RESEARCH ARTICLE

Effects of Simulated Nitrogen Deposition on Soil Net Nitrogen Mineralization in the Meadow Steppe of Inner Mongolia, China

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Abstract

Effects of simulated nitrogen (N) deposition on soil net nitrogen mineralization (NNM) were examined *in situ* during two growing seasons, using the resin-core technique in the semiarid meadow steppe in Inner Mongolia, China. The aim of this study is to clarify the effect of N levels (0, 10, and 20 kg N ha^{-1}yr^{-1}) and forms (NH$_4^+$ and NO$_3^-$) on soil mineral N and NNM. Our results showed that N levels had no significant differences on soil mineral N and NNM. In the first year, three N treatments ((NH$_4$)$_2$SO$_4$, NH$_4$Cl and KNO$_3$) increased soil NH$_4^+$ concentrations but had no significant effects on soil NO$_3^-$ concentrations. In the second year, (NH$_4$)$_2$SO$_4$ treatment increased soil NO$_3^-$ concentrations, NH$_4$Cl and KNO$_3$ treatments decreased them. Three N treatments significantly decreased soil NH$_4^+$ concentrations in the later stages of the second year. As for the soil NNM, three N treatments had no significant effects on the rates of soil NNM (R_m) and net nitrification (R_n) in the first year, but significantly decreased them in the second year. The contribution of N addition to R_m was higher from (NH$_4$)$_2$SO$_4$ than from NH$_4$Cl and KNO$_3$. However, Soil R_m was mainly affected by soil water content (SWC), accumulated temperature (T$_a$), and soil total N (TN). These results suggest that the short-term atmospheric N deposition may inhibit soil NNM in the meadow steppe of Inner Mongolia.

Introduction

The global nitrogen (N) deposition has increased from 34 to 105 Tg yr^{-1} since before 1860 and is expected to double in the next 25 years because of the anticipated increase in human activities [1–4]. In China, the average annual dry and wet N deposition is estimated at 12.9 kg N ha^{-1} yr^{-1}, and it has been increasing at a rate of 0.17 kg N ha^{-1} yr^{-1} or 1.47% per year [5]. Elevated atmosphere N deposition has been considered as an important factor influencing the N cycle processes especially on mineralization, nitrification and denitrification in terrestrial

ecosystems [2,3,6]. Therefore, it is important to better understand the impacts of N deposition on N transformation in N-limited natural ecosystem, and to accurately evaluate the changes in ecosystem productivity and capability to sequester N induced by elevated N deposition.

Soil organic N mineralization, as one of the major processes of N cycle plays an important role in determining soil N availability and net primary productivity [7,8]. Net nitrogen mineralization (NNM) is the outcome of two concurrent and opposite processes: gross N mineralization and gross N immobilization turnover. The actual availability of inorganic N depends on the rate of NNM and its transport through the soil [9,10].

Simulated atmospheric N deposition experiments on soil NNM have been conducted in some forest ecosystems. Several prior studies have discussed whether N additions have a positive [11–16] or a negative effect on NNM [17–19]. Some of them were measured by laboratory incubation, which did not agree well with the results from the field observation. Most of N inputs were mixed type of N fertilizer, NH_4NO_3, which will not help understanding of the single effects of different N forms on NNM. The response of soil NNM to N deposition depends largely on the input of N forms and concentrations, the balance between production processes of H^+ (nitrification, plant uptake, and the fixation of NH_4^+) and its consumption (denitrification, plant uptake, and the fixation of NO_3^-), and the buffering capacity of soils [6,8,20]. However, the effects of N deposition on NNM remain far from certain especially in China's temperate grassland which comprises nearly 12.5% of the global grassland areas.

To improve our understanding of the responses of NNM to the elevated atmosphere N deposition in China's temperate grassland, we conducted an *in situ* N fertilization addition experiments in the semiarid meadow steppe in Inner Mongolia, China in May 2008. The experiment lasted for two years, and it involved different forms and levels of N. The objective of these experiments was to explore the effects of the amounts and forms of N addition on soil inorganic N and rates of NNM *in situ* in the meadow steppe of Inner Mongolia, China.

Materials and Methods

Site description

The simulated N deposition experiment was conducted in one of the grazing-excluded experimental plots of the meadow steppe at the Inner Mongolia Grassland Ecosystem Research Station of the Chinese Academy of Agricultural Sciences. The site is located in Hulun Buir City, Inner Mongolia, China, 49°19′–49°20′N latitude, 119°55′–119°58′E longitude, 628–649 m above the sea level. The average annual dry and wet N deposition is estimated at 8.5 kg N ha^{-1} yr^{-1}. The selected plot was fenced in 1997. The mean annual precipitation at the site is 350–400 mm. Precipitation occurs mainly from June to September. Mean annual temperature ranges from –5 to –1°C. The ground is covered by snow in most of the winter season from November to April of the following year. Annual cumulative temperature of ≥10°C is 1680–1800°C, and the frost-free period is around 100 days. Air temperature and precipitation in 2008 and 2009 are shown in Fig 1.

