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Warming and increased precipitation individually influence soil carbon sequestration of Inner Mongolian grasslands, China

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ABSTRACT

The impact of climate change (i.e. warming and changes in precipitation patterns) on carbon (C) sequestration in the continental steppe is currently disputed and uncertain. We conducted a six-year field experiment in the temperate grassland ecosystem in Inner Mongolia, China. We found that C storage in the 0–30 cm soil layer significantly decreased by 129.3 g C m^{-2} under warming (W) but significantly increased by 145.9 g C m^{-2} with increased precipitation (P), with no apparent interaction of W × P. The effects of W and P on soil C sequestration varied in different soil fractions (labile vs. recalcitrant soil organic matter) and layers (topsoil vs. subsoil), which complicated the prediction of the short- and long-term effects of soil C sequestration in climate change scenarios. Furthermore, C:nitrogen (N) ratios in soils with increasing C and N were asynchronous under W, P, and W + P treatments, suggesting that the limiting effect of N on soil C sequestration would be intensified under W with P conditions. Our findings suggest that, without an increase in precipitation or concurrent P and W, the semiarid Inner Mongolian grasslands may potentially act as a net C source in the future.

1. Introduction

Global mean temperature is predicted to increase by 1.8–4.0 °C by the end of 21st century, and precipitation regimes are simultaneously predicted to change regionally and globally (IPCC, 2007). Synchronous changes in temperature and precipitation can have profound effects on regional and global carbon (C) cycles (Luo et al., 2008). It is estimated that more than 2190 Pg C have been stored in soils and plants in terrestrial ecosystems, making those ecosystems major C sinks that could partially offset anthropogenic CO_2 emissions (Schimel, 1995). The stability of soil organic matter (SOM) during perturbations such as global climate change, which would cause major changes in temperature and precipitation, is critically important to global C cycles. Therefore, understanding the effects of changes in temperature and precipitation on soil C sequestration in terrestrial ecosystems is important.

Approximately 32% of the earth's natural vegetation is in temperate grasslands (Adams et al., 1990). These ecosystems represent a significant component of the global C cycle and have enormous potential as sinks for atmospheric CO_2 (Jones and Donnelly, 2004). Soil C sequestration depends on the balance between inputs from aboveground and belowground biomass and losses through the decomposition of residues, all of which could be affected by climate change. Warming (W) or increased precipitation (P) influences the C cycle of grasslands by altering net primary productivity (Andresen et al., 2010; Arnone et al., 2008) and soil respiration (Allison et al., 2010). In semi-arid grasslands, soil moisture is especially influential on the rates of plant growth and SOM decomposition, thereby influencing SOM accumulation from litter and plant root inputs. Simulations with parameters for photosynthetic rate, soil respiration, and root turnover have predicted a positive effect of W and P on soil C sequestration in grasslands (Luo et al., 2008). However, the combined effects of W and P on potential soil C sequestration in the semiarid Inner Mongolian grasslands remains unverified by long-term field experiments.

Because the composition of particle-size fractions in soil is critical for SOM turnover (Christensen, 2001; Olk and Gregorich, 2006), we therefore selected soil particle-size fractions as an indicator with which to evaluate SOM changes under various perturbations (Olk and Gregorich, 2006; He et al., 2009, 2011). The content information of the labile (particle size > 50 μm) and recalcitrant (particle size < 50 μm) fractions allow better detection and prediction of changes in soil C and nitrogen (N) dynamics, which cannot be readily determined using traditional indicators of total C and N contents or net gas exchange measurements. Changes in the C and N content in the soil fraction in response to climate change may have

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a considerable effect on long-term terrestrial C and N storage and sequestration (Belay-Tedla et al., 2009).

Herein, we present the results of a six-year manipulative field experiment under control (CK), W, P, and W + P conditions in a temperate grassland in northern China. Our findings addressed three questions: (1) whether W and P individually or interactively affect soil C storage in the semiarid Inner Mongolian grasslands, (2) whether responses to W and P are consistent between bulk soil and soil fractions (labile SOM and recalcitrant SOM), and (3) whether asynchronous changes in soil C and N occur.

