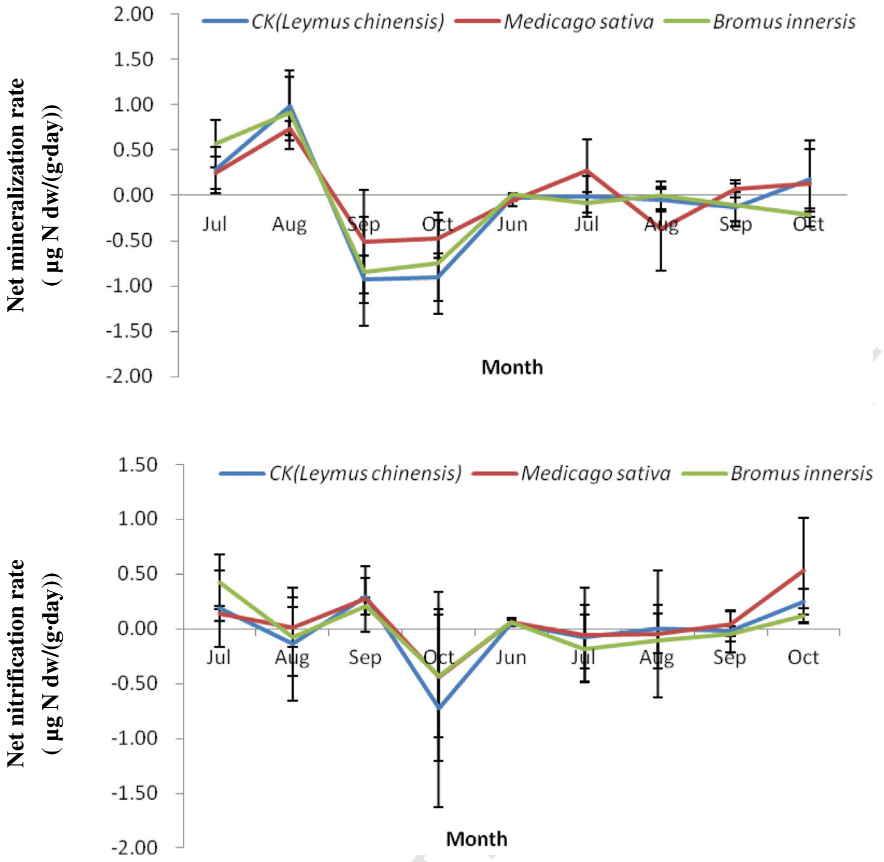

**Fig. 3 – Seasonal dynamics of net mineralization rate and nitrification rate in different grassland types. The values are average ± standard error of three replicates.**

33.1% lower. However, differences in $N_2O$ emissions between growing seasons and vegetation types were found insignificant ($p > 0.05$; Table 2).

### 2.5. *Correlation analysis between $N_2O$ emissions and environmental factors*

The influence of environmental factors on $N_2O$ emissions was tested using correlation and PCA analyses. Correlations between $N_2O$ and $NO_3^-$–N or nitrification rate ($N_{nin}$), temperature were found positively significant ($p < 0.01$) (Table 3, Fig. 5). Positive correlations were also found between $N_2O$ and MBN, net mineralization rate or net nitrification rate ($p < 0.05$). There was a highly significant negative correlation between $N_2O$ and $NH_4^+$–N ($p < 0.01$). However, no correlation was found between $N_2O$ and precipitation or soil moisture ($p > 0.05$). The PCA results (Fig. 5) indicated that $NO_3^-$–N, MBN, and $N_{nit}$ were the main factors influencing $N_2O$ emissions. Among them, $NO_3^-$–N, $N_{nin}$ or MBN was the most significant, followed by $NH_4^+$–N, $N_{min}$ and temperature.