The soil type at the site is classified as chestnut according to the Chinese classification, or Haplic Arenosol according to the FAO classification. Total N, inorganic N, C/N ratios, pH values and soil bulk density are shown in Table 1. One of the main plant community types (the climax community) in the area is the *Stipa baicalensis*. The grass community is mainly composed of *S. baicalensis*, *Leymus chinensis*, *Carex pediformis*, *Artemisia tanacetifolia*, *Melissitus ruthenica*, *Bupleurum scorzonerifolium*, *Vicia amoena*. Among them, *S. baicalensis* and *L. chinensis* are the dominant species.
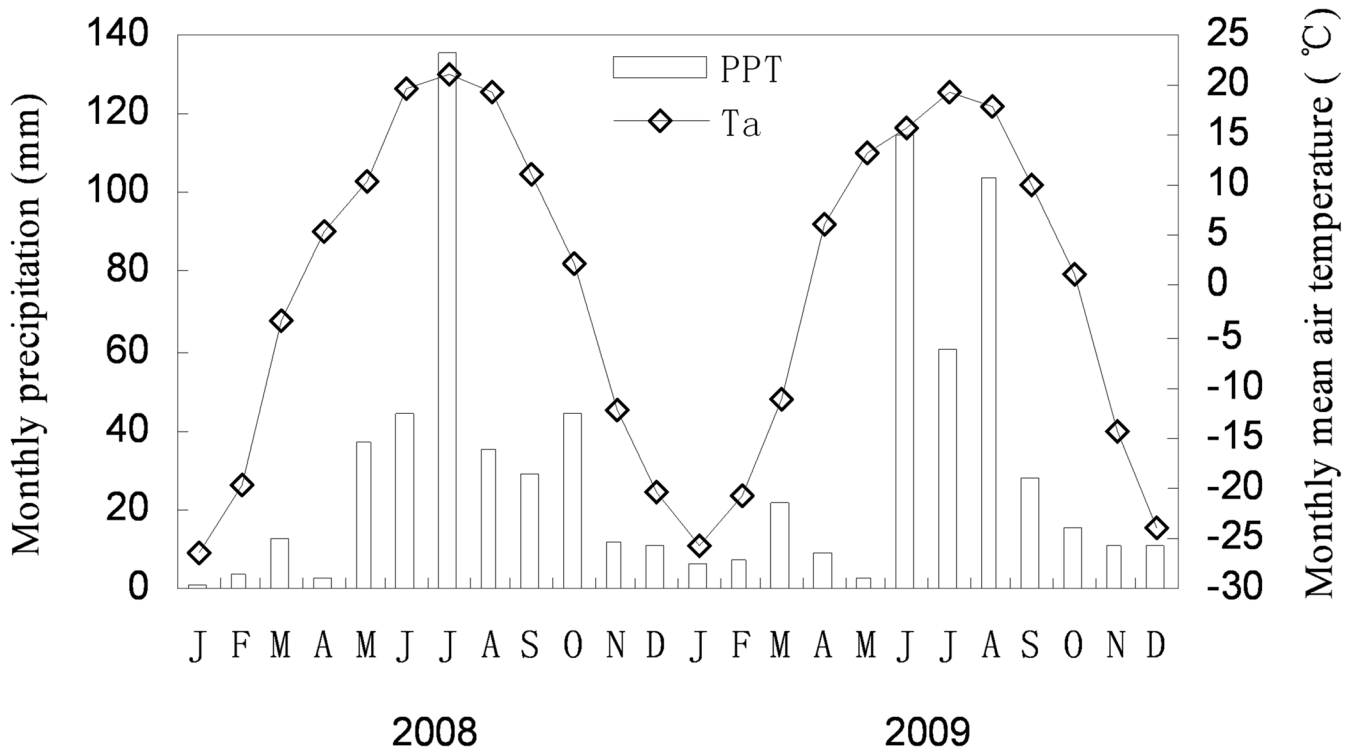
Fig 1. Air temperature and precipitation at the meteorological station close to the study area in 2008 and 2009.

Experimental design

In May 2008, a plot was selected for N deposition experiment in the fenced meadow steppe. Three N fertilizers ($(NH_4)_2SO_4$, NH_4Cl, and KNO_3) were used as three levels of control (CK, 0 kg N ha^{-1} yr^{-1}), low N (LN, 10 kg N ha^{-1} yr^{-1}), and high N (HN, 20 kg N ha^{-1} yr^{-1}). The fertilized amount was set to simulate a future increase in the atmospheric N deposition by 1- and 2- fold. Each N treatment was replicated three times, a total of 21 sub-plots of 3 m × 4 m in size were set up in a randomized block design. The adjacent sub-plots were separated by a 2 m wide buffer strip to minimize the disturbance from neighboring treatments. One year's amount of added N was divided into two equal portions: one portion in the growing season (from May to October) and the other portion in the non-growing season. Over the growing season, N fertilizers were added on around 10th of every month as 6 equal doses. Duo to the severe weather conditions in the non-growing season, N fertilizers were all applied in one time in November. N fertilizer was dissolved in 1 L deionized water, and sprayed on each sub-plot, and the control plots received 1 L deionized water only. So 7 L additional water was added to each sub-plot per year. No significant differences in general soil properties were observed between the treatments before N application.

Table 1. Soil physiochemical properties of 0–20 cm soil in the sampling site.

Total Organic C(%)	DOC(mg kg^{-1})	Total N(%)	NH_4^+-N(mg kg^{-1})	NO_3^-N(mg kg^{-1})	C/N	pH	Soil bulk density(g cm^{-3})
5.1	122.9	0.20	13.4	4.6	25.5	5.9	1.22

In situ net N mineralization

The experiment was carried out from May 2008 to October 2009 (2 integral growing seasons) using the resin-core technique [21,22], which was similar to that described by Liu et al. [23]. The method confined soil cores *in situ* in an open tube, with an anion exchange resin bag at the bottom to intercept any leached N. At each sampling, four duplicate samples of two paired samples of soil were taken in each subplot. The PVC tubes were sharpened in the outside at the bottom to avoid compression of grassland soil. After the aboveground vegetation was removed, one of a pair of PVC tubes (5 cm in diameter and 17 cm long) was inserted 15 cm into the soil. The soil was taken to the laboratory for the determination of the extractable N. The other tube was inserted 15 cm into the soil to confine a soil core, and then 2 cm soil layer was excavated from the bottom of the tube. The excavated soil was put into a filter paper, one resin bag, filter paper, and one cylindrical block of gypsum in order (the height of resin bag and plaster is about 2 cm). The resin bag was made of nylon and containing 10 g of anion exchange resin beads. The tube was then returned to its original position, and left for *in situ* incubation for about 4 weeks. When it was removed and further tubes were driven into the soil to repeat the cycle until the experiment ended on 15 October. All the initial and incubated soil samples and the collected resin bags were stored in refrigerator at 4°C temporarily, and extracted within 24 h.

