Effects of water and nitrogen addition on vegetation carbon pools in a semi-arid temperate steppe

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Abstract Global change will lead to increases in regional precipitation and nitrogen (N) deposition in the semi-arid grasslands of northern China. We investigated the responses of vegetation carbon (C) pools to simulated precipitation and N deposition increases through field experiments in a typical steppe in Inner Mongolia. The treatments included NH_4NO_3 addition at concentrations of 0 (CK), 5 (LN, low nitrogen), 10 (middle nitrogen, MN), and 20 (HN, high nitrogen) ($\text{g m}^{-2} \text{a}^{-1}$) with and without water. After three consecutive years of treatment, from 2010 to 2012, water addition did not significantly change the size of the

total vegetation C pools, but it significantly decreased the ratio of root:shoot (R:S) ($P = 0.05$) relative to controls. By contrast, N addition significantly increased the total vegetation C pools. The C pools in the LN, MN and HN treatments increased by 22, 39 and 44 %, respectively. MN produced the largest effect among the N concentrations, although differences between N-added treatments were not significant ($P > 0.05$). N addition significantly reduced the ratio of root:shoot (R:S) ($P = 0.03$). However, there were no significant interactive effects of water and N addition on the vegetation C pools.

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Introduction

Photosynthetic carbon (C) fixation by vegetation is one of the major processes controlling the C input to terrestrial ecosystems (Joiner et al. 2011). Soil organic C, the largest C reservoir in many terrestrial ecosystems (Schlesinger 1977), is primarily plant derived (Kuzakov and Domanski 2000; Ryals and Silver 2013). Over the past 50 years, terrestrial vegetation has absorbed 25–30 % of anthropogenic CO_2 emissions, with the C stored in vegetation and soil (Reichstein et al. 2013). Terrestrial gross primary production, which is approximately $(123 \pm 8) \text{ Pg a}^{-1}$, is the largest global component of C flux and directly influences the C budget of terrestrial ecosystems (Malhi 2012; Beer et al. 2010). Understanding changes in plant C-fixation capacity and its main influencing factors are important areas of C cycle research.

At present, the rapid changes in atmospheric CO₂ concentration, temperature, precipitation, and nitrogen (N) deposition have drawn global attention (Galloway and Cowling 2002; IPCC 2007). As described in the fifth IPCC assessment report, the response and feedback of the C cycle to future climatic and environmental changes have been fully accounted for in most of the earth system models of the Coupled Model Intercomparison Project Phase 5 (CMIP5) (Yu and Piao 2014). Among all the global change factors, water and soil nutrient availability are important limiting factors for plant growth in most terrestrial ecosystems, particularly in arid and semi-arid ecosystems (Belnap 2001; Austin et al. 2004; Guo et al. 2006; Zhao et al. 2009). However, to date, most studies of the C cycle processes of terrestrial ecosystems occurring under the scenario of global change have focused on soil C sequestration and emission (Craine and Gelderman 2011; Jin et al. 2007; Asner et al. 2004; Groenigen et al. 2011), and few have focused on vegetation C fixation. An improved understanding of the response and sensitivity of terrestrial vegetation C fixation to global environmental change can increase our ability to accurately predict the C budgets of terrestrial ecosystems.

In recent years, increasing N deposition and shifts in precipitation regimes have become global trends (Galloway and Cowling 2002; IPCC 2007). Asia, including China, is one of the three regions with the highest N deposition rates in the world, due to high levels of fertilizer application, fossil fuel combustion, and other human activities (Dentener et al. 2006; Zhang et al. 2008). Meanwhile, a global increase of approximately 0.5–1 % in precipitation per decade is predicted over this century (IPCC 2001), and in northern China, annual precipitation is predicted to increase by 14–155 mm by the end of the century, according to the simulations of 13 IPCC global climate models (Jiang et al. 2008). Changing patterns of N deposition and precipitation can greatly change plant growth and vegetation C pools in terrestrial ecosystems (Potts et al. 2006; Yu et al. 2009; Thomas et al. 2010); however, there remains considerable uncertainty regarding the effects of increasing water and N inputs. For example, many studies have reported that additional N should increase the aboveground C pools in grasslands (Wedin and Tilman 1996; Foster and Gross 1998; Xia and Wan 2008). Gough et al. (2000) found that the aboveground biomass increased by 50 % under N fertilization in North American herbaceous ecosystems; however, others studies reported either no effects (Bai et al. 2010) or negative effects (Vitousek and Howarth 1991). Water availability is critical to all life (Wu 2011) and is another important factor influencing the primary production of ecosystems. In an Amazon rainforest, Phillips et al. (2009) found that before 2005, plots exhibited a long-term net increase of approximately