2. Materials and methods

2.1. Study site

The study site was located in a temperate steppe in Duolun County, Inner Mongolia Autonomous Region, China (42°20′N, 116°11′E). The climate at the study site was a typical semiarid continental climate with a mean annual temperature of 2.1 °C, a monthly mean temperature ranging from 18.9 °C in July to −17.5 °C in January, and a mean annual precipitation of 385.5 mm (with 80% of all precipitation occurring between June and September; Bai et al., 2010). Soil is of the chestnut type in Chinese soil classifications (i.e. Calcic Kastanozems, the equivalent of Calcis-orthic Aridisol in the U.S. soil classification system) with a pH of 6.84. The dominant vegetation consisted of perennial grassland plants including *Stipa krylovii* Roshev., *Artemisia frigida* Willd., *Potentilla acaulis* L., *Cleistogenes squarrosa* Keng., and *Allium bidentatum* Fisch.

2.2. Experimental design

Detailed information about our experimental design has appeared in previous studies (Bai et al., 2010; Liu et al., 2009; Niu et al., 2008). We designed our experiment with six replicates of four treatments (CK, W, P, W + P) for a total of 24 subplots, each measuring 3 m × 4 m. Beginning in April 2005, the W subplots were heated continuously using 165 cm × 15 cm MSR-2420 infrared radiators (Kalglo Electronics, Bethlehem, PA, USA), which were suspended 2.5 m aboveground. Long-term monitoring data showed that the mean soil temperatures at a depth of 10 cm were 1.39 °C and 1.02 °C higher in all W subplots compared to those in the CK and P subplots, respectively. In the P subplots, 15 mm of water was added weekly in July and August, and a total of 120 mm precipitation (approximately 30% of the mean annual precipitation at the study site) was added annually. Based on the results of previous studies in the experimental area, P significantly increased soil moisture by an average of 3.13% and 4.33% (v/v) in 2006 and 2007, respectively (Liu et al., 2009; Bai et al., 2010).

2.3. Field sampling

At the end of our six-year experiment (September 2010), we diagonally established two sampling points in each plot. We established one quadrat (0.5 m × 0.5 m) at each sampling point and investigated plant community cover and height. Subsequently, aboveground biomass was limited to ground level with all plant species combined. Within each quadrat, three soil cores (30 cm apart) were collected from three layers at depths of 0–10 cm, 10–20 cm, and 10–30 cm. Samples from the same plot were then mixed at the same depths of 0–10 cm, 10–20 cm, and 10–30 cm via sieving through a 2-mm mesh. Soil bulk density was measured at depths of 0–10 cm, 10–20 cm, and 20–30 cm using the core method (volume 100 cm^3).

2.4. Particle size fractionation and chemical analysis

We fractionated soil samples into sand (particle size 50–2000 μm), silt (2–50 μm), and clay (<2 μm) fractions with ultrasonic energy to disrupt aggregates using methods described in a previous manuscript (He et al., 2011). In brief, after manually removing visible root remnants, 50 g of soil (particles, 2 mm) was dispersed in 250 mL of distilled water using a KS-600 probe-type ultrasonic cell disrupter system (Shanghai Precision and Scientific Instrument, Shanghai, China) operating for 32 min in continuous mode at 360 W. Under these conditions, the real power input was 56.02 W, and the value of applied energy was 430 J mL^{-1} suspension. Sand (50–2000 μm) and coarse silt (20–50 μm) were separated with wet sieving. To further separate fine silt (2–20 μm) and clay (<2 μm), samples were centrifuged repeatedly at 150 × *g* for five min. The supernatants were collected in 250-mL centrifuge bottles and centrifuged at 3900 × *g* for 30 min; the precipitated fraction was referred to as clay. All of the fractions were dried at 50 °C and ground for further chemical analysis.

Organic C content (%) of the samples was measured using wet combustion. For this procedure, 0.5 g of soil sample was digested with five mL each of 1 N $K_2Cr_2O_7$ and concentrated H_2SO_4 at 180 °C for 5 min, followed by titration of the digests with standardized $FeSO_4$