Soil samples and resin bags extraction and chemical analysis

After stones had been removed by hand, all soil samples were thoroughly mixed, homogenized, and sieved through a 2 mm sieve. Mineral N was extracted with 0.01 M $CaCl_2$ solution. The resin bags were extracted with 1 M NaCl solution (resin: NaCl 1: 5 w/v). The filtrates of soil and the solution of extracted resin bags were kept frozen before they were analyzed on an automated flow injection analysis (Braun & Lübbe, Norderstedt, Germany). Rates of net ammonification (R_a), net nitrification (R_n), and net mineralization (R_m) were calculated from measured changes in inorganic N content divided by the time interval [23]. Soil water content (SWC) was determined gravimetrically by oven-drying at 105°C for 24 h. Soil temperature at 10 cm depth was measured with Model SN 2202 digital thermo detector at the time of sampling.

Total soil organic carbon (TOC) and TN were analyzed using a Multi N/C 2100 analyzer (Analytik Jena, Germany). Soil dissolved carbon (DOC) was assayed following the procedures presented by Ghani et al. (2003) [24]. Soil pH values were determined in water (water: soil = 2.5: 1) suspension. Soil bulk density was measured using the core method.

The peak aboveground and root biomasses were measured in mid-August in both years. Aboveground biomass was measured by harvesting vegetation over a 25 cm × 25 cm area (n = 5). Root biomass was measured by a 10 cm diameter soil cores collected to 40 cm depth, because more than 95% of the root biomasses were in this depth. At each sampling, 10 cores were collected on each plot. Live roots, carefully removed from the soil cores, were rinsed with water. All plant materials were dried in an oven at 60°C to constant weight.

Statistical analysis

Statistical analyses were conducted using the SPSS 16.0 package. Means (n = 3) and standard errors (SE) were calculated. We used three way repeated measures multivariate analysis of variance (MANOVA) with N forms, N levels and sampling time as main effects to test differences in NH_4^+-N, NO_3^--N, R_m, R_n, and R_a. Relationships between R_m and soil parameters were tested using Pearson correlation analysis. Statistical significant differences were set at P values <0.05 unless otherwise stated.
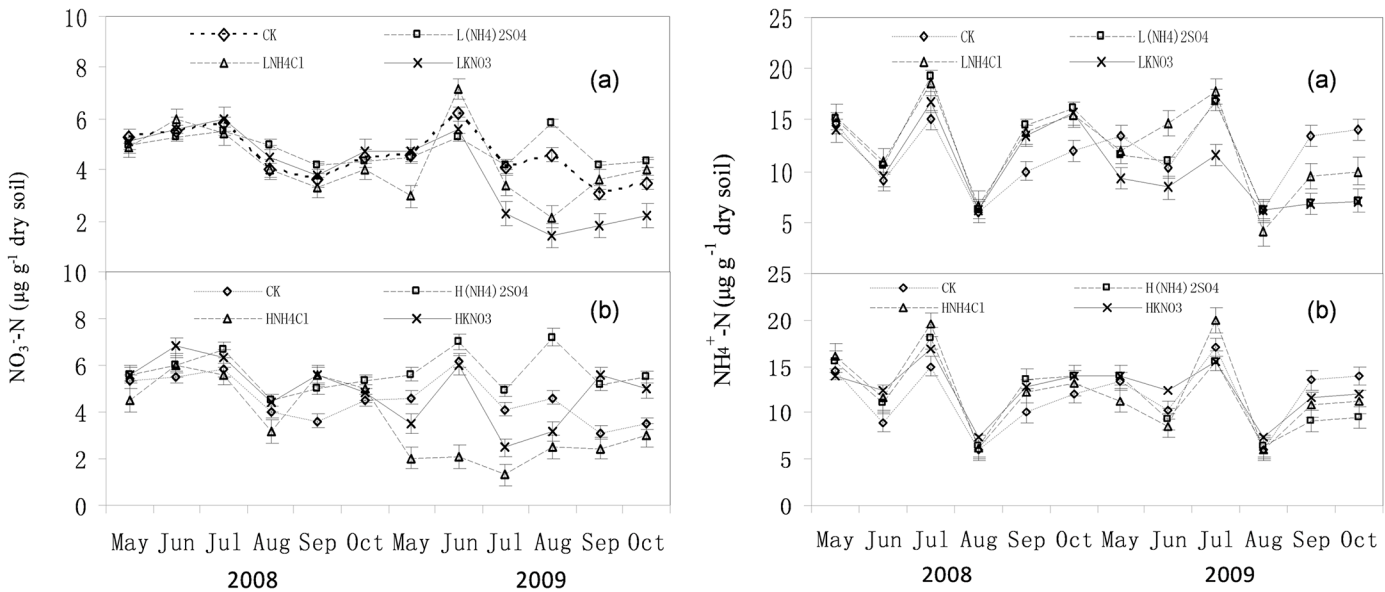
Fig 2. The seasonal variations of NO$_3^-$-N and NH4+-N in LN (a) and HN treatments (b) during the growing seasons of 2008 and 2009.

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Results

Seasonal dynamics of mineral N concentration

The seasonal patterns of NO$_3^-$N were similar between LN (a) and HN (b) treatments with one peak on 15 July 2008 and two peaks on 15 June and 12 August 2009 (Fig 2). Levels rather than forms of N addition had subtle effects on soil NO$_3^-$N (Table 2, P>0.05) except for NH$_4$Cl (P<0.05) in 2009. In 2008, there were no significant differences between N addition treatments and the control. In 2009, H(NH$_4$)$_2$SO$_4$ treatment increased NO$_3^-$N concentrations compared to CK, while HNH$_4$Cl and HKNO$_3$ treatments decreased them significantly (P<0.05). The reduction effect of HNH$_4$Cl on NO$_3^-$N concentrations was more obvious (P<0.05).

