Managed grassland alters soil N dynamics and N2O emissions in temperate steppe

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ABSTRACT

Reclamation of degraded grasslands as managed grasslands has been increasingly accelerated in recent years in China. Land use change affects soil nitrogen (N) dynamics and nitrous oxide (N2O) emissions. However, it remains unclear how large-scale grassland reclamation will impact the grassland ecosystem as a whole. Here, we investigated the effects of the conversion from native to managed grasslands on soil N dynamics and N2O emissions by field experiments in Hulunber in northern China. Soil (0–10 cm), nitrate (NO3−), ammonium (NH4+), 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 conversion of L. chinensis grassland to M. sativa or B. inermis grasslands decreased concentrations of NO3−, but did not change NH4+. Soil microbial N was slightly decreased by the conversion of L. chinensis grassland to M. sativa, but increased by the conversion to B. inermis. The conversion of L. chinensis grassland to M. sativa (i.e., a legume grass) increased N2O emissions by 26.2%, while the conversion to the B. inermis (i.e., a non-legume grass) reduced N2O emissions by 33.1%. The conversion from native to managed grasslands caused large created variations in soil NO3− and NH4− N concentrations. Net N mineralization rates did not change significantly in growing season or vegetation type, but to net nitrification rate. These results provide evidence on how reclamation may impact the grassland ecosystem in terms of N dynamics and N2O emissions.

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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 (NH4+) and nitrate (NO3−), are important N sources for plants, although organic N in the form of free intact amino acids also can be taken up by

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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 (N₂O) is released during N transformation, primarily via processes of denitrification and nitrification (Skiba and Smith, 2000; Lan et al., 2014). N₂O 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 N₂O 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, NH₄⁺ is converted to nitrite (NO₂⁻) and then to nitrate (NO₃⁻), while in denitrification, NO₂⁻ is gradually reduced to NO and further to nitrogen gases (NO, N₂O, and N₂) (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 (Raiiesi 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 N₂O emissions (Pihlatie et al., 2004; Mørkved et al., 2007; Wang et al., 2013). N₂O emissions increased with increasing water filled pore space (WFPS), the maximal N₂O 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 × 10⁴ km² in 2001 to 12.8 × 10⁴ km² 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 N₂O 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 N₂O emissions are still lacking. Therefore, we conducted field experiments to assess the influences of grassland conversion on soil N dynamics and N₂O emissions in temperate grassland ecosystems in Inner Mongolia, China. We aimed to test the following hypotheses: land use change from native to managed grasslands promotes net N mineralization (Attard et al., 2016); conversion to the legume M. sativa increases N₂O emissions because biological N fixation provides more NH₄⁺ (Bernard et al., 2007); and soil moisture has stronger impacts on N₂O emissions than temperature in this semiarid region (Bedar-Haughn et al., 2006).

## 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″E) 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.

<table>
<thead>
<tr>
<th>Grassland type</th>
<th>M. sativa</th>
<th>B. inermis</th>
<th>L. chinensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH value</td>
<td>6.96 ± 0.68a</td>
<td>6.88 ± 1.05a</td>
<td>6.72 ± 0.98a</td>
</tr>
<tr>
<td>Alkali hydrolyzable nitrogen (mg/kg)</td>
<td>135.57 ± 7.79a</td>
<td>124.37 ± 8.36b</td>
<td>125.42 ± 5.55b</td>
</tr>
<tr>
<td>Available potassium (mg/kg)</td>
<td>307.88 ± 21.87a</td>
<td>205.81 ± 67.97c</td>
<td>235.49 ± 34.45b</td>
</tr>
<tr>
<td>Total nitrogen (g/kg)</td>
<td>2.40 ± 0.67a</td>
<td>2.29 ± 0.24a</td>
<td>2.31 ± 0.15a</td>
</tr>
<tr>
<td>Soil organic carbon (g/kg)</td>
<td>16.17 ± 3.01a</td>
<td>15.83 ± 2.65b</td>
<td>16.02 ± 2.76b</td>
</tr>
<tr>
<td>Texture</td>
<td>Sandy soil</td>
<td>Sandy soil</td>
<td>Sandy soil</td>
</tr>
<tr>
<td>Soil depth (cm)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Average aboveground biomass (kg/ha)</td>
<td>5843.36 ± 250.17a</td>
<td>6044.91 ± 309.05a</td>
<td>3044.85 ± 167.16b</td>
</tr>
</tbody>
</table>
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 metestation, 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$_2$O 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$_2$O 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 Polyvinyl chloride (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 each pair of cores (initial sample) was removed and sent to the laboratory and stored in a refrigerator at 4°C for the following measurement. No fertilizer was applied.