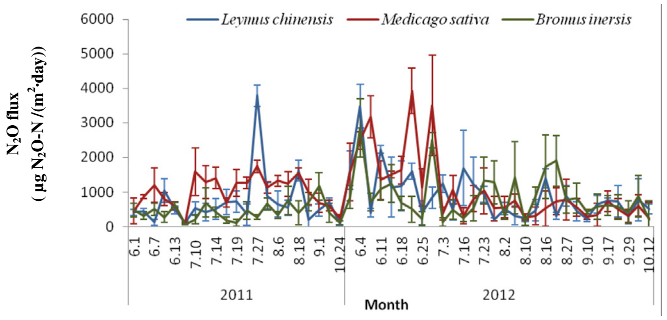

**Fig. 4 – Seasonal dynamics of $N_2O$ emissions in different grassland types. The values are average ± standard error of three replicates.**

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**Table 3 – Multiple linear regression analysis between $N_2O$ emission and $NH_4^+$, $NO_3^-$, $N_{nin}$, air temperature, microbial biomass nitrogen, and soil moisture.**

| Species | Multiple linear regression analysis | $R^2$ | *p* value |
|---|---|---|---|
| *L. chinensis* | $N_2O$ emission = (6.23 ± 1.05) + (20.5 ± 2.45) × $[NO_3^-]$ + (4.67 ± 0.35) × $N_{nin}$ − (9.68 ± 1.25) × Moisture | 0.47 | 0.02 |
| *M. sativa* | $N_2O$ emission = (−22.47 ± 9.8) + (3.1 ± 0.6) × $[NO_3^-]$ + (5.78 ± 0.22) × $N_{nin}$ + (38.7 ± 13.6) × $N_{nit}$ | 0.40 | 0.03 |
| *B. inermis* | $N_2O$ emission = (5.65 ± 3.6) + (6.3 ± 1.4) × $[NO_3^-]$ + +(6.12 ± 0.27) × $N_{nin}$ + (19.6 ± 7.9) × $N_{min}$ | 0.55 | 0.04 |

## 3. Discussion

### 3.1. Effects of land use change on soil N dynamics

Anthropogenic factors play a major role in atmospheric N deposition into natural soil systems, while human activities exert both intentional and unintentional alterations (Houlton et al., 2013). From a global budget perspective, agricultural emissions of NOx or $NH_3$ comprise a large-scale recycling, representing a new N input to downwind ecosystems (Houlton and Morford, 2015; Zhou et al., 2016). Land use change often alters soil physical properties such as bulk density and porosity, which affect soil N mineralization *via* effects on microbial activities (Gardner and Drinkwater, 2009; Meagan and Laurie, 2012). In the current study, mineralization was increased by land use change (Fig. 2), which causes significant changes in soil $NO_3^-$–N and $NH_4^+$–N concentrations. This may be due to increased aeration and porosity in managed soils following plowing (Tibisay et al., 2007). The conversion from native grasslands to managed grasslands may also increase the aboveground biomass (Table 2). Different ecosystems or species show differential preferences for nitrogen (Oliveira et al., 2016; Gerschlauer et al., 2016; Macduff et al., 1997; Attard et al., 2016). Legumes are considered a high quality forage due to their high protein content (Mortenson et al., 2004). More importantly, it is well known that legumes can increase N availability in soils by fixing atmospheric N in an available form (Spehn et al., 2002). Increases in soil total N and available N concentrations were observed when cropping systems involved more legume crops (Li et al., 2015). Changes in soil $NO_3^-$–N and $NH_4^+$–N were also reflected in plant N accumulation, suggesting responses to enhanced soil N mineralization (Bernard et al., 2007; Davies et al., 2001; Nevens and Reheul, 2002). In this paper, the higher $NO_3^-$–N concentrations observed in 2011 than in 2012 may be related to rainfall patterns; the earlier rainfall supplied sufficient water for soil in 2011, which promoted N transformations (Fig. 1). Booth et al. (2005) suggested that the temperature–nitrification relationship depends in part on the availability of $NH_4^+$.