The seasonal patterns of NH$_4^+$-N were similar between LN (a) and HN (b) treatments, with two peaks occurred in July in both years. N levels had subtle effects on soil NH$_4^+$-N (Table 2, P>0.05), except for KNO$_3$ treatments in 2009. LKNO$_3$ treatment decreased NH$_4^+$-N concentrations significantly compared to CK (P<0.05), but HKNO$_3$ treatment had no significant effects on them. In 2008, there were no significant differences among treatments, while in the

Table 2. Results of F tests based on repeated measures ANOVA on effects of N level, N forms, and their interactions on soil mineral N, R_m, R_n, and R_a.

Variations	Growing season of 2008					Growing season of 2009				
	NH$_4^+$-N	NO$_3^-$N	R_m	R_n	R_a	NH$_4^+$-N	NO$_3^-$N	R_m	R_n	R_a
Time (T)N level (L)	5.75*2.11	4.25*1.56	6.11*2.83	6.35*2.56	5.87*3.01	5.78*1.98	5.09*2.02	7.23*2.71	6.58*2.62	6.41*3.51
N form (F)	1.07	2.33	1.88	1.76	2.23	3.56	3.01	4.89*	5.07*	5.88*
T×L	1.10	0.96	1.23	1.07	0.99	1.26	1.55	0.89	1.42	0.76
T×F	2.05	1.67	2.00	1.54	1.76	1.87	2.13	1.17	1.53	0.75
L×F	1.05	1.34	1.32	1.85	1.11	1.52	2.26	0.99	1.06	1.63
T×L×F	1.18	0.93	0.87	0.56	0.32	1.76	1.64	0.52	0.39	0.47

*: statistically significant at P < 0.05

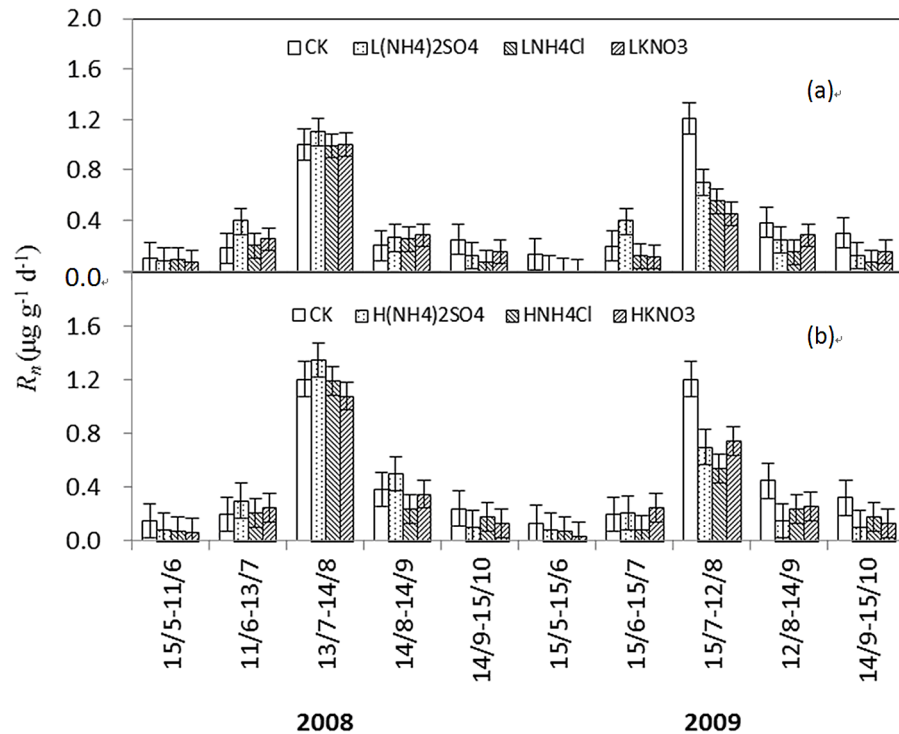doi:10.1371/journal.pone.0134039.t002

Fig 3. The seasonal variations of R_n in LN (a) and HN treatments (b) during the growing seasons of 2008 and 2009.

later stages of 2009, three LN treatments decreased NH_4^+-N concentrations. The suppression effect of $LKNO_3$ was the most obvious among the three LN treatments. In 2008, the three HN treatments tended to increase NH_4^+-N concentrations, but there were no significant differences between N addition treatments and control (P>0.05). In the later stages of 2009, the three HN treatments all decreased NH_4^+-N concentrations. Contrary to the LN treatments, ammonium N fertilizers addition had more reduction effects than nitrate N fertilizers on soil NH_4^+-N concentrations.

Net nitrification rate

The seasonal patterns of daily R_n were similar between LN and HN treatments with two peaks in July in both years (Fig 3). In the LN treatments, R_n ranged from 0 to 1.21 µg g^{-1} d^{-1} and varied significantly during the two growing seasons. The incubation period had significant effects on R_n (P<0.05). In 2008, the three LN treatments had no significant effects on R_n, while in the later stages of 2009, they decreased R_n significantly. $L(NH_4)_2SO_4$, LNH_4Cl, and $LKNO_3$ treatments decreased R_n by 12.1–42.6%, 13.3–54.5%, and 13.0–62.8%, respectively, compared to CK. The reduction effect was most obvious in the warm and rainy July of 2009. However, there were no significant differences in R_n among each LN treatment.

In the HN treatments, R_n ranged from 0.03 to 1.35 µg g^{-1} d^{-1} and varied significantly during the two growing seasons (Fig 3). In 2008, $H(NH_4)_2SO_4$ treatment tended to increase R_n during the incubation period from 11 June to 13 July, 13 July to 14 August, and 14 August to 14 September, but HNH_4Cl and $HKNO_3$ treatments had no significant effects on R_n. In the later stages of 2009, the three HN treatments had significant reduction in R_n. $H(NH_4)_2SO_4$, HNH_4Cl, and $HKNO_3$ treatments decreased R_n by 14.1–66.2%, 13.2–55.4%, and 10.0–63.7% compared to

CK, respectively. The reduction effects were most obvious in July of 2009. $H(NH_4)_2SO_4$ treatment had a stronger inhibition on R_n than HNH_4Cl and $HKNO_3$ treatments.

