Higher quantity and lower frequency of N addition and mowing improved gross N turnover in a temperate steppe of Northern China

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Abstract

1. Anthropogenic effects, such as nitrogen (N) enrichment and mowing, are constantly changing the function and structure of grassland ecosystems. In order to test whether the magnitude and frequency of N addition, as well as occurrence of mowing, affects gross N turnover. We designed a long-term field experiment which included 5 levels of N addition (0, 2, 10, 20, and 50 g N m-2 yr-1) and mowing in a typical grassland of northern China. 2. To test the effects of N addition frequency, the amount of N applied was separated evenly by two times (twice a year, low frequency) or twelve times (monthly, high frequency) that results were compared against a control site where none of the treatments were applied. Mowing effects were also considered at each N treatment levels. 3. Our results showed that the N level, the frequency of N addition, and mowing significantly influenced gross ammonification (GA) and nitrification (GN) rates. Specifically, the effect of N addition frequency was significantly different under the highest N addition level (50 g N m-2 yr-1), lower frequency (twice a year) significantly increased N turnover rates. Mowing significantly increased the GA rate, while decreased GN rate both under the highest N addition level (50 g N m-2 yr-1) and lower N addition frequency. Further long-term study of the effects of the interactions between N addition and mowing on N turnover will be needed for understanding the mechanisms by which nutrient cycling in typical grassland ecosystems may change in the future.

Keywords

Gross mineralization; Microbial biomass C and N; Mowing; Nitrogen addition; Steppe

Introduction

Global atmospheric nitrogen (N) deposition had increased 5 to 20-fold since the preindustrial period, with average deposition rates of 2 g m^{-2} yr^{-1} in recent years, and the deposition of reactive N will continue to increase over the next century (Galloway et al., 2008; Sutton & Bleeker, 2013), which greatly influenced main components of ecosystem N pool (i.e., plant, soil and microbe N pools) of grassland ecosystems (Galloway et al., 2008; Gruber & Galloway, 2008; Lebauer & Treseder, 2008). Most studies simulated N deposition were designed to supply N fertilization once or twice a year (Bustamante et al., 2012; Haugwitz & Michelsen, 2011) which cannot reflect the natural N deposition with dry or wet N deposition. Moreover, grazing or mowing is the main land use way for maintaining the natural grassland ecosystem in northern China. Both N deposition and mowing have significant effects on the N cycling in grassland ecosystem by changing nutrient availability and leaching within them (Collins, Knapp, Briggs, Blair, & Steinauer, 1998; Isbell et al., 2013; Wang et al., 2011).

The experimental results from various individual studies are different which have been conducted to explore how N deposition or fertilization affects N turnover processes in ecosystems, especially in soil N pool (i.e. N ammonification, nitrification, denitrification and immobilization) (Bhatti, Ahmad, Qasim, Riaz, & Cresser, 2018; Lu et al., 2011). There were studies showed that N addition might increase, decrease or have minor

effect on soil N turnover (Brenner, Boone, & Ruess, 2005; Kowaljow & Mazzarino, 2007; Riley, 1998). Most of previous studies focused on the effects of N input on soil net N mineralization rates, these researches about the responses of soil gross N turnover to N enrichment are still limited. The growing season of most of the northern natural grassland begins in May, the rapid growth period of plants is from June to July, so fertilization once or twice a year could not reflect the effects of the N deposition pattern under natural conditions.

Previous studies showed that grazing or mowing (Collins et al., 1998; Giese et al., 2013) also strongly affects nutrient cycling processes because mowing removed aboveground biomass and decreased litter biomass, which could alter soil surface temperature and moisture and further affect soil microbial activity and N turnover (Baptist et al., 2013; Ma, Guo, Xin, Yuan, & Wang, 2013; Robson, Lavorel, Clement, & Roux, 2007; Wang et al., 2015). And some researches indicated that mowing reduced root and soil N concentrations (Kitchen, Blair, & Callaham, 2009; Turner, Seastedt, & Dyer, 1993), which likely due to loss of N with aboveground biomass removal, the change of soil N concentrations would directly or indirectly effects on soil N turnover. However, mowing effects in grassland ecosystems on soil N turnover are the response mechanisms of soil gross N turnover are the response mechanisms of soil gross N turnover and the interactions of N deposition remain unclear.