Land use change influences nitrification and consequently $NO_3^-$–N production in grasslands (Fig. 2). Moreover, soil $NO_3^-$–N concentrations in native grasslands are higher than in managed grasslands, indicating that native grasslands have higher nitrification rates and release more $NO_3^-$–N than managed grasslands. Burton et al. (2007) reported that it is possible that the conversion from a mixed species forest to a single species forest has changed the quality of organic matter input and subsequently microbial population and diversity, which has ultimately resulted in higher nitrification rates in the native forest soils compared to the plantation soils, the truth could analogized to grassland appropriately. Moreover, the high microbial N demand in native grassland and consequently low substrate ($NH_4^+$) availability for nitrification and most of the $NO_3^-$–N produced was probably taken up by cultivated forages or

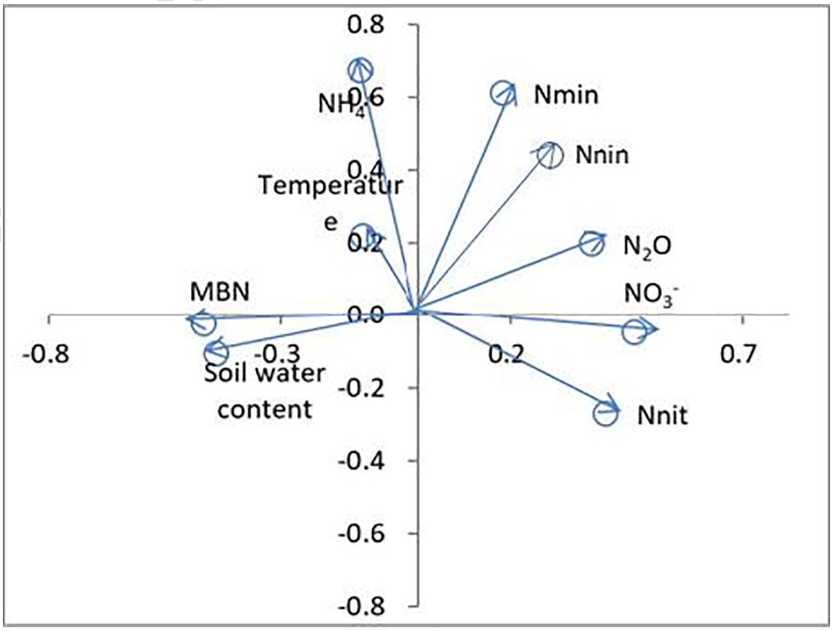

Fig. 5 – Principal component analysis between $N_2O$ emissions and their related impact factors.

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lost by high $NO_3^-$–N leaching may also contribute to low $NO_3^-$–N concentration in the managed soils (Xu and Xu, 2015). Meanwhile, we only collected 2 year data, we will continue to do the experiment to reveal the relative regular in further in the following days. Given the absence of plant uptake and leaching in the incubation cores, $NO_3^-$ immobilization and reduction were likely the dominant $NO_3^-$ consumption pathways. Nitrogen producing and consuming processes may be simultaneously activated by enzymes when the intracellular N content of associated microorganisms reaches critically low levels (Bedard-Haughn et al., 2006; Bengtson et al., 2005). At the early stages of litter decomposition, microbes immobilize N from the soil to facilitate their own growth, whereas at later stages of decomposition when the C:N ratio of the litter materials has substantially decreased, N is released to the soil (Cookson et al., 2007). Warming can stimulate net N mineralization under optimal moisture conditions and thereby leads to microbial growth and increased secretion of $NH_4^+$–N. We found that MBN content reached peak levels in August, perhaps as a result of this process (Fig. 2c). Because the soil columns were incubated under aerobic conditions, the relatively higher pH in native grassland soil than in managed grassland soil (Table 1) may have promoted nitrification in the native grassland. Such differences in nitrification activities between different land use types were also observed elsewhere, (*e.g.*, Sun et al., 2013).

### 3.2. Effects of land use change on $N_2O$ emissions

Previous research suggested that land use change may cause $N_2O$ emissions to increase (MacDonald et al., 2011; Velthof et al., 2010). In this paper, we found that the conversion to newly-planted *M. sativa* resulted in higher emissions, but the conversion to *B. inermis* resulted in lower $N_2O$ emissions than the native grasslands (Fig. 3). This confirms our second hypothesis that *M. sativa* grassland increases $N_2O$ emissions because biological N fixation promotes N inputs into the soil. Although increased $N_2O$ emissions are often attributed to soil disturbances and substrate incorporation by tillage, the difference between results obtained from *M. sativa* and *B. inermis* plots suggests that the effects of land use change on $N_2O$ emissions are more complex than previously stated.