Net ammonification rate

The seasonal patterns of daily R_a were similar between LN and HN treatments with three negative values in July 2008 and May and July 2009 (Fig 4). In the LN treatments, R_a ranged from -0.19 to 0.30 µg g^{-1} d^{-1} and varied significantly during the two growing seasons. The incubation period also had significant effects on R_a in all treatments (P<0.05). During the two growing seasons, the LNH_4Cl treatment significantly increased R_a, except for the negative values during the period from 13 July to 14 August 2008 and from 15 July to 12 August 2009. The promotion effects of LNH_4Cl on R_a in 2009 were greater than that in 2008, and the effects were most obvious in June and August. LNH_4Cl treatment increased R_a by 42.8–122.0% in 2008 and 114.0–288.0% in 2009. $L(NH_4)_2SO_4$ and $LKNO_3$ treatments had no significant effects on R_a.

In the HN treatments, R_a ranged from -0.47 to 0.44 µg g^{-1} d^{-1} and varied significantly during the two growing seasons. The HNH_4Cl treatment significantly increased R_a, except for the negative values during the periods from 13 July to 14 August 2008 and from 15 July to 12 August 2009. Contrary to the LNH_4Cl treatment, the promotion effect of HNH_4Cl on R_a was greater in 2008 than in 2009. In the later period of 2009, there was no significant difference in R_a between treatments.

Net N mineralization rate

Given that R_n was far greater than R_a in values, the seasonal variations of R_m were similar to R_n. The seasonal patterns of daily R_m were also similar between LN and HN treatments with two peaks in July of 2008 and 2009. In the LN treatments, R_m ranged from -0.08 to 1.02 µg g^{-1} d^{-1}, and the incubation period had significant effects on R_m in all treatments (Fig 5, P<0.05).
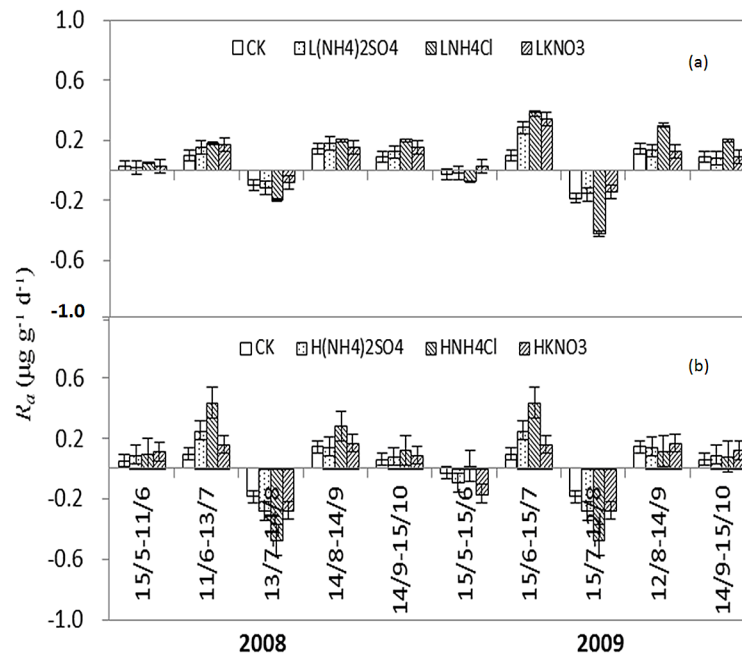

Fig 4. The seasonal variations of R_a in LN (a) and HN treatments (b) during the growing seasons of 2008 and 2009.