The NH$_4$+–N and NO$_3$–N concentrations were expressed on a dry weight basis. The NO$_3$–N concentrations were measured using a 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:

$$N_{\text{min}} = \left( [\text{NH}_4^+] + [\text{NO}_3^-] \right) - \left( [\text{NH}_4^+] + [\text{NO}_3^-] \right)/t_{j-i}$$

where $N_{\text{min}}$ is the net N mineralization rate (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 $i$ days, and $t_{j-i}$ is the soil incubation from $i$th to $j$th day. The net N nitrification rate ($N_{\text{nitr}}$, µg N dw/(g·day)) is given by:

$$N_{\text{nitr}} = \left( [\text{NO}_3^-] - [\text{NO}_3^-] \right)/t_{j-i}.$$
chromatograph (Chemical Engineering Department Dalian bright chemical industry research Institute, Dalian, China) equipped with a 63Ni 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 N2O production (Wang et al., 2014). Prior report showed that the increase in N2O 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).

The N2O emissions were calculated as the slope of the linear regression of the N2O concentration change over time. The N2O emissions inside the chambers were estimated using the following equation:

$$F = \frac{M \times V \times (C_2 - C_1 \times 273 \times P_2)/(P_0 \times T_2) - C_1 \times 273 \times P_1/(P_0 \times T_1)}{A \times (T_2 - T_1) \times 22.4}$$

where, $F$ ($\mu$g N2O-N/(m$^2$·day)) is the N2O flux; $M$ (g/mol) is the molecular weight of N2O-N; $V$ (0.2 m$^3$) is the volume of the chamber; $A$ (0.25 m$^2$) is the area from which N2O was emitted into the chamber; $T_1$ and $T_2$ (day) are the sampling and end times, respectively; $C_1$ and $C_2$ are the concentrations of N2O (cm$^3$/m$^3$) at the beginning and end of gas collection, respectively; $T_1$ and $T_2$ are the soil absolute temperatures (K) at the beginning and end of gas collection, respectively; $P_1$ and $P_2$ are the atmospheric pressures inside the chamber (Pa) at the beginning and end of gas collection, respectively; and $P_0$ is the standard atmospheric pressure at 273 K (Pa). We assumed that $P_2 = P_1 = P_0$.

The cumulative N2O fluxes were calculated by interpolating the N2O fluxes that were measured during the sampling periods (Dong et al., 2000; Peng et al., 2011).

1.7. Statistical analyses

Correlation analysis was used to evaluate the relationships between N2O emissions and temperature, soil moisture content, NO$\_\text{3}$–N, NH$\text{4}$–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 N2O emissions, and analysis of variance was used to analyze the differences in N2O 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 40°C.
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](image-url) - 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).
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 NO3-N produced by soil nitrification remained as residual NO3 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

N2O emissions varied greatly across treatments. The average N2O 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 N2O internal fluctuation was clear in the L. chinensis grassland. The seasonal pattern of N2O 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 N2O-N/(m2·day)). The N2O emissions showed a net positive flux throughout the growing season (272.6 ± 39.46–3931.8 ± 101.37 μg N2O-N/(m2·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 N2O-N/(m2·day) during the whole season.

Overall, the average rates of N2O emissions were different between grassland types from 2011 to 2012, decreasing in the order M. sativa (1038.7 ± 89.19 μg N2O-N/(m2·day)) > L. chinensis (823.3 ± 46.35 μg N2O-N/(m2·day)) > B. inermis (695.0 ± 78.29 μg N2O-N/(m2·day)). Compared with the L. chinensis grassland, the average N2O emissions of the M. sativa grassland were 26.2% higher, while emissions of the B. inermis grassland were
33.1% lower. However, differences in N₂O emissions between growing seasons and vegetation types were found insignificant (p > 0.05; Table 2).