When mineralization is increased by soil temperature, nitrification will also be increased, as long as no other $NH_4^+$-assimilating or consuming processes dominate (Bedard-Haughn et al., 2006). Chu and Grogan (2010) indicated that nitrification was not limited by $NH_4^+$ availability alone; however, we found no significant correlation between $N_2O$ and $NH_4^+$. The origin of $N_2O$ emissions from nitrification is still on debate. Ritchie and Nicholas (1972) suggested that $NH_4^+$ oxidizers reduced $NO_2^-$ to $N_2O$ to minimize intracellular accumulation of $NO_2^-$ which is toxic. Remde and Conrad (1990) showed that $N_2O$ could originate from nitrite produced inside the cells. Khalila et al. (2004) showed that $N_2O$ production during nitrification was usually very low.

Many studies have suggested that temperature and soil moisture played an important role in $N_2O$ emissions (Liu et al., 2015; Lu et al., 2015; Wang et al., 2016). Our results were largely in line with this finding. No significant linear correlation was found between $N_2O$ emissions and soil moisture ($p > 0.05$), whereas a significantly positive correlation was found between $N_2O$ emissions and temperature. This indicates that soil moisture conditions are not a limiting factor on $N_2O$ emissions during the growing season in temperate grassland in Hulunber, showing conflicts with our third hypothesis.

Zheng et al. (2002) and Houlton and Morford (2015) suggested that nitrification dominated $N_2O$ emissions when air temperature was between 15 and 35°C. However, when temperature was lower than 5°C or higher than 40°C, nitrification may be inhibited. During the entire growth season in Hulunber, the atmospheric temperature was between 15 and 25°C, with an average temperature of 18.3°C. This indicates that nitrification could be responsible for $N_2O$ emissions at our research site. A significantly positive correlation between $N_2O$ emissions and soil $NO_3^-$–N ($R^2 = 0.56$, $p < 0.05$) suggests that nitrification is a dominant process here, otherwise the higher $NO_3^-$ concentrations as substrate for denitrification will lead to higher denitrification. However, Jiao et al. (2015) found that soil temperature had no effect on $N_2O$ emissions ($R^2 = 0.003$, $p > 0.05$). Furthermore, Du et al. (2000) and Xu (2003) noted that $N_2O$ emissions in Inner Mongolia grasslands were not sensitive to temperature changes because soil moisture may counterbalance the effect of soil temperature on $N_2O$ emissions. It is clear that further research is needed on this important subject matter of $N_2O$ emissions by involving, among other factors, soil temperature and soil moisture.

## 4. Conclusions

Conversion of *L. chinensis* grasslands to *M. sativa* or *B. inermis* grasslands lowered the concentration of soil $NO_3^-$–N, but caused little change in $NH_4^+$–N. MBN decreased with conversion of *L. chinensis* grasslands to *M. sativa* grasslands, but to the contrary, conversion of *L. chinensis* grassland to *B. inermis* grasslands strongly raised MBN. Conversion of native grasslands to managed grasslands influenced variations in the $NO_3^-$–N and $NH_4^+$–N concentrations. Net mineralization rates did not change in growing seasons or vegetation type. However, the growing season had a significant impact on net nitrification rate, and thus elevated $N_2O$ emissions in *M. sativa* grasslands. In contrast, conversion to *B. inermis* grasslands lowered $N_2O$ emissions. The effects of land use change on $N_2O$ emissions depend on the type of managed grasslands. Conversion from native grasslands to legume pastures caused 26.2% increase in $N_2O$ emissions, while conversion to the non-legume grasslands reduced $N_2O$ emissions by 33.1%. These results provide new insights into the mechanisms regulating the impact of grassland land use change on nitrogen conversion, and potentially facilitate the protection and restoration of the grassland ecosystems.

## Uncited reference

Wakelin et al., 2009

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