The area of Inner Mongolia steppe is about 1.18 million km^2, and it is of great importance for ecological conversation and local farmer livelihood. We performed the experiment of N addition amounts with different N addition frequency and mowing to determine their individual and potential interactive effects on the gross N mineralization rate of the soil using a ^{15}N isotope dilution technique (Müller, Stevens, & Laughlin, 2004) and other N pool components (i.e., plants, soil and microbes). There is a lack of understanding of the interaction between the annual N deposition pattern (i.e. N supply frequency in different months) and mowing affect the N turnover processes limits our ability to predict the effects of future climate change for natural mowed grassland ecosystems. Consequently, our study hypothesis that fertilization during different months of the year can increase soil gross N mineralization and plant productivity, because N addition during different months in N-deficient grassland can stimulate soil microbial activities and meet the N demand of different plants at different stages.

The objectives of this study are: (i) to investigate how gross N turnover responds to levels and frequencies of N addition and mowing; (ii) to examine how N addition level and frequency and mowing impact the N and C pool within plants, soil, and microbes in the temperate steppe; and (iii) to find the relationship between gross N turnover and the size of the plant and soil N pool under different N addition levels.

Materials and methods

Study Site

The study site of this experiment was located in the Xilin River Basin, a temperate steppe (116°14'E, 43deg13'N) near the Inner Mongolia Grassland Ecosystem Research Station (IMGERS) in the Inner Mongolia Autonomous Region, China. The experimental area is about 100 ha natural grassland with an elevation up to1250 m, which has been fenced since 1999 to exclude large animal grazing. Based on a long-term observational dataset (1980-2013), the mean annual temperature here was 0.9 degC, with mean monthly temperatures ranging from -21.4 degC in January to 19.7 degC in July, and the mean annual precipitation was about 350.00 mm, approximately 70%-80% of which fell between May and August. The soil at the site is either a Haplic Calcisol or Calcic-Orthic Aridisol, as classified by the FAO and the US soil classification system, respectively. The dominant plant species in the community are *Stipa grandis* and *Leymus chinensis* , which together account for more than 60% of the total peak aboveground biomass, the other 40% of vegetation are forbs.

Experimental design

Eighty experimental plots were established, with a randomized complete block design in September 2008 (Y. Zhang et al., 2016). Each plot was measured 10 m x 10 m, and there were 2-m walkways between adjacent

plots. Each plot was treated with one of five different levels of N addition, i.e., 0, 2, 10, 20 and 50 g N m^{-2} yr^{-1} (designated as N-0, N-2, N-10, N-20, and N-50, respectively), at one of two N addition frequencies (twice a year vs. monthly), and with one of two management regimes (un-mowing vs. mowing). Hence, there were 20 treatments (5 x 2 x 2) in total, each with 4 replicates. The form of N added to the plots was purified NH$_4$NO$_3$(> 99%). N additions started on 1 November and were continued on the first day of June of the same year and November of the next year for the low frequency treatments (2 N addition yr^{-1}), or started on 1 November and continued on the first day of each month for the high frequency treatment (monthly, or 12 N addition yr^{-1}). Fertilizer was applied referring to the method of Zhang et al. (2016). Mowing treatments were carried out two times a year, with 10 cm stubble height left after mowing, on 25 June to 30 June and 25 August to 30 August in every year from 2009 to 2012.