0.89 Mg ha⁻¹ a⁻¹) in aboveground biomass, but after encountering intense drought in 2005, this rate declined by 2.39 Mg ha⁻¹ a⁻¹ in the 28 plots suffering the greatest water deficits. In arid and semi-arid ecosystems, the limitations of precipitation on plant growth will be even more pronounced (Sims and Singh 1978; Le Hourou et al. 1988; Bai et al. 2004; Dalglish and Hartnett 2006; Swemmer et al. 2007; Van Aukn 2009). However, more rainfall does not necessarily enhance growth. Wang and Zhou (2004) found that as annual rainfall exceeded 350 mm, aboveground net primary productivity (ANPP) in *Leymus chinensis* grassland declined. These studies indicate that there are complex effects of increasing N deposition and increasing precipitation on terrestrial vegetation. In addition to the main effects of water or N, global change involves concurrent changes in multiple factors, which could yield complex interactive effects (Dukes et al. 2005; Bell et al. 2008). However, little information exists on the interactive effects of water and N on vegetation C pools in arid and semi-arid ecosystems. Thus, study of the vegetation C pool responses to changes in water and N availability is urgently needed.

In China, grassland is the largest terrestrial ecosystem, covering 40 % of the total land area (Chen and Wang 2000). The total biomass of grassland vegetation in China is approximately 1044.76 Tg (Piao et al. 2004). Approximately 78 % of grasslands in China are located in temperate arid and semi-arid regions where the fragile environments and intensive human activity increase the sensitivity of local ecological processes to environmental change relative to other ecosystems (Warren et al. 1996; Wen 1996). We studied the main and interactive effects of increasing precipitation and N deposition on vegetation C pools in a temperate, semi-arid typical steppe in Inner Mongolia, China. Our objectives were to (1) identify the differential responses of vegetation C pools to increased N and water availability and (2) determine whether there are significant interactive effects of water and N on vegetation C pools.

Materials and methods

Experimental site

The experiment site was a *Leymus chinensis* steppe (4333'10"N, 11640'29"E; 1265 m above mean sea level) in Baiinxile pasture, Xilin River Basin, Inner Mongolia, China. The site was near the Inner Mongolian Grassland Ecosystem Research Station of the Chinese Academy of Sciences (Fig. 1). The Xilin River Basin is in the core area of the Northeast China Transect (NECT) for the International Geosphere-Biosphere Program (IGBP) for the study

Fig. 1 Map of the sampling site in the Xilin River Basin of Inner Mongolia, China

of global change. The temperate typical steppe along this transect is representative of grasslands in China and Eurasia (Zhang et al. 1997; Qi et al. 2014). The climate is semi-arid, temperate with mean annual temperature of -0.4°C , ranging from -21.41°C in January to 18.53°C in July. Mean annual precipitation is approximately 354 mm (70 % falls between July and September). The plant community was dominated by *L. chinensis*, *Stipa grandis*, *Agropyron michnoi*, and *Cleistogenes squarrosa*. The experimental site had been used for grazing prior to enclosure, with a grazing intensity of approximately 2.25 sheep per hectare. The soil is classified as chestnut soil according to Chinese soil classification or as Calcic-orthic Aridisol according to soil taxonomic classification. The soil is comprised of 60 % sand, 21 % clay, and 19 % silt. The depths of the soil profiles were 100–150 cm, with a 20–30 cm humus layer. Characteristics of the soils in the experimental plots are listed in Table 1.

Experimental design

In May 2010, an area of steppe 51×78 m with flat topography and relatively uniform vegetation distribution

was fenced. Forty $8 \text{ m} \times 8 \text{ m}$ plots separated by 1 m buffers were demarcated, with eight treatments and five replicates assigned at random among the plots to examine the main and interactive effects of water and N addition. The eight treatments included two water treatment levels (added/not added) and four N treatment levels; CK, low nitrogen (LN, $5 \text{ g m}^{-2} \text{ a}^{-1}$), middle nitrogen (MN, $10 \text{ g m}^{-2} \text{ a}^{-1}$), and high nitrogen (HN, $20 \text{ g m}^{-2} \text{ a}^{-1}$).