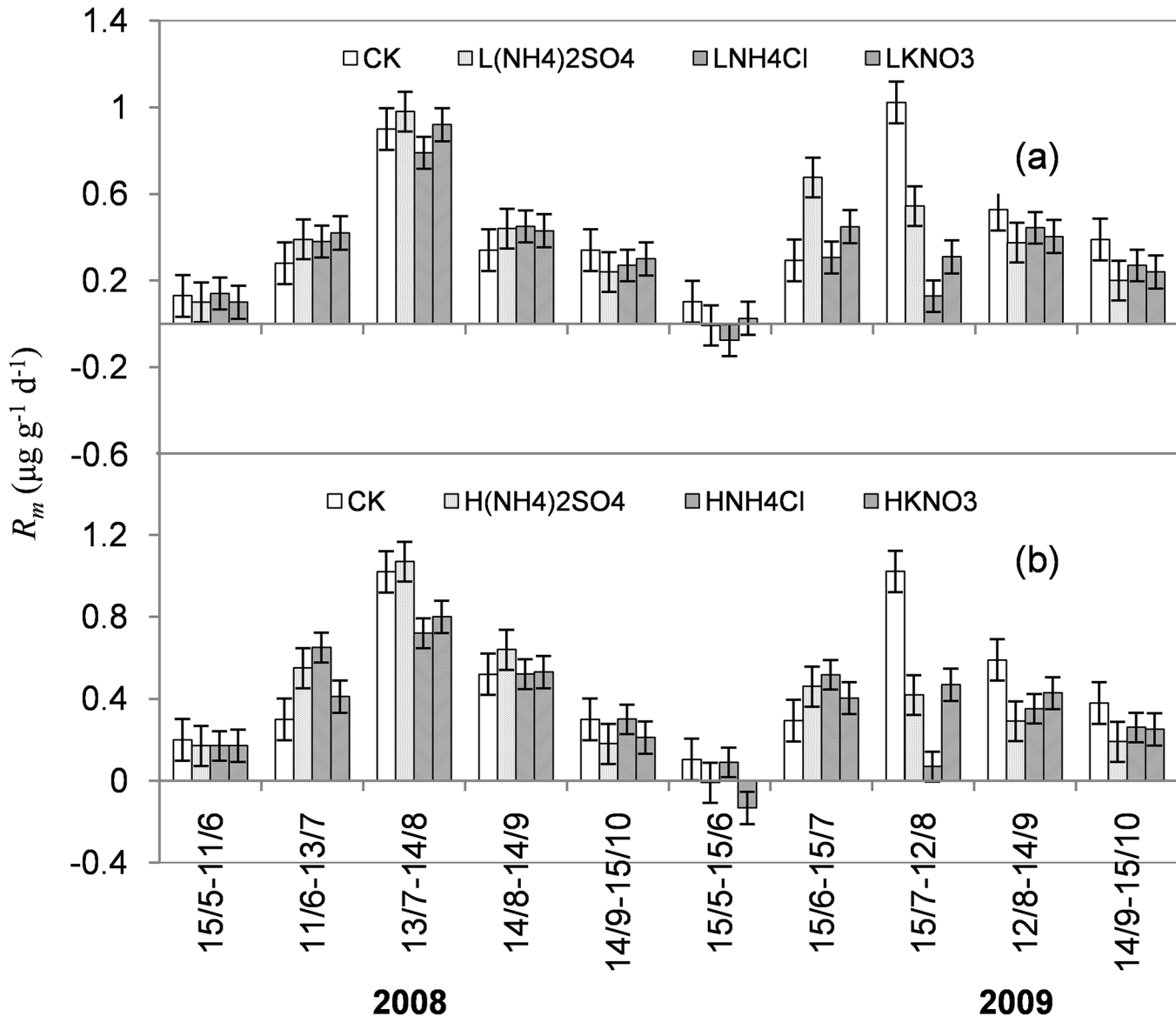doi:10.1371/journal.pone.0134039.g004

Fig 5. The seasonal variations of R_m in LN (a) and HN treatments (b) during the growing seasons of 2008 and 2009.

In 2008, the three N treatments had no significant effects on R_m. In the later period of 2009, they significantly decreased it. $L(NH_4)_2SO_4$, LNH_4Cl, and $LKNO_3$ treatments decreased R_m by 12.1–110.2%, 17.0–180.0%, and 24.5–70.3%, respectively, compared to CK. The most obvious reduction effects also occurred in July. However, there were no significant differences in R_m among the LN treatments except for the incubation period from 15 June to 15 July.

In the HN treatments, R_m ranged from -0.13 to 1.07 $\mu g\ g^{-1}\ d^{-1}$, and varied significantly during the two growing seasons (Fig 5, P<0.05). In 2008, $H(NH_4)_2SO_4$ treatment tended to increase R_m except for the beginning and the end of the growing season. HNH_4Cl and $HKNO_3$ treatments showed the same trend in R_m, with increased R_m in June, and decreased R_m in July, at comparable magnitude to that in CK. In 2009, the three HN treatments significantly decreased R_m except for the period from 15 June to 15 July. $H(NH_4)_2SO_4$ treatment had the most obvious reduction effect, especially in July. $H(NH_4)_2SO_4$, HNH_4Cl, and $HKNO_3$
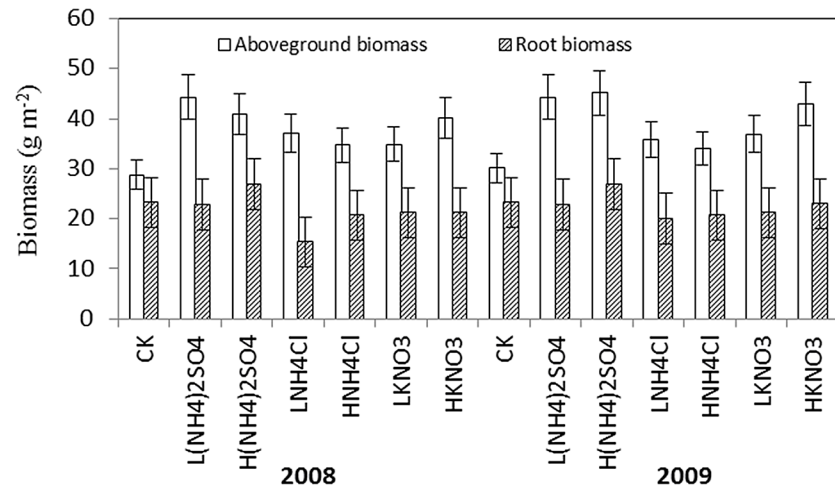
Fig 6. The aboveground biomass and root biomass of different treatments in August of 2008 and 2009.

treatments reduced R_m by 36.7–58.8%, 13.0–93.1%, and 16.7–53.9%, respectively, compared to CK. However, there were no significant differences between HNH_4Cl and $HKNO_3$ treatments.

Aboveground biomass and root biomass

In either the LN or HN treatments, N addition caused significant increases in aboveground biomasses (Fig 6, P<0.05), but not root biomasses. In 2008, $L(NH_4)_2SO_4$, $H(NH_4)_2SO_4$, LNH_4Cl, HNH_4Cl, $LKNO_3$, and $HKNO_3$ treatments increased aboveground biomasses by 53.8%, 41.7%, 28.8%, 20.5%, 21.2%, and 39.6%, respectively. In 2009, they increased aboveground biomasses by 47.2%, 49.8%, 18.9%, 13.0%, 22.6%, and 42.9%, respectively. However, there were no significant differences between N levels (P>0.05). The increases in aboveground biomasses of $(NH_4)_2SO_4$ treatments were greater than that of NH_4Cl and KNO_3 treatments, but there were no significant differences between LN and HN treatments.

Relationships between R_m and environmental factors

Our results showed that soil R_m was significantly positively correlated with soil SWC and the effective accumulated temperature (T_a) in each treatment (Table 3). The variations of soil SWC and T_a could explain more than 58.1% and 80.5% of the variation in R_m, respectively (Table 3, P<0.05). Also, soil R_m was positively correlated with the soil TN rather than TOC. The variation of TN could explain more than 53.4% of the variation in R_m. Soil R_m was negatively but not significantly correlated with C/N of soil organic matter (SOM) (Table 3, P>0.05). There were positive but insignificant correlations between R_m and soil pH values (Table 3, P>0.05).