Soil and plant sampling and measurements

Plant and soil samples were collected in mid-August 2012. To investigate vegetation biomass and collect plant and soil samples, a 2 m x 0.5 m quadrat was randomly placed in each plot, but at least 50 cm inside the border of each plot to avoid edge effects. The aboveground biomass (AGB; g m^{-2}) was measured by clipping all plants above the soil surface, oven-drying the combined clippings at 65 degC for 48 h, recording the sample's dry weight, and then finely crushing the sample in a mill to determine the C and N content of plants (C$_{plant}$ and N$_{plant}$; mg kg^{-1}). Three root cores were collected using a 7 cm diameter root auger at 10 cm intervals in the plots to a soil depth of 30 cm. These core samples were then mixed and soaked in water to remove the mineral components of the soil, then treated as described above to measure the belowground biomass (BGB; g m^{-2}). Soil samples were collected with a soil augur (3 cm in diameter), and in each plot three cores were taken with 0-10 cm depth and at least 50 cm apart. Soil samples were then mixed and sieved through 2-mm mesh, stored at 4 degC for laboratory analysis of soil ammonium (NH$_4^+$-N; mg kg^{-1}), nitrate (NO$_3^-$-N; mg kg^{-1}), and moisture (SM %) content, as well as microbial biomass carbon and N (MBC and MBN; mg kg^{-1}). Soil subsamples were air-dried for analysis of soil pH, and soil C and N (C$_{soil}$ and N$_{soil}$; mg kg^{-1}). The C$_{plant}$, C$_{root}$, and C$_{soil}$ were determined by a H$_2$SO$_4$-K$_2$Cr$_2$O$_7$oxidation method (X. T. Lu, Lu, Zhou, Han, & Han, 2012). The N$_{plant}$, N$_{root}$, and N$_{soil}$were determined by using the Kjeldahl acid-digestion method with an Alpkem auto-analyzer (Kjektec System 1026 distilling unit, Sweden). Soil NH$_4^+$-N and NO$_3^-$-N were analysed using a flow injection auto analyzer (FLAstar 5000 Analyzer; Foss Tecator, Hillerod, Denmark), in which these substances were extracted from 10 g of fresh soil sample with 50 ml of 0.5 M K$_2$SO$_4$solution. MBC and MBN were analysed with a fumigation extraction method (Vance, Brookes, & Jenkinson, 1987). Soil pH was determined by measuring pH of a soil-water suspension (soil: water = 1: 2.5) with a pH meter (FE20-FiveEasy).

Gross ammonification and nitrification rates

The ^{15}N pool dilution technique was used to determine the gross ammonification rate (GA; mg kg^{-1} SDW d^{-1}) and gross nitrification rate (GN; mg kg^{-1} SDW d^{-1}) in intact soil cores with one from each experimental plot. Soil in the cores were labelled with^{15}N-(NH$_4$)$_2$SO$_4$or ^{15}N-KNO$_3$ (30 atom%^{15}N enrichment and 3 mL 100 g^{-1} dry soil equivalent) for quantification of gross N ammonification or nitrification. A custom-made multi-injector, which consisted of 10 simultaneously operated syringes with custom-made side-port cannulas, was used to ensure homogenous labelling. Each injection was equivalent to 0.1 mL, 100 g^{-1} soil, and then was labelled with 3 mL of ^{15}N-enriched solution that corresponded to 2 mg N kg^{-1} dry soil; 3 replicate labels were made from each core. When soil was sampled, the fresh soil core was labelled immediately with the apparatus above mentioned (3 times at 0.3 cm, 1.5 cm, and 2.5 cm depth). About one hours later (t1), the first soil core was collected and extracted by 0.5 M K$_2$SO$_4$ solution with a soil: solution ratio of 1:2 as described by Dannenmann et al. (2009). Forty hours after labelling (t2), the second soil core was extracted using the same procedure as at t1.

Soil extracts were frozen for further processing. The diffusion technique described by Dannenmann et al. (2006) was used to collect NH$_4^+$and NO$_3^-$ on acid filter traps, which were then analysed for ^{15}N enrichment with an isotope ratio mass spectrometer (Delta Plus XP; Thermo) at the Center of Stable Isotopes of KIT-IMK-IFU. Subsamples of the soil extracts were analysed to determine their NH$_4^+$ and NO$_3^-$ concentrations by

a commercial laboratory in order to avoid bias. Gross ammonification and nitrification rates were calculated with the equation provided by Kirkham & Bartholomew (Kirkham & Bartholomew, 1954)(1954).

Statistical analyses

All results are presented in figures as mean +- SE (standard error). Repeated measures analysis of variance (ANOVA) for a randomized complete block design was performed, and Tukey's HSD post hoc test was used to test for differences in the response variables between different treatments if the ANOVA revealed an overall significant difference. If $P < 0.05$, differences were concluded to be statistically significant. Linear regression was also performed to examine the relationships of GA and GN rates with soil pH and NH_4^+-N + NO_3^--N. All statistical tests were performed with SAS 9.0, except for regression analyses, which were performed in the SIGMAPLOT software (SIGMAPLOT 12.5 for windows; Systat Software Inc., San Jose, CA, USA) that was also used to produce graphs. Structural equation modelling (SEM) was carried out with the software package IBM SPSS Amos 21.0.