2.5. Correlation analysis between N₂O emissions and environmental factors

The influence of environmental factors on N₂O emissions was tested using correlation and PCA analyses. Correlations between N₂O and NO₃⁻ or nitrification rate (N_nit), temperature were found positively significant (p < 0.01) (Table 3, Fig. 5). Positive correlations were also found between N₂O and MBN, net mineralization rate or net nitrification rate (p < 0.05). There was a highly significant negative correlation between N₂O and NH₄⁺ (p < 0.01). However, no correlation was found between N₂O and precipitation or soil moisture (p > 0.05). The PCA results (Fig. 5) indicated that NO₃⁻, MBN, and N_min were the main factors influencing N₂O emissions. Among them, NO₃⁻, N_nit or MBN was the most significant, followed by NH₄⁺, N_min and temperature.

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

Fig. 4 – Seasonal dynamics of N₂O emissions in different grassland types. The values are average ± standard error of three replicates.
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 NH3 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 NO3–N and NH4+–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 NO3–N and NH4+–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 NO3–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 NH4+.

Land use change influences nitrification and consequently NO3–N production in grasslands (Fig. 2). Moreover, soil NO3–N concentrations in native grasslands are higher than in managed grasslands, indicating that native grasslands have higher nitrification rates and release more NO3–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 (NH4+) availability for nitrification and most of the NO3–N produced was probably taken up by cultivated forages or

Fig. 5 – Principal component analysis between N2O emissions and their related impact factors.

Table 3 – Multiple linear regression analysis between N2O emission and NH4+, NO3, Nmin, air temperature, microbial biomass nitrogen, and soil moisture.

<table>
<thead>
<tr>
<th>Species</th>
<th>Multiple linear regression analysis</th>
<th>R²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. chinensis</td>
<td>N2O emission = (6.23 ± 1.05) + (20.5 ± 2.45) × [NO3] + (4.67 ± 0.35) × Nmin − (9.68 ± 1.25) × Moisture</td>
<td>0.47</td>
<td>0.02</td>
</tr>
<tr>
<td>M. sativa</td>
<td>N2O emission = − (22.47 ± 9.8) + (3.1 ± 0.6) × [NO3] + (5.78 ± 0.22) × Nmin + (38.7 ± 13.6) × Nmin</td>
<td>0.40</td>
<td>0.03</td>
</tr>
<tr>
<td>B. inermis</td>
<td>N2O emission = (5.65 ± 3.6) + (6.3 ± 1.4) × [NO3] + (6.12 ± 0.27) × Nmin + (19.6 ± 7.9) × Nmin</td>
<td>0.55</td>
<td>0.04</td>
</tr>
</tbody>
</table>
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$_4^+$. 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$_2$O emissions

Previous research suggested that land use change may cause N$_2$O 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$_2$O emissions than the native grasslands (Fig. 3). This confirms our second hypothesis that M. sativa grassland increases N$_2$O emissions because biological N fixation promotes N inputs into the soil. Although increased N$_2$O 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$_2$O 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$_2$O and NH$_4^+$. The origin of N$_2$O emissions from nitrification is still on debate. Ritchie and Nicholas (1972) suggested that NH$_4^+$ oxidizers reduced NO$_3^-$ to N$_2$O to minimize intracellular accumulation of NO$_2^-$ which is toxic. Remde and Conrad (1990) showed that N$_2$O could originate from nitrite produced inside the cells. Khalil et al. (2004) showed that N$_2$O production during nitrification was usually very low.

Many studies have suggested that temperature and soil moisture played an important role in N$_2$O 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$_2$O emissions and soil moisture ($p > 0.05$), whereas a significantly positive correlation was found between N$_2$O emissions and temperature. This indicates that soil moisture conditions are not a limiting factor on N$_2$O 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$_2$O emissions when air temperature was between 15 and 35°C. However, when temperature was lower than $5\degree$C or higher than $40\degree$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 $18.3\degree$C. This indicates that nitrification could be responsible for N$_2$O emissions at our research site. A significantly positive correlation between N$_2$O 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$_2$O emissions ($R^2 = 0.003$, $p > 0.05$). Furthermore, Du et al. (2000) and Xu (2003) noted that N$_2$O emissions in Inner Mongolia grasslands were not sensitive to temperature changes because soil moisture may counterbalance the effect of soil temperature on N$_2$O emissions. It is clear that further research is needed on this important subject matter of N$_2$O 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$_2$O emissions in M. sativa grasslands. In contrast, conversion to B. inermis grasslands lowered N$_2$O emissions. The effects of land use change on N$_2$O emissions depend on the type of managed grasslands. Conversion from native grasslands to legume pastures caused 26.2% increase in N$_2$O emissions, while conversion to the non-legume grasslands reduced N$_2$O 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.

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