Discussion

The effects of N addition on mineral N

Our results showed that in both the HN and LN treatments, the additions of $(NH_4)_2SO_4$ increased soil NO_3^- concentrations, NH_4Cl and KNO_3 decreased soil NO_3^- concentrations in the second year. These results are very similar to the results from the long-term fertilization experiment in the neutral purple paddy soil ecosystem reported by Qin et al. [25], and also similar to the results of Zhang et al. [26] from experiments with three different soil types in

Table 3. Pearson's linear correlation coefficient (r) between environmental factors and R_m under different treatments.

Item	CK	H(NH$_4$)$_2$SO$_4$	L(NH$_4$)$_2$SO$_4$	HNH$_4$Cl	LNH$_4$Cl	HKNO$_3$	LKNO$_3$
SWC (%)T$_s$ (°C)T$_a$ (°C)TN (%)TOC (%)Soil C:N	0.628*0.3410.791* 0.615*0.485–0.490	0.661*0.4530.818* 0.634*0.543–0.432	0.654*0.4320.821* 0.65*0.511–0.516	0.595*0.5210.805* 0.534**0.327–0.509	0.581*0.4110.887** 0.651** 0.153–0.498	0.621*0.4540.876** 0.647** 0.138–0.671	0.609*0.4910.855** 0.621*0.012–0.457
Soil pH	0.381	0.468	0.381	0.474	0.597*	0.405	0.313

T$_s$: soil temperature; T$_a$: accumulated temperature

*, **: statistically significant at P<0.05, 0.01.

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grassland. Besides the specific NH_4^+ or NO_3^- effects, the salt effects of SO_4^{2-} and Cl^- may be a reasonable explanation for the increase or decrease in NO_3^- concentrations. We found SO_4^{2-} promoted nitrification, and Cl^- had significant inhibitory effects on nitrification. Application of different NH_4^+-N fertilizer, soil chemical and physical properties would change because of salt effects (such as osmotic pressure, pH value). $(NH_4)_2SO_4$ in soil solution is easier to produce more H^+ than NH_4Cl, but the osmotic pressure of NH_4Cl in soil solution is greater than that of $(NH_4)_2SO_4$ [27]. Many researches have indicated that NH_4Cl has direct inhibitory effects on nitrification [27,28]. Few researches, however, has not examined the inhibition mechanism. Therefore, the mechanism of the inhibitory effects of NH_4Cl on nitrification needs to be further studied.

Our study also showed that the promotion of $(NH_4)_2SO_4$ fertilizer to soil NO_3^--N concentration was higher than KNO_3 fertilizer, and the inhibition of KNO_3 fertilizer on soil NH_4^+-N concentration was higher than $(NH_4)_2SO_4$ fertilizer (Fig 2). Generally, NO_3^--N and NH_4^+-N fertilizers should have opposite effects on soil mineral N because of their opposite ion charges [29]. The less sensitivity of soil NO_3^--N concentration to KNO_3 amendment is because that NO_3^- are very mobile in soil owing to their negative charge[30]. Moreover, NO_3^- may not be present in the treated plots as long as NH_4^+, which are positively charged and are more strongly adsorbed onto exchange sites in the soil matrix [29]. Also, the K^+ can exchange NH_4^+ from the exchange sites in the soil and then release NH_4^+ [27]. This explanation is supported by our results of the accumulation of soil NH_4^+-N and NO_3^--N with N addition (Fig 2).

In this study, we did not observe significant effects of N addition on inorganic N concentrations in the first year, and N levels on inorganic N concentrations in the second year. Generally, the dynamics of soil NO_3^--N and NH_4^+-N concentrations under N additions are mainly determined by the inorganic N input (N addition and mineralization) and losses to plant uptake. The addition of N fertilizers significantly increased aboveground biomasses in our experiment (Fig 6, P<0.05), previous study also reached the same conclusion [31]. These results suggested that the loss of soil inorganic N through the uptake of vegetation offsets the input N and soil organic N mineralization. On the other hand, the meadow steppe soil is N-limited and has a strong capacity to immobilize exogenous N, perhaps the N level in our experiment is not enough to affect the mineral N concentration in the soil. We attributed the no responses to the short-term phenomenon. In a short term, the abundance and activity of soil microorganisms are regulated more by plant species than by direct effect of N input [31,32]. The effects of N input on soil N pool are decided by two processes: 1) the increased nitrification would increase the activity of soil N, accelerate the NO_3^- loss, and reduce the soil N pool [19]. 2) The increased available N would inhibit production of lignin degrading enzyme, allowing NO_3^--N and NH_4^+-N to combine with lignin and phenolic compounds to form stable compounds not easy for decomposition. This process would reduce N release rate, and thereby improve soil N accumulation [20,33]. The soil N accumulation varies with N deposition according to the balance of the processes 1 and 2.

The effects of N addition on R_m, R_n, and R_a

In the later period of 2009, $(NH_4)_2SO_4$, NH_4Cl, and KNO_3 treatments all showed significant decreases in R_m and R_n (P<0.05). The most obvious reduction effect occurred in the warm and rainy July. High level of $(NH_4)_2SO_4$ addition had a stronger inhibition on R_m and R_n than NH_4Cl and KNO_3 addition. The slight promotion effect of $(NH_4)_2SO_4$ in the early stages and inhibitory effect in the later stages of 2009 were also consistent with some previous results [12, 34, 35]. Zhang et al. [36] have reported that 3-year N addition (0–640 kg N ha^{-1} yr^{-1}) caused gradual or step increases in soil NNM and nitrification in the early growing season. In a typical

