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ORIGINAL PAPER

Carbon and nitrogen storage in plant and soil as related to nitrogen and water amendment in a temperate steppe of northern China

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Abstract We conducted a field-manipulated experiment to assess whether changes in precipitation and nitrogen (N) deposition alter ecosystem carbon (C) and N storage. Both C and N pools of plant and soil were monitored when urea-N (17.5 g N m^{-2}) and water (increasing mean annual precipitation by 50%) were added to a temperate steppe. After 2 years of treatments, both N and water addition significantly increased soil inorganic N availability by 125% and 62% during the growing season. While water addition significantly increased ecosystem C storage by 6% and N storage by 8%, N addition showed significant effects on neither of them. There were no interactions between N and water addition to affect both total C and N storage in this ecosystem, though they did interact to affect several individual pools (e.g., aboveground biomass N pool, litter C, and N pool). Results from the present study indicate that water availability is more important than N availability for C sequestration and that increasing precipitation will favor C sequestration in this semi-arid grassland.

Keywords Carbon stock · Nitrogen deposition · Precipitation regimes · Soil moisture · Soil nitrogen availability · Semi-arid grassland

Introduction

Anthropogenic-driven global change has caused dramatic changes in ecosystem structure and function by affecting resource availability and disturbance for plants (Weltzin et al. 2003), and thus may adversely affect global C and N cycles (Falkowski et al. 2000; Gruber and Galloway 2008). One of the major challenges for ecologists today is to predict how ecosystems will respond to predicted environmental changes (Knapp et al. 2008). In the past few decades, extensive works have been conducted to understand the distribution of global C and N stocks (Schimel 1995) and the effects of global change factors on C and N storage in various ecosystems (Mack et al. 2004; Xu et al. 2004; Shi et al. 2010). Moreover, it has been realized that global change factors can interact to affect several ecosystem properties and processes (Zavaleta et al. 2003; Harpole et al. 2007), which potentially can influence ecosystem C and N inputs and outputs. Thus, global change factors may interact to affect ecosystem C and N storage, especially in an ecosystem that is co-limited by N and water availability, such as the semi-arid grassland.

Human activities have dramatically altered the cycling of nitrogen on Earth, doubling the N input into terrestrial ecosystems over the past century (Gruber and Galloway 2008). Elevated N inputs into terrestrial ecosystems are causing major changes in ecosystem functioning. Nitrogen deposition or addition has been reported to have variable influences over ecosystem C and N pools. Although

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increased N availability has been shown to enhance plant growth and net primary productivity (Bai et al. 2008), varying responses of soil C and N stocks to N addition have been reported. For example, results from a prairie ecosystem showed soil C and N stocks are significantly higher under N fertilization treatment (10 g m^{-2} $year^{-1}$), and can be explained by the higher arbuscular mycorrhizal (AM) fungi abundance (Wilson et al. 2009). In a dry meadow community of alpine tundra in Colorado, Neff et al. (2002) observed no significant changes in soil C stocks in response to N addition. Moreover, N addition may have different effects on C and N stocks in various pools of a certain ecosystem, such as plant, organic and mineral soil pools (Mack et al. 2004). Therefore, the effects of N deposition/addition on ecosystem C and N storage remain controversial.

Global climate models predict that precipitation will be increased at the midlatitude regions (IPCC 2007). In water-limited ecosystems, altered precipitation regime will impact ecosystems to an even greater degree than increases in atmospheric CO_2 and temperature (Weltzin et al. 2003). Altered precipitation regimes can influence ecosystem C and N storage through several pathways. Aboveground net primary productivity is positively related with annual precipitation in grasslands (Bai et al. 2008). Water addition stimulates gross ecosystem productivity, ecosystem respiration and net ecosystem exchange (Niu et al. 2009). Increased soil water availability can enhance the nutrient concentrations of senesced leaves (Lü and Han 2010) and soil N availability (Wang et al. 2006), which have great implications for litter decomposition and consequently for C and N cycling. Furthermore, an altered precipitation regime can change plant community composition and diversity (Weltzin et al. 2003), which are important controls for ecosystem C sequestration and N storage (Oelmann et al. 2007).

Grassland, one of the most widespread vegetation types worldwide, accounts for 40% of the national land area in China (Kang et al. 2007). Aboveground biomass C stocks for China's grasslands averaged 145.5 Tg C and with an annual increase of 0.7% during 1982–1999 (Piao et al. 2007). The temperate steppe in northern China is a typical vegetation type in the Eurasian continent. In this ecosystem, primary production is tightly linked to both long-term average and inter-annual precipitation patterns (Bai et al. 2004, 2008; Xia et al. 2009), suggesting that future climate changes may have significant consequences for ecosystem function. Moreover, many grassland ecosystems all over the world are experiencing simultaneous increase in both N and water availability (Harpole et al. 2007). However, changes in C and N content and storage in both plant and soil components of grasslands, and their relations with N and water availability are not well known. In grassland ecosystems, less than 10% of organic C is stored in the aboveground biomass while the remainder is in belowground. Thus, it is important to consider not only aboveground storage but also belowground (Kitchen et al. 2009).