Each year, the equivalent of 51.7 mm rainfall, representing approximately 15 % of the mean annual rainfall at the study site, was applied to the added water treatment plots. This amount was based on the predicted future increase of 12–18 % by the end of the 21st century, according to 13 IPCC-AR4 models for northern China (Jiang et al. 2008). From 2010 to 2012, the water was manually added each month from June to September using backpack sprayers. The water input each month was calculated as the proportion of rainfall in that month relative to the total amount over the 4 months. This amount was then divided by two and applied twice per month (usually mid-month and late in the month) to ensure each addition was equivalent to the median amount of the daily rainfall in this area. For the N treatments, NH_4NO_3 was ground into

Table 1 Characteristics of *L. chinensis* steppe soil

Soil sampling depths (cm)	Total organic C (g kg^{-1})	Total N (g kg^{-1})	C/N ratio	$\text{NH}_4^{+}\text{-N}$ (mg kg^{-1})	$\text{NO}_3^{-}\text{-N}$ (mg kg^{-1})	Bulk density (g cm^{-3})
0–10	16.52 ± 1.05	1.43 ± 0.27	11.55 ± 1.51	3.98 ± 0.03	6.54 ± 0.50	1.28 ± 0.01
10–20	14.05 ± 1.41	1.35 ± 0.28	10.40 ± 1.17	3.91 ± 0.08	3.38 ± 0.14	1.34 ± 0.03
20–30	13.83 ± 1.65	1.21 ± 0.29	11.43 ± 1.47	3.84 ± 0.52	3.85 ± 0.21	1.35 ± 0.03

Data are means ($\pm$ SEs, $n = 3$)

fine powder, weighed, and then spread evenly within each plot. The fertilizer was applied twice annually, usually in late June and in late July. The N addition levels were chosen based on the current atmospheric N deposition level and its possible change in the next 50 years, simultaneously referred to the existing experiments in China and abroad (Chen et al. 2005; Arens and Sullivan 2008; Tu et al. 2009; Thomas et al. 2010). We applied large doses of N and water over a short period based on the assumption that this method effectively mimicked small inputs of N and water over longer periods that would result from climate change.

Vegetation sampling and analysis

Vegetation surveys were conducted in mid-August 2012, the time of peak biomass, after 3 years of water and N addition. There were five repetitions of every treatment, and we randomly sampled three quadrats in each repeated plot at each sampling period, so there were 15 samples for each treatment. For measurements of aboveground biomass, vegetation was cut at ground level and sorted into living plants and standing dead plants. Surface litter was subsequently collected. Belowground biomass was measured by digging soil pits ($40 \times 40 \times 40 \text{ cm}^3$) at the same locations where the aboveground biomass had been removed. Roots within 0–40 cm depth accounted for 80–90 % of the total belowground biomass in our study area (Xiao 2010). Root samples were collected separately from two layers at 0–20 and 20–40 cm depth in each sampling plot. For each layer, the soil was removed from the root samples by gently washing with tap water using a 2 mm sieve.

All plant samples (aboveground living plant, aboveground standing dead plant, surface litter, and root) were oven-dried at 80°C to constant weight to determine the dry biomass and then were pulverized to test C content. Organic C content was determined using a total organic C analyzer (Elementar vario TOC cube, Germany). The vegetation C pools for the aboveground components (aboveground living plant, standing dead plant and surface litter) and the belowground root for each plot were estimated by multiplying the sample biomass by the organic C concentration for each component.

Statistical analyses were performed using SPSS 17.0 software. Statistically significant differences between N addition treatments were investigated using one-factor analysis of variance (ANOVA). A two-way ANOVA was used to test for significant effects of the main factors; i.e., N addition (N), water addition (W), and their interaction (N $\times$ W). Graphs were prepared using Excel (2010).