Results

Aboveground and belowground biomass

Nitrogen addition treatments, regardless of adding N levels and frequencies, significantly increased aboveground biomass (AGB) and belowground biomass (BGB) ($P < 0.01$). However, mowing showed no significant effect on AGB ($P > 0.05$) but significantly decreased BGB ($P < 0.001$). Besides, the levels and frequency of N addition and mowing interactively affected AGB and BGB, respectively ($P < 0.01$, Table 1).

Across all N addition levels, AGB increased by 30.92% on average in control (no mowing, $P < 0.05$) and by 40.80% in mowed plots ($P < 0.05$) at the lower frequency N addition (twice a year) (Fig. 1a). AGB also increased by 10.67% in control plots ($P > 0.05$) and by 74.81% in mowed plots ($P < 0.05$) at the higher frequency of N addition (monthly) (Fig 1a). At the highest N addition level (N-50), AGB tended to be higher in mowed versus control plots, and AGB at higher N addition levels (N-20, N-50) was significantly higher in mowed than un-mowed plots at the higher frequency of N addition ($P < 0.05$, Fig. 1a). However, no significant effect of lower-frequency N addition was found on AGB (Fig. 1a). BGB at the lower frequency of N addition was significantly lower than that at high frequency of N addition under relatively lower N addition levels (N-2, N-10) ($P < 0.05$, Fig. 1b) while opposite trends were observed under relatively higher N addition levels (N-20, N-50) (Fig. 1b). BGB was significantly increased by 41.46% ($P < 0.05$) under N addition, and it increased with increasing N addition levels at lower N addition frequency ($P < 0.05$).

Gross nitrogen ammonification and nitrification rates

Nitrogen addition level and frequency had significant effects on both gross ammonification (GA, $P < 0.05$) and gross nitrification rates (GN, $P < 0.05$) while mowing showed no significant effects on GA and GN. Besides, the interaction of N addition level and N addition frequency was significant for GA ($P < 0.001$), but not for GN. In contrast, there were significant interactions between mowing and N addition level or N addition frequency on GN ($P < 0.01$), but not on GA (Table 2).

Across the N addition levels, the highest N addition (N-50) significantly increased GA by 278.45% ($P < 0.05$). For the lowest addition level (N-2), GA was significantly decreased by 44.61% ($P < 0.05$), but was not significantly changed at higher N addition levels (N-10, N-20) with mowing (Fig. 2a, Table 2). Higher N addition levels (N-10, N-20) significantly increased GN by 398.61% and 548.75% ($P < 0.05$, Fig. 2b). N addition with mowing significantly increased GN ($P < 0.05$, Fig. 2b). However, the frequency of N addition and mowing had no interactive effects on GN (Fig. 2b).

The relationships between gross N turnover and soil and plant N content

Soil pH values were negatively correlated with soil GA ($R^2 = 0.63$, $P < 0.0001$) and GN ($R^2 = 0.41$; $P < 0.01$) (Fig. 3a). However, GA ($R^2 = 0.87$, $P < 0.0001$; $R^2 = 0.23$, $P < 0.05$) and GN ($R^2 = 0.55$, $P < 0.001$; $R^2 = 0.56$, $P < 0.001$) (Fig. 3b, 3c) were positively correlated with the soil NH_4^+-N and NO_3^--N concentrations, respectively.

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Based on regression analyses, the SEM suggested that N addition directly affected plant biomass and N content and soil N and GA, while it indirectly affected GN through modifying soil N (Fig. 4). Overall, the SEM results suggested that N addition level and soil N pool were the most important factors affecting soil GA and GN.

Discussion

Aboveground and belowground biomass

Our results found that nitrogen (N) addition directly increased aboveground biomass in semi-arid steppe ecosystem, and on average N addition significantly increased the aboveground and belowground biomass (AGB and BGB) in this system, the results are consistent with most studies using N addition experiments (Bustamante et al., 2012; Kim & Henry, 2013; T. Zhang, Guo, Gao, Guo, & Sun, 2015; Y. Zhang et al., 2016). Because N is a major limiting factor for regulating the plant growth and biodiversity of terrestrial ecosystems, it often determines the net primary productivity of grassland ecosystems (Baer, Blair, Collins, & Knapp, 2004; Bobbink et al., 2010; Hutchison & Henry, 2010; Lebauer & Treseder, 2008). In addition, our results demonstrated that comparing with N addition, either the level of N added or the frequency of N addition, mowing had little effects on AGB and BGB. This was likely due to the fact that N addition significantly changed soil nutrient availability, especially the amount of N available in the soil, which would have greatly improved the growth of plants. Moreover, mowing significantly decreased vegetation cover, which led to increase in the surface temperature and decrease in soil water content. As a consequence, the growth of plants might be restricted in mowed plots even though N was added (Bardgett, Wardle, & Yeates, 1998; Tix, Hebberger, & Charvat, 2006).