In order to examine the effects of N and water addition and their interactions on ecosystem C and N storage, we conducted a manipulative experiment in a temperate steppe of northern China. In general, this area is both N and water deficient as indicated by the strong stimulation of ecosystem primary productivity in response to N addition (Bai et al. 2010; Niu et al. 2010) and higher precipitation in the wet years (Bai et al. 2004, 2008; Xia et al. 2009). The specific questions addressed in this study are (1) how do ecosystem C and N storage and soil N availability respond to N and water amendments in this semi-arid grassland? (2) Are there any interactive effects between N and water addition on ecosystem total C and N storage and individual pools (aboveground biomass, root, litter, and soil) in this ecosystem?

Materials and methods

Study site

Our study was conducted in a typical steppe ecosystem (43º33′00″ N, 116º40′20″ E, 1,250 m asl) located in proximity to the Inner Mongolia Grassland Ecosystem Research Station. The study area is located in a gently rolling landscape. Topographic relief exhibits little variation, with elevation ranging from 1,250 to 1,260 m in the studied site. The long-term mean annual, minimum and maximum air temperatures for this area are 1.1°C, −20.8°C, and 16.4°C. Mean annual precipitation is 345 mm with 90% distributed from May to September. Soil in the top 40-cm layer is classified as calcic-orthic aridisol according to the US soil classification (He et al. 2008), below 40 cm a mixture of sandy soil and gravels. The study site had been fenced to exclude grazing disturbance from large mammals since 1999. The community is characterized by the dominance of perennial species *Leymus chinensis* Tzvel., *Stipa grandis* Smirn., *Achnatherum sibiricum* (Linn.) Keng., *Cleistogenes squarrosa* (Trin.) Keng., and *Agropyron cristatum* (L.) Gaertn.

Experimental design

The experiment consisted of four treatments (control (C), N addition (N), water addition (W), and both N and water addition (NW)) replicated five times, resulting in twenty 4×4-m plots, with a 1-m buffer between each plot. Treatments were randomly assigned to plots within each block. The distance between the experimental setup and the next fence was more than 50 m. On 10 May 2006, we compared litter biomass (within a quadrat of 0.5×0.5 m in each plot), root biomass (four cores with the diameter of 8 cm in each 0.5× 0.5-m quadrat, 0–40 cm) and soil total N and inorganic N in the plots and found no significant difference ($P>0.05$). We added N (urea in dry form) during the rainy days of mid-May and early July 2007 and 2008, totaling 17.5 g N m^{-2} $year^{-1}$. This amount was selected because it is in the range found to have a significant effect on primary productivity in this area (Bai et al. 2008). For water addition treatments, 10 mm of tap water was manually applied with a sprayer every week throughout the growing season (May–September) in 2007 and 2008. The aim of the water-addition treatment was to reduce water stress and to increase long-term mean annual precipitation by approximately 50%. Water was always applied after 16:00 p.m. to prevent rapid loss by evaporation. In total, water was added 18 times each year, amounting to 180-mm water $year^{-1}$. The annual precipitation was 240 mm (192 mm for May–September) in 2007 and 362 mm (295 mm for May–September) in 2008.

Field sampling and laboratory analysis

Field sampling was conducted in mid-August 2008, which is the typical period when aboveground biomass attains its peak value in this ecosystem (Bai et al. 2010). Aboveground vegetation was sampled by clipping all plants at the soil surface using a 1×1-m quadrat, which was placed at least 50 cm inside each plot to avoid edge effects. This quantity could be considered approximately equal to the aboveground net primary productivity of the current year (He et al. 2008; Bai et al. 2010). Litter samples were collected subsequently from each 1×1-m quadrat. The plant tissues were oven-dried at 70°C for 48 h.

Root biomass was determined by soil coring method. Soil cores from four random locations were collected within each 1×1-m quadrat established for aboveground biomass sampling by using an 8-cm diameter soil core sampler. The samples were separately collected from four layers at the depths of 0–10, 10–20, 20–30, and 30–40 cm. Root material from each layer of one quadrat were separated from soil using a 1-mm sieve followed by wet sieving using a 0.3-mm sieve. Dry mass of roots was determined by oven-drying at 70°C for 48 h. Soil sampling was conducted using a 4-cm diameter soil sampler, and the samples were separately collected from four layers (10 cm for each layer). All soil samples were air-dried in a ventilation room, and cleared of roots and plant debris before being prepared for chemical analysis. Soil bulk density was measured within each quadrate for the four layers.