Results

Responses of vegetation C pools to water addition

After 3 years of simulated increasing rainfall, each component of the vegetation C pools for the control (CK) and water-added treatment (WCK) were calculated (Fig. 2). The C pools of the aboveground living plants and litter were 12.55 and 25.09 % higher, respectively, in the water-addition plots than in the CK treatment plots (Fig. 2a), while the standing dead C pool in the WCK treatment was 5.52 % lower than in the CK treatment. For the belowground components, water addition reduced the C pools of roots at 0–20 and 20–40 cm by approximately 5.19 and 4.56 %, respectively, relative to controls. Differences in the C pools of the plant components between the treatments

Fig. 2 Effect of added water on C pools by plant component. Bars with different small letters denote treatments that differ significantly at $P < 0.05$. Error bars indicate the standard errors of the mean ($n = 15$). Aboveground C pool is the sum of the living plant C pool, the standing dead C pool and the litter C pool; belowground C pool is the sum of the root C pools at 0–20 and 20–40 cm depth. The aboveground C pool plus the belowground C pool equals the total vegetation C pool. R20 is Root (0–20 cm); R40 is Root (20–40 cm); LP living plant, SD standing dead, L litter

with and without water addition were not significant. The C pool of all aboveground components combined was 11.46 % higher in the water-added plots than in the control plots, whereas the belowground and total C pool was 5.09 and 1.81 % lower, respectively (Fig. 2b). These differences were not significant.

Effects of N addition on vegetation C pools

The vegetation C pools of the different plant components in the fertilized and unfertilized plots are shown in Fig. 3. The plots receiving the CK, LN, MN and HN treatments had 0, 5, 10 and 20 $\text{g m}^{-2} \text{a}^{-1}$ added, respectively, and no water added. The C pool of the aboveground living plants was significantly higher ($P < 0.05$) in fertilized plots than in unfertilized plots. The C pools of the aboveground living plants were 181.75 g m^{-2} (HN), 176.56 g m^{-2} (MN), and 154.28 g m^{-2} (LN), values 84, 79, and 56 % higher, respectively, than the corresponding CK value (98.83 g m^{-2}). However, the C pools did not differ significantly by added-N treatment level. N addition had a negligible effect on the C pools of the standing dead, litter and 20–40 cm root components. However the C pool of roots at 0–20 cm depth (709.43 g m^{-2}) in the MN treatment was significantly higher ($P = 0.02$) than that of the CK plots (521.80 g m^{-2}). The C pools at this root depth were also higher in the LN (597.31 g m^{-2}) and HN (663.37 g m^{-2}) treatments than in the control treatment but not significantly so. Moreover, the root C pool at 0–20 cm depth did not differ significantly between the N-added treatments.

The aboveground, belowground and total C pool values showed a similar pattern among the different N treatments, decreasing in the order $\text{MN} > \text{HN} > \text{LN} > \text{CK}$; the highest amount of C pool was observed in the MN, not HN, treatments (Fig. 4). The mean values of total C pool in the LN, MN and HN treatments were 953.81, 1090.10 and

Fig. 3 Vegetation C pools by N treatment. Bars with different small letters denote treatments that differ significantly at $P < 0.05$. Error bars indicate standard errors of the mean ($n = 15$). R20 is Root (0–20 cm); R40 is Root (20–40 cm); LP living plant, SD standing dead, L litter

Fig. 4 Effects of added N on aboveground, belowground and total vegetation C pools. Bars with different small letters denote treatments that differ significantly at $P < 0.05$. Error bars indicate the standard errors of the mean ($n = 15$)

1047.77 g m^{-2} , respectively; values 1.22, 1.39 and 1.34 times higher, respectively, than that of the CK treatment (782.64 g m^{-2}). The mean values of aboveground C pool in the CK, LN, MN and HM treatments were 154.86, 221.27, 255.02 and 250.61 g m^{-2} , respectively. Moreover, both the total C pool and the aboveground C pool were significantly higher ($P < 0.05$) in the three fertilized plots than in the unfertilized plots, but neither differed significantly between fertilizer treatments. Belowground C pool was significantly higher in the MN (835.08 g m^{-2}) and HN (797.16 g m^{-2}) treatments ($P < 0.05$) than in the CK treatment (627.78 g m^{-2}), with no significant differences observed between LN (732.53 g m^{-2}) and any of the other treatments.

Interactive effects of N and water addition on vegetation C pools

The results of the two-way ANOVA are shown in Table 2. The C pools of each plant component were not significantly affected by the addition of water, although the P values of the living plant (0.07) and 0–20 cm root layer components (0.06) approached significance. The C pools of the standing dead plant, litter and 20–40 cm root components were not significantly ($P > 0.05$) affected by N addition. However, there were significant effects of N addition on the C pools of the living plant and 0–20 cm root components, as well as on aboveground, belowground and total C pool ($P < 0.05$). The effects of the interaction between N and water on the total C pool and on the C pool of individual plant components were insignificant.