Gross nitrogen ammonification and nitrification rates

In our study, the degree of the response of gross ammonification rates (GA) to N addition was lower than that of gross nitrification rates (GN) under lower levels of N addition, and GA and GN were strongly affected by the higher levels and frequencies of N addition tested, suggesting that GA and GN were closely related to the concentration of NH_4^+-N and NO_3^--N in soils. Few studies had examined how N addition affected gross N turnover in grassland ecosystem. Our results indicated that gross N turnover responded positively to N addition, which was in agreement with numerous studies (Barnard, Leadley, & Hungate, 2005; Niboyet et al., 2010). However, gross N mineralization does not always increase linearly with increasing N deposition levels. This has been observed in studies of forest ecosystems (Corre, Beese, & Brumme, 2003; Gao, Yang, Kou, & Li, 2015; Tietema, 1998) in which the rates of gross N mineralization increased up to an intermediate ambient level of N enrichment, but then dropped somewhat at N-enriched conditions higher than it in these systems. Differences between their results and ours are likely due to differences between the two different types of ecosystems studied and environmental conditions. Walecka-Hutchison & Walworth (2007) found that gross nitrification was stimulated by the lower N addition, while was inhibited by the higher N application, which disagreed with our results. Such discrepancy may be caused by the differences of soil nutrient availability, microbial diversities and soil enzyme activities. In addition, we found that soil pH decreased with increasing levels of N addition, and gross N turnover rates significantly decreased with increasing soil pH. This result was inconsistent with the findings of Cheng et al. (2013), in which GA and GN were positively correlated with soil pH in forests, which likely due to differences in the gross N turnover processes of different ecosystem types, and the major limiting factors are different in forest and grassland ecosystems. Furthermore, many studies indicated that GA and GN were influenced by some other factors aside from soil N availability, e.g., vegetation and soil types (Gerschlauer et al., 2016; Xu & Xu, 2015), soil moisture (Y. Chen, Borken, Stange, & Matzner, 2011), and other human or environmental factors(Wang, Dannenmann, Meier, & Butterbachbahl, 2014; West, Hobbie, & Reich, 2006). However, studies about the effects of N addition frequency on soil gross N turnover were very limited, so further studies are still needed.

In addition, we found that mowing had negligible effects on GA and GN under any N addition conditions, which is different from the findings of Wang et al. (2015), who found that mowing increased nitrification rate and did not affect ammonification rate. This difference between our study and theirs may be due to

the possibility that the response of GA and GN to mowing was counteracted by the level and frequency of N addition. On the other hand, mowing might also affect soil gross turnover by changing the environmental conditions (i.e. soil moisture, temperature and so on) in grassland ecosystems (Bremer, Ham, Owensby, & Knapp, 1998; Han, Zhang, Wang, Jiang, & Xia, 2011; Owensby, Hyde, & Anderson, 1970). And previous studies indicated that mowing might increase soil total carbon and nitrogen by increasing plant diversity (Cong et al., 2014; Fornara & Tilman, 2008) and change the compositions of soil microbial communities (Li et al., 2017), which would directly or indirectly impact soil gross N turnover. However, few studies have evaluated the response of gross N turnover to mowing, so as a consequence, our knowledge of the response mechanism involved in such relationships is very limited.

5 Conclusion

Soil N mineralization and availability are crucial for ecosystem productivity and carbon sequestration, especially in the context of global climate change or anthropogenic disturbances. The frequency of N addition and mowing had only occasional effects on those variables, which likely due to the effects of the frequency of N addition and mowing were offset by the effects of the levels of N addition, or that interactions of these with other human or environmental factors that we did not examine occurred. Nevertheless, GA and GN were significantly affected by N addition frequency and mowing especially at the highest N addition level. Consequently, long-term research on the response of N cycling to human activities or environmental variables is needed to further improve our understanding of the response mechanism of N cycling to climatic change.

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