The top 10 cm of soil from each plot were sampled with a soil corer (5 cm in diameter) and analyzed for gravimetric water moisture and inorganic N ($NH_4^+ - N$ and $NO_3^- - N$) every month from June to October 2008. Gravimetric water moisture was determined by drying at 105°C for 24 h. To analyze inorganic N, 50 ml of 2 M KCl solution was added to a 10 g soil sample. The mixture of soil and extractant was shaken for 1 h on a reciprocal shaker, and then the soil suspension was filtered (Whatman No. 1 filter paper). Soil solutions were kept frozen prior to analysis for $NH_4^+ - N$ and $NO_3^- - N$ on a FIAstar 5000 Analyzer (Foss Tecator, Denmark).

Organic C contents of plant and soil samples were determined by a modified Mebius method (Nelson and Sommers 1982). Total plant and soil N were measured using the modified Kjeldahl wet digestion procedure (Gallaher et al. 1976) with $NH_4^+ - N$ analyzed colorimetrically by the alkali method with a 2300 Kjeldahl Analyzer Unit (FOSS, Sweden).

We calculated the total soil organic C density (TSOC; g C m^{-2}) and total soil N density (TSN; g N m^{-2}) on a ground area basis as follows:

$$\mathrm{TSOC} = \sum D_i \times P_i \times \mathrm{OM}_i \times S \times R$$

$$\mathrm{TSN} = \sum D_i \times P_i \times \mathrm{TN}_i \times S \times R$$

where D_i, P_i, OM_i, TN_i, and S represent the soil layer thickness (cm), bulk density (g cm^{-3}), organic C concentration (%), total N concentration (%), and cross section area (cm^{-2}) of soil core of the ith layer, respectively; and i= 1, 2, 3, and 4; R=(1−weight of rocks/weight of soil)× 100%, as the soils contained no rocks, here R=1.

Statistical analysis

All data were expressed as mean±1 SE. Data were tested for normality using the Kolmogorov–Smirnov test and for homogeneity of variances using Levene's test. Repeated measurement analysis of variance (ANOVA) was used to determine the effects of N and water addition on soil KCl-extractable N pools. Three-way ANOVAs were used to determine the effects of N, water addition, soil depth and their interactions on soil C and N concentrations. Two-way ANOVA was used to determine the effects of N and water addition on plant and soil C and N pools. All statistical analyses were conducted with SPSS (Version13.0 for windows, SPSS Inc., Chicago, IL, USA), and in all cases used a significance level of 0.05.

Results

Inorganic N in the soil

Both N and water addition significantly enhanced soil mineral N availability ($P<0.001$; Table 1; Fig. 1). Across

Table 1 Results (*F* values) of repeated measures ANOVAs on the effects of N addition (N), water addition (W), sampling month (M), and their interactions on soil $NH_4^+ - N$, $NO_3^- - N$, and total inorganic N (In-N) concentrations

	$NH_4^+ - N$	$NO_3^- - N$	In-N
N	26.34*	52.63*	76.91*
W	0.06	115.44*	58.545*
N×W	1.41	0.05	0.50
M	13.60*	17.61*	21.77*
N×M	15.42*	13.67*	20.33*
W×M	1.17	3.05***	0.83
N×W×M	0.99	2.21****	0.11

*$P \leq 0.001$; ***$P \leq 0.05$; ****$P \leq 0.1$

the growing season of 2008, N addition increased soil $NH_4^+ - N$, $NO_3^- - N$ and inorganic N concentrations by 143%, 112%, and 125% (all $P<0.001$; Table 1; Fig. 1), respectively. Sampling date interacted with N addition to affect soil mineral N concentrations (Table 1), in that N addition significantly enhanced mineral N concentrations in the first 2 months but not in the next 3 months during the growing season. Water addition enhanced soil $NO_3^- - N$ and inorganic N concentration by 109% and 62% (all $P<0.001$), whereas it had no impact on soil $NH_4^+ - N$ (Table 1). There was no interaction between N and water addition to affect soil mineral N concentration (Table 1).

Carbon and N in different soil depth increments

Results from three-way ANOVAs indicated significant main effects of N addition, water addition and depth on both soil C and N concentration and content except that there was no significant effect of N addition on soil C content (Table 2). Nitrogen and water addition significantly interacted to affect soil C concentration and content but did not affect soil N concentration and content. A significant interaction between water addition and depth occurred to affect soil C concentration. The effects of N and water addition on soil N concentration depended on soil depth, as indicated by the significant interactions between them.

Bulk soil C and N content declined exponentially with depth (Fig. 2a, b). In the top 10 cm, soil C and N content were significantly enhanced under elevated water conditions (Fig 2a, b).There were no significant main effects of N addition, water addition and depth and no interactions among them on soil C/N ratios (Table 2). However, separate one-way ANOVA by depth increment revealed significantly lower C/N in the N and water combined plots than control plots in 0–10 cm depth (Fig. 2c).