The ratio of root:shoot (R:S) is a key parameter in many global terrestrial C cycling models (Wang et al. 2010). Comparing R:S among the different water and N treatments, it is apparent that within each N treatment, R:S was

Table 2 Results (*F*- and *P* values) of the two-way ANOVA testing for effects of N addition (N), water addition (W), and their interaction ($W \times N$) on vegetation C pools (15 replicates)

Factors	Living plant		Standing dead		Litter		Root (0–20 cm)	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
W	3.61	0.07	0.21	0.65	1.33	0.27	3.84	0.06
N	18.98	0.00	1.09	0.38	1.67	0.21	5.84	0.01
$W \times N$	0.11	0.95	0.00	1.00	0.42	0.74	0.15	0.93
Factors	Root (20–40 cm)		Aboveground		Belowground		Total	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
W	0.71	0.41	3.26	0.09	2.75	0.11	0.75	0.40
N	0.34	0.80	15.46	0.00	6.62	0.00	16.23	0.00
$W \times N$	0.89	0.47	0.06	0.98	0.36	0.79	0.28	0.84

Table 3 Mean ratio of root:shoot (R:S) by water and N treatments

Treatments	R:S ratio		Without water/with water
	Without water addition	With water addition	
CK	6.07 ± 0.71a*	5.29 ± 0.51a	1.15
LN	4.97 ± 0.53b*	3.91 ± 0.38b	1.27
MN	4.51 ± 0.42b*	3.85 ± 0.36b	1.17
HN	4.52 ± 0.33b*	3.74 ± 0.34b	1.21
Average	5.02	4.19	1.20
CV (%)	14.56	17.42	

* Values followed by the same letter within the same column are not significantly different at $P < 0.05$; values in the ‘Without water addition’ column followed by an asterisk are significantly different from their corresponding (same row) values in the ‘With water application’ at $P < 0.05$

consistently lower in the water-added treatments than in treatments without added water (Table 3). The R:S of the different N treatments without water were 19.61 % higher, on average, than those with added water. Water addition reduced the R:S in each N treatment. Moreover, water addition increased the variation coefficient (CV) of the R:S among the different N treatments. Regardless of water addition, R:S values for fertilized plots were all significantly lower than for CK plots. Similarly, N addition also reduced R:S values of the vegetation.

These analyses showed that the effect of N addition on R:S varied with water treatment and suggested that water and N availability may have had an interactive effect. However, two-way ANOVA indicated that the R:S ratio was significantly ($P < 0.05$) affected by both water and N addition, but not by the interaction of N and water (Table 4).

Discussion

Response of vegetation C pools to water addition

Addition of water had no significant effect on aboveground, belowground or total C pools (Table 2). Water

Table 4 Results (*F*- and *P* values) of two-way ANOVA of the effects of N addition (N), water addition (W), and their interaction ($W \times N$) on R:S ratios (15 replicates)

Factors	<i>F</i>	<i>P</i>
Water addition	4.67	0.05
N addition	3.71	0.03
Interaction ($W \times N$)	0.01	1.00

addition significantly reduced R:S (Table 3). This result may be due to the higher-than-average level of natural precipitation in our experimental year. Wang and Zhou (2004) studied the same area and reported that the cumulative precipitation from October to the subsequent August was the most important factor influencing annual ANPP, and that when accumulated precipitation approached 350 mm, additional precipitation no longer promoted aboveground plant growth and could even inhibit growth. The accumulated precipitation at our study site between October 2011 and August 2012 was 404.5 mm, and in 2012, annual precipitation was 514.1 mm, both exceeding the 350 mm threshold. The increase in natural rainfall alleviated water restrictions on plant growth and led to the insignificant effect of increasing artificial precipitation.

Although the responses of plant component C pools were not affected by added water, the insignificant increases in aboveground C pools and the decrease in belowground C pools together caused significant reduction in R:S values when water was added. Similar results were reported by Dukes et al. (2005) for a California grassland. This decrease in R:S reflects the change in plant growth in response to the change in soil moisture. When soil moisture content is increased by natural or artificial rainfall, plants allocate more energy to aboveground growth, acquiring more light energy to satisfy the requirements of plant photosynthesis. In addition, root respiration also increases under favorable soil moisture conditions, reducing the root biomass accordingly (Xu and Zhou 2005; Tian et al. 2010; Wen et al. 2010).