steppe dominated by a *Leymus chinensis* community, however, N addition also showed dose effects. Zhang et al.[37] found that low N addition (17.5 kg N ha^{-1} yr^{-1}) stimulated N mineralization but high N addition (280 kg N ha^{-1} yr^{-1}) inhibited N mineralization. Unfortunately, we did not measure soil R_m in the non-growing season of 2008 because of very low temperature and the deep snow, and only observed the slightly increased R_m in May 2009. N addition raised initial R_m, because combination of the increased N with organic matter would decrease soil C/N ratio, accelerate the decomposition of SOM, and release nutrients [34]. Under the conditions of long-term fertilization, the total soil N mineralization would increase while the R_m would decline gradually [35,38]. Gundersen [39] also found that N input could elevate the R_m only in N-limited ecosystem. On the other hand, the inhibition might be that N input changes the chemical properties of SOM, and slows extracellular enzyme activity in the decomposition process [40,41]. The inhibited generation of humus degrades enzyme for the soil with elevated available N [12]. Liu et al. [42] found no effect of N fertilization on soil microbial indices in the first growing season in temperate steppe in northern China, which was not consistent with the results of N enrichment ecosystem [43]. Therefore, the responses of soil microbial activities to N forms should be further investigated in the N-limited steppe.

We found that KNO_3 treatments decreased R_m and R_n in the later stages of 2009 (P<0.05). On the one hand, the addition of NO_3^- directly inhibited nitrification. On the other hand, such decrease maybe correlated with the fact that NO_3^- was added as KNO_3 supplying with K^+ ions. This K^+ replaced H^+ in soil exchange sites, leading to increase in the concentration of H^+ in soil solution [44]. Recent research also showed that in N-limited ecosystems, NO_3^- addition could cause slow decrease in soil pH [30]. N mineralization, especially nitrification, decreases linearly with the soil pH value [24,36]. Therefore, the effects of NH_4^+-N on the increment of soil N mineralization in the temperate semi-arid grassland is greater than that of NO_3^-N.

The reduction effects of N addition on R_m and R_n was most obvious in the warm and rainy July of 2009. The potential reason maybe that the optimal hydrothermal conditions favor soil denitrification, therefore decreased the R_m and R_n.

In the two growing seasons, in either the LN or HN treatments, $(NH_4)_2SO_4$ and KNO_3 treatments did not significantly increase soil R_a in most of the period. However, the NH_4Cl treatments significantly increased soil R_a, except for some negative values (13 July-14 August 2008, 15 May-15 June, and 15 July-12 August 2009). Those negative values could be explained by N immobilization, or by gaseous losses at nitrification for higher moisture content due to the absence of plants in the field incubation experiment [21,45,46]. The negative R_a also indicates that the available NH_4^+-N could have been oxidized rapidly into NO_3^-N under the nitrification of autotrophic bacteria, or fixed by microorganism [3,47]. The promotion effects of NH_4Cl on R_a maybe explained by the inhibition of Cl^- on the activity of soil microbial nitrification [28]. With the increase of NH_4Cl, the promotion effect of HNH_4Cl on R_a in 2008 was gradually disappeared in the later stages of 2009.

The correlations between soil parameters and R_m

While soil temperature and moisture are the main controlling factors of N mineralization, our results showed that R_m is positively correlated with SWC (P<0.05) but not significantly correlated with soil temperature (P>0.05). An explanation of these results is that our study area is located in a semi-arid climatic zone where SWC is relatively low. The mean air temperature of the area is higher than 10°C in most of the growing season. Some previous studies have shown that SWC limits NNM when SWC is less than 15%, and R_m rises with soil temperature in the temperature range from 5–35°C [48,49]. In our experiments, the SWC was less than 15% in

most of the incubation period while daily mean air temperature was between 5–23°C (Fig 1), In such conditions, NNM was likely subjected to SWC.

There was a positive significant correlation between R_m and soil TN in our experiment results, this relationship was well documented and highlight the great impacts of TN on NNM [50]. Also, there was a highly significant relationship between soil R_m and T_a in different treatments of our experiments (Table 3, P<0.05). Some previous studies have also indicated that T_a could explain NNM by more than 83% [51]. The R_m was negatively but insignificantly correlated with C/N of SOM (P>0.05). N input did not change the soil C/N in short period in our experiments. A reason behind this result could be the fertilization time and N levels in soils. Vourlitis and Zorba [13] indicated that there was a significant negatively correlation between R_m and C/N of SOM. When the C/N of SOM is greater than 30:1, it may not provide N for plant in the initial stage of mineralization. When the C/N of SOM was less than 15:1 at the beginning of its mineralization, effective N provided by SOM will exceed the amounts of microbial assimilation, which makes it possible for plants to absorb the N from SOM mineralization [52].There is a positive but not significant correlation between R_m and soil pH values in our experiment results. Prior studies have shown that soil pH controls the nitrification in soil [25,36]. Because soil pH could influence the dissolved organic matter, and the substances rich in carbon and N groups for microbial life activities, and therefore influence the N mineralization [6,47]. The correlations between R_m and environmental factors changed little in response to N addition (Table 3).

Conclusions

With designed field experiments, this study investigated the short-term effects of N addition on soil mineral N and NNM. The results demonstrated that continuous two-year N addition had significantly affected soil mineral N and NNM. $(NH_4)_2SO_4$ treatments increased soil NO_3^- concentrations, while NH_4Cl and KNO_3 treatments decreased them. Three N treatments all decreased soil NH_4^+ concentrations. As for the NNM, three N fertilizer additions significantly decreased soil R_m over the short term, and the contribution of N addition to soil R_m was higher from $(NH_4)_2SO_4$, than from NH_4Cl and KNO_3. However, we can't expect the changes of soil NNM under the increased N deposition in the future. The long-term observations of N deposition effects on soil N transformation and the complex biochemical inhibition or promotion mechanisms by which inorganic N affects soil NNM are necessary.

Author Contributions

Conceived and designed the experiments: XRL SGL. Performed the experiments: XRL JQR. Analyzed the data: XRL QWZ. Contributed reagents/materials/analysis tools: XRL JQR QWZ. Wrote the paper: XRL JQR QWZ.

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