Ecosystem C and N storage

Water addition significantly increased ecosystem total C storage by 10% (Fig. 3). Neither N addition nor its interaction with water addition had significant effects on ecosystem total C storage. The C storage varied substantially among different pools (Fig. 3), with 90% of the total storage distributed in soil. Water addition increased aboveground biomass and litter C pools, whereas showed no effects on roots C and soil C (Fig. 3). Nitrogen addition failed to show a significant effect on ecosystem total C

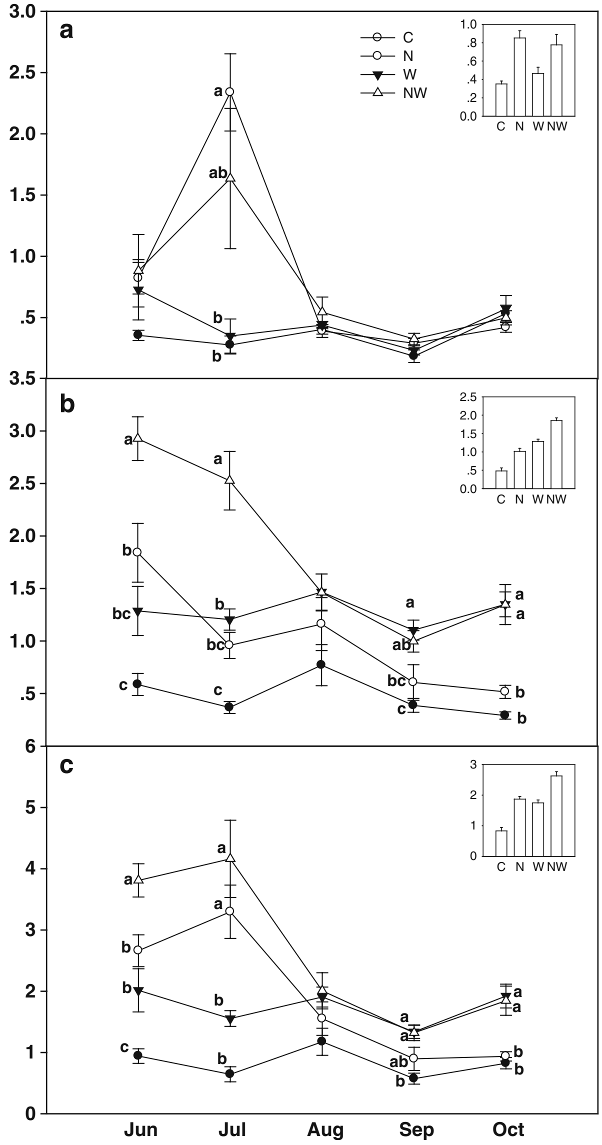

Fig. 1 Seasonal dynamics and means (columns, means±1 SE; $n=5$) of soil $NH_4^+ - N$ (**a**), $NO_3^- - N$ (**b**), and total inorganic N (**c**) concentrations in 2008. *Bars* represent SE values. *Different letters* indicate significant differences ($P \leq 0.05$) among treatments within the same month. *C* control, *N* N addition, *W* water addition, *NW* N and water addition

Table 2 Results (*F* values) of three-way ANOVAs on the effects of N addition (N), water addition (W), soil depth (D), and their interactions on soil C (C%) and N concentration (N%), soil C and N content, and soil C/N ratio (C/N)

	C%	N%	C content	N content	C/N
N	14.33*	15.47*	0.05	4.11***	1.29
W	34.19*	24.99*	5.43**	15.52**	0.69
D	1194.28*	325.79*	145.18*	140.41*	2.33****
N×W	5.74***	1.41	6.74***	0.01	3.57****
N×D	2.60****	3.57***	0.17	1.52	0.73
W×D	5.79**	7.72*	1.20	5.57**	0.42
N×W×D	1.60	0.94	0.96	1.29	0.66

**P*≤0.001; ***P*≤0.01; ****P*≤0.05; *****P*≤0.1

storage. Water and N addition interacted to affect C storage in litter, whereby N addition increased litter C pool under ambient water conditions but decreased it under elevated water conditions (Fig. 3).