Response of vegetation C pools to N addition

N is a major limiting nutrient for plant growth in many terrestrial ecosystems (Carvalho et al. 2011; LeBauer and Treseder 2008). In our study, addition of N increased aboveground C pools. Relative to the control treatment, the aboveground C pools of LN, MN and HN increased by approximately 43, 65, and 62 %, respectively; however, aboveground C pools did not differ by N treatment. Similar results have been reported in other studies (Foster and Gross 1998; Xia and Wan 2008; Qi et al. 2011). Qi et al. (2011) found that *L. chinensis* aboveground biomass increased by 39 % under N fertilization in a potted field control experiment. Among the above-ground plant components, the living plant C pool increased significantly, whereas the C pools of standing dead and litter did not. These results might be due to the lag in response of standing dead and litter to increasing N deposition relative to living plants, although increasing living plant biomass will, over time, lead to higher biomass of standing dead and litter. The relatively rainy weather conditions of 2012 also accelerated litter decomposition and the leaching of easily-decomposed litter compounds (Peng et al. 2014).

In many terrestrial ecosystems, added N decreases root primary production because plants allocate more resources to the canopy to compete for light when soil nutrients are more available (Bardgett et al. 1999; Hautier et al. 2009). Zeng et al. (2010) found that N fertilization resulted in lower root C pools, but Harpole et al. (2007) found that N addition had no effect on root biomass production. However, our results are not fully consistent with previous findings. Our belowground C pools were greater in all N treatments than in the control treatment, and significantly greater for MN and HN (Fig. 4). Similar results were reported by Pan et al. (2005) and Qi et al. (2011). These differences among studies may be due to different soil moisture conditions. Some studies have found that water

availability is the most important limiting factor for plant growth in arid and semi-arid grasslands (Bai et al. 2010; Rao and Allen 2010). Under drought stress conditions, N addition cannot effectively promote plant growth because drought severely restricts the mobility of N in soil (Chen et al. 2005), although it may improve water use to mitigate the effects of decreased plant photosynthesis (Bardgett et al. 1999). However, N availability will become an important limiting factor when water availability is not restricted. Additional N will have a positive effect on plant growth in most N-limited terrestrial ecosystems, but progressive N application generally increases shoot growth more consistently and to a greater extent than root growth, leading to a decrease in the R:S ratio (Dukes et al. 2005). In our experimental year of 2012, the total precipitation from May to August was 32 % higher than the average annual rainfall during that period. Therefore, water was no longer a factor restricting the positive effects of N on plant growth, which led to an insignificant positive effect on plant biomass (Fig. 2), and an insignificant interaction between water and N (Table 2). The R:S ratios of the N-added plots were lower than those of controls, indicating that the internal distribution ratio of the vegetation C pool changed due to increasing N input. Because decomposition rates of aboveground and belowground plant components differ (Giese et al. 2009), this change in the internal distribution of plant biomass due to N addition will inevitably affect the C input from plants to soil.

The highest levels of aboveground, belowground and total vegetation C pools were recorded for the MN treatment, rather than the HN treatment (Fig. 4). Addition of optimal amounts of N should promote leaf photosynthesis and plant growth but addition of excessive N might reduce plant photosynthetic capacity because of the self-shading effect (Brown et al. 1996) or because of nutrient imbalances in N, P, Mn and Mg, which would decrease leaf protein concentrations and Rubisco activity, resulting in inhibited leaf photosynthesis (Nakaji et al. 2001). Some researchers have also found that addition of large quantities of anthropogenic N may increase soil acidity, leading to increased loss of cations and decreased availability of phosphorus and other limiting nutrients, ultimately reducing plant production (Maston et al. 1999; Li et al. 2003; Song et al. 2010).

Conclusions

Addition of water did not have a significant impact on total or plant component C pools. N addition significantly increased total vegetation C pools and MN addition had the most significant promoting effect among the N treatments. Addition of both water and N significantly reduced R:S

ratios. Changes in soil moisture and N availability with increasing precipitation and N deposition on China's temperate grasslands could change the distribution of vegetation C pools and accordingly change the C input from plants to soil. Interactive effects between water and N addition on vegetation C pools were not significant. R:S ratios of treatments with addition of both water and N were, however, lower than those with addition of N alone. To explore the micro-scale effects of water and N addition on plant C fixation potential and to more accurately predict grassland C budgets, studies conducted at the leaf scale on plant photosynthesis under scenarios of anticipated global change should receive more attention in future research.

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