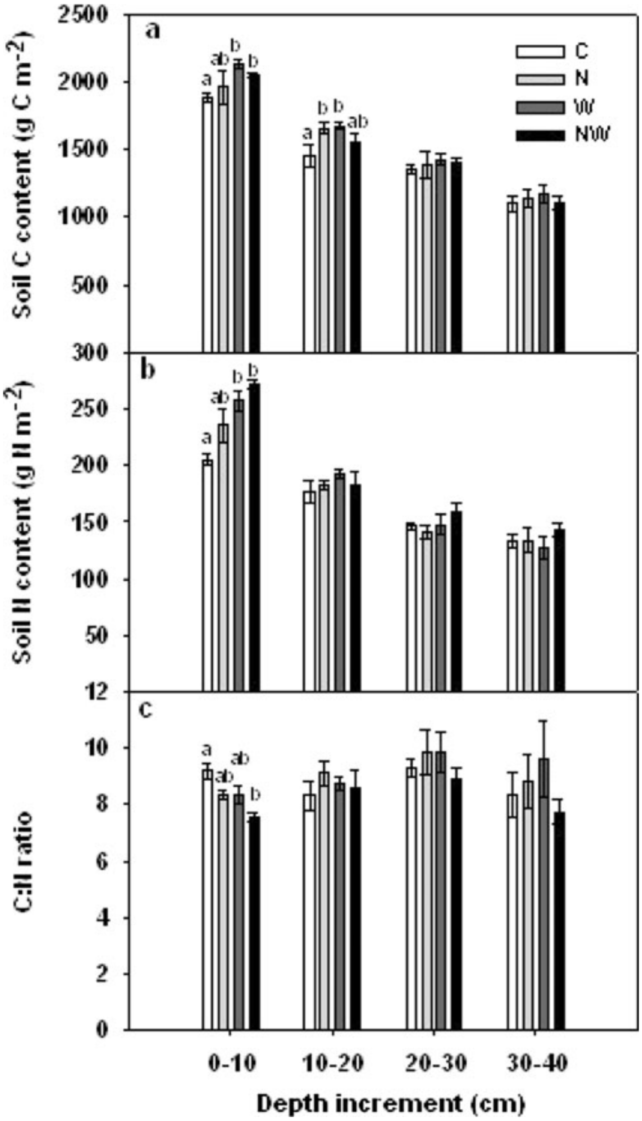

Fig. 2 Effect of N and water addition on soil C (**a**), N (**b**) contents, and soil C/N ratio (**c**) (mean±1 SE; *n*=5) at different soil depths. *Letters* indicate significant differences ($P \leq 0.05$) among treatments *C* control, *N* N addition, *W* water addition, *NW* N and water addition

Water addition significantly increased ecosystem total N storage and N pools in aboveground biomass, litter, and soil (Fig. 4). Nitrogen addition enhanced aboveground biomass and litter N pools but only under ambient water condition (significant N×W interactions; Fig. 4). In contrast, N addition did not affect the N storage in roots, soil, and the entire ecosystem (Fig. 4).

Discussion

Effects of N addition

Results from this study showed that N addition had no significant effect on either total ecosystem C storage or individual C pool (aboveground biomass, litter, roots and soil) in this semi-arid grassland ecosystem over the first 2 years of the treatments (Fig. 3). We attributed the unchanged C storage in aboveground biomass and litter in response to N addition to the limited effect of N addition on aboveground productivity in 2007 and 2008 (Lü, unpublished data). In a recent study, Bai et al. (2010) found that N addition, with a rate up to 17.5 gm^{-2}, usually showed no effects on aboveground productivity in the first 2 years of treatments in this semi-arid grassland. Nitrogen fertilization will lead to shifts in plant functional group composition in this area (Bai et al. 2010), with perennials being replaced by fast-growing annuals. However, this replacement may have not occurred in the short-term. In fact, N addition showed neutral or negative effects on the aboveground biomass of dominant perennial grasses in the mature grassland ecosystem in this region (Bai et al. 2010). Moreover, we found that C storage in roots did not change after 2 years of N addition. This may largely be accounted for by unchanged root biomass in response to N addition (Two-way ANOVA, $F=1.849$; $P=0.193$). These results suggest that unchanged aboveground biomass in response to N amendment in the initial 1 or 2 years after experimental N addition may be mirrored by similar responses of C storage.

The C stored in soil organic matter generally accounts for ~90% of total C storage in this region (He et al. 2008). The change of soil C storage in response to global change

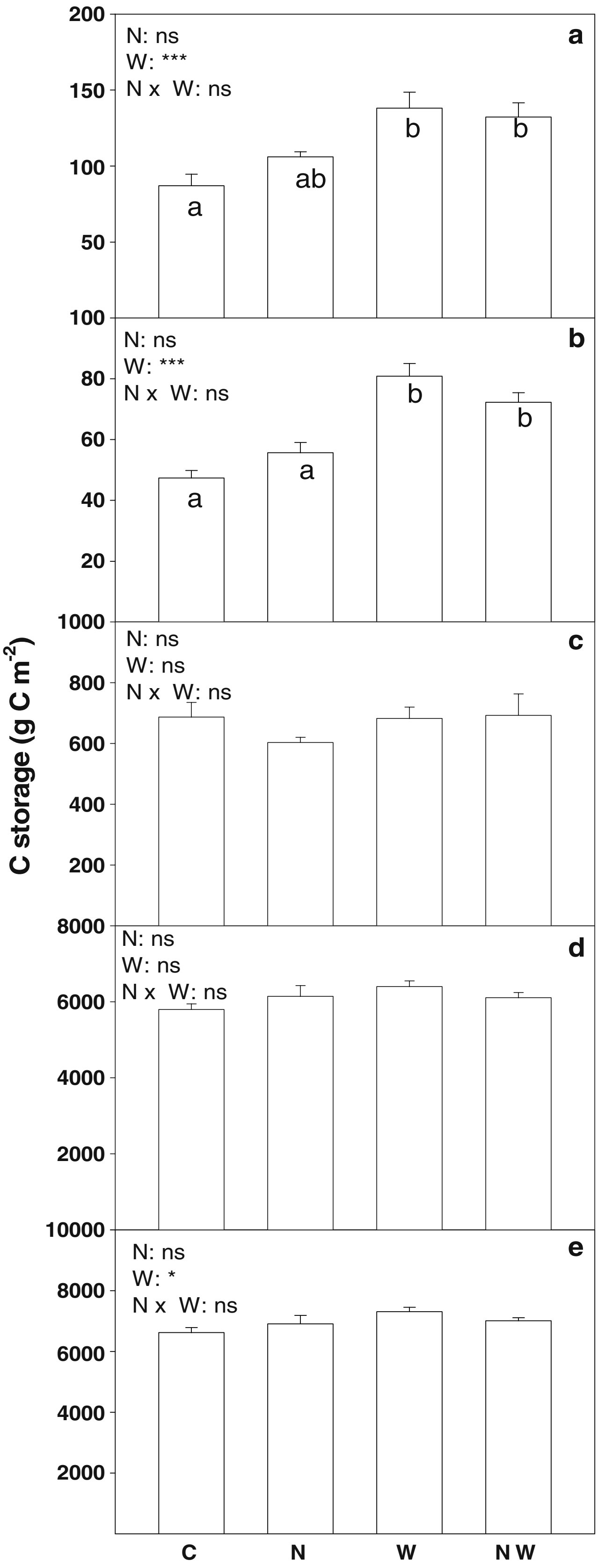

Fig. 3 Effect of N and water addition on C pools in aboveground biomass (**a**), litter (**b**), roots (**c**), soil (**d**), and ecosystem total C storage (**e**) in a semi-arid grassland of northern China. Data are represented as mean±1 SE (*n*=5). *Letters* indicate significant differences ($P \leq 0.05$) among treatments. *C* control, *N* N addition, *W* water addition, *NW* N and water addition. *$P \leq 0.05$; **$P \leq 0.01$, ***$P \leq 0.001$

factors depends on the balance between C inputs to soil by net primary productivity and C outputs by soil organic matter decomposition (von Lützow and Kögel-Knabner 2009). Results from this study show that the short-term effect of N addition on this large C pool was not significant. Given the failure of N addition to affect plant C pool in this ecosystem, our results indicate that the short-term effects of N addition on microbial biomass and activities may be limited. In fact, results from another experiment that focused on soil microbial community in this region showed no statistically significant differences between the N addition plots (16 gN m^{-2} $year^{-1}$) and the control plots with respect to microbial biomass C, microbial biomass N and soil organic matter concentration after 3 years of N addition (Zhang et al. 2008). Furthermore, a recent study in the same area reported that N addition had no significant effect on total soil respiration over two growing seasons (Yan et al. 2010). It is well known that AM fungi are important belowground C sinks and sensitive to soil N availability. However, the responses of fungal communities to N addition depend on plant C inputs (Allison et al. 2007), and N addition may have no significant effects on fungi biomass in some grasslands (Murray et al. 2006). Considering that the soil C sequestration should be a slow process, long-term experiments are needed to evaluate the role of increased N availability in determining the changes of soil C stocks.

It is reasonable to expect that N addition with a rate up to 17.5 gm^{-2} $year^{-1}$ would significantly enhance ecosystem N storage. Nitrogen addition did significantly increase soil mineral N concentrations during the growing season in this ecosystem, which have also been found in other studies (Zhang et al. 2008; Bai et al. 2010). However, N addition had no significant effects on either total N storage or individual N pools in this ecosystem except for aboveground biomass N pool (Table 2). Similarly, Vourlitis et al. (2009) showed that short-term N addition (4 years) failed to stimulate ecosystem N storage in Mediterranean-type shrubland. The enhanced aboveground biomass N pool in response to N addition in this study largely resulted from increased N concentration of plant tissues (Lü and Han 2010) but not from the improvement of biomass. It is notable that the significant effect of N addition on aboveground biomass N pool was only observed under ambient water conditions, indicating this ecosystem was more water limited than N limited. Soil C/N ratio has been suggested as a sensitive indicator of N impacts on soil (Evans et al. 2006). A decline in soil C/N ratio with N

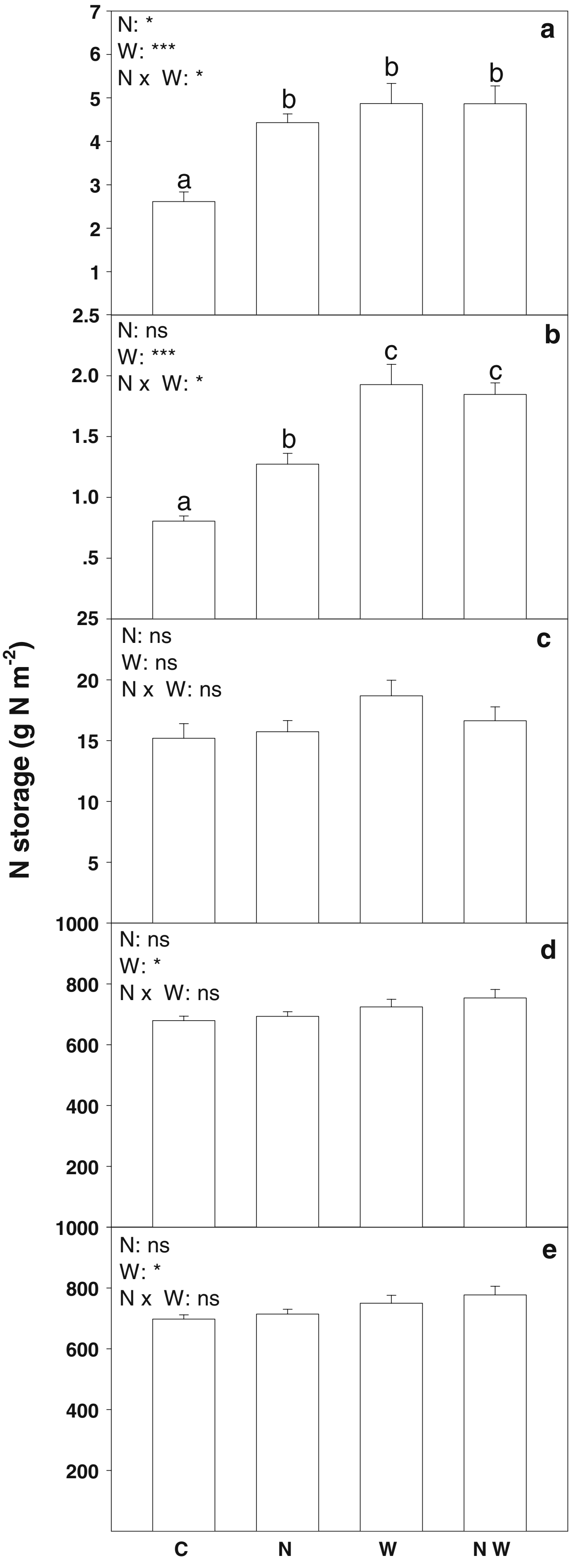

Fig. 4 Effect of N and water addition on N pools in aboveground ▶ biomass (**a**), litter (**b**), roots (**c**), soil (**d**), and ecosystem total N storage (**e**) in a semi-arid grassland of northern China. Data are represented as mean±1 SE ($n=5$). *Letters* indicate significant differences ($P \le 0.05$) among treatments. *C* control, *N* N addition, *W* water addition, *NW* N and water addition. *$P \le 0.05$; **$P \le 0.01$; ***$P \le 0.001$

addition has been widely observed, resulting from either N enrichment (Baron et al. 2000) or soil C loss (Vourlitis et al. 2007). In this study, N addition did not affect both soil C and N content, consequently the soil C/N ratio. These results indicate that the temperate steppe in this area had limited ability to retain additional N inputs, at least in the short-term. It is important to underline that it is well established that measurements of organic C and total N contents of soil are not sensitive to changes in environmental factors and agricultural management.

There are several possible pathways for the loss of added N in this ecosystem. Firstly, the soil depth beyond 40 cm may respond to aboveground environmental variation, as suggested by Kitchen et al. (2009), who found that sampling to a depth of 90 cm can also reveal important root responses to treatment in prairie ecosystem. Secondly, N leaching represents a potentially significant pathway of N loss. Nitrate-N, the most mobile form of N, usually dominates the components of soil inorganic N in the temperate steppe (Wang et al. 2006). In the urban grasslands of Maryland, Groffman et al. (2009) found that annual $NO_3^- - N$ leaching can reach up to 4.1 g N m^{-2} year^{-1}. The temporal trend of soil inorganic N in this study showed that inorganic N concentration was much lower in the late (September and October) than the early stage of the growing season (Fig. 1). We attribute this to the heavy rain during the end of July and the beginning of August (117 mm from 31st July to 11th Aug and 63.3 mm in 31st July), because precipitation intensity would significantly enhance $NO_3^- - N$ leaching (Sugita and Nakane 2007). Thirdly, N loss from NO and N_2O emissions has been found to be substantial in the grassland ecosystems that received additional N input (Mosier et al. 1998), even in a relatively short-term (Dittert et al. 2005). Furthermore, N may return directly to the atmosphere via abiotic pathway (solar radiation drives N gas loss) in this semi-arid ecosystem due to the high soil-surface temperature as that has been observed in the arid environment (McCalley and Sparks 2009).

Effects of water addition

Water addition significantly enhanced plant and soil C storage in this semi-arid grassland ecosystem and the increased aboveground biomass and litter C pools in response to water addition may have accounted for this change (Fig. 3). Water addition significantly increased aboveground productivity by 60% (Lü, unpublished data), which may explain the increased aboveground biomass C pool under elevated water conditions. Previous studies also reported that water addition can significantly stimulate gross ecosystem productivity in this semi-arid grassland (Niu et al. 2009). Water addition failed to affect soil C storage in the present study, we assume it is because the amount of precipitation is not as important as precipitation distribution in influencing carbon sequestration in this area as suggested by Chen et al. (2009). The result also indicates that gains in productivity may translate into increased soil C inputs, but not necessarily into increased soil C sequestration. As primary productivity is positively related with annual precipitation (Bai et al. 2008) and water addition usually enhance net ecosystem exchange in this area (Niu et al. 2009), it is reasonable to expect water addition will increase soil C storage in the long-term.

Both plant available soil N concentration and total ecosystem N storage showed a positive response to water addition in this study. Similar results have been found in a regional scale experiment carried out in the grasslands of the Central Great Plains, in which McCulley et al. (2009) observed that ecosystem N storage increased across the precipitation gradient. The positive effects of water addition on soil inorganic N concentrations in this ecosystem can be attributed to the increased microbial activities in response to increased water availability as reported by Liu et al. (2009). The simultaneous alleviation of water and N limitation in the watered plots resulted in increased aboveground biomass and enhanced N concentrations in plant tissues (Lü and Han 2010), and consequently higher N storage in both aboveground biomass and litter. Water addition stimulated not only N pools related with plant but also the soil N pool, probably due to altered N cycling under elevated water environment. Many studies have demonstrated that water additions affect litter decomposition dynamics (Liu et al. 2006; Xiao et al. 2007), stimulate litter mass loss and nutrient releasing, especially for plants with higher litter quality (Liu et al. 2006). Thus, more N was given back to soil pool in the watered plots than that in the control plots. On the other hand, increased plant material supply under elevated water conditions will stimulate N immobilization and reduce denitrification losses (Crasweel 1978). In fact, water-initiated increases of soil N concentration and content only happened in the surface layer (Fig. 2), indicating that the changes in the soil N pool may have resulted from litter decomposition and microbial immobilization.

The interactive effects of N and water addition

Partially consistent with the results of a study that found no interactive effects of N and water addition on ecosystem C fluxes in semi-arid grassland (Niu et al. 2009), we observed no significant interactions between N and water addition to affect ecosystem total C and N storage. However, N and water addition interacted to affect the litter C and N pool and aboveground biomass N pool. For the litter pool, N addition tended to increase C and N storage under ambient water conditions, whereas it tended to decrease them under elevated water environment. This may be partly explained by the shifts of water or N limitation under these treatments. In the plots that received only additional N, the decomposition may be limited by water availability (Liu et al. 2006). In contrast, when water and N were added in combination, the rate of litter decomposition may be higher and resulting in lower litter biomass storage. As for the effects of N and water addition on aboveground biomass N storage, N addition significantly increased it under ambient water conditions but not under elevated water conditions. A probable explanation is that water addition can significantly enhance soil inorganic N concentrations and plant tissue N concentration (Lü and Han 2010), so the effects of N addition may be limited when it was added together with water. These results suggest that water availability may be relatively more important than N availability in regulating plant C and N pools in this semi-arid grassland. Similarly, Seagle and McNaughton (1993) also reported primary limitation by water instead of N in a modeling study, though other studies observed co-limitation of water and N in different grassland ecosystems (Harpole et al. 2007). All these differences may be resulted from the relative importance of N and water in regulating ecosystem processes in different locations (Niu et al. 2009).

Conclusions

With a field manipulative experiment, this study investigated the short-term effects of N and water addition on grassland ecosystem C and N storage. Admittedly, this study suffers from several caveats that limit the potential for a complete understanding of how N deposition and increased precipitation will influence C and N storage in the temperate steppe of northern China. Firstly, N input from atmospheric deposition cannot be well simulated as urea fertilizer was used. Secondly, the amount of water added is much higher than the potential increase of precipitation in this area in the near future. Third, this study is based on a limited temporal (the second year of treatments) and spatial (in one site with mature community) perspective. Both soil organic C and total N were not sensitive to the 2-year treatments of N and water addition. It is well known that at least 5–10 years are needed to detect significant changes in both pools. It remains unknown whether the results from the present study can be generalized over longer temporal and larger spatial scale. Despite all these possible limitations, our results highlights the importance of water availability in regulating responses of ecosystem C and N storage in semi-arid grassland in the short-term. Based on the results from this study and those from a recent study by Bai et al. (2010), we suggest that longer term responses of this semi-arid grassland ecosystem to N addition should be included in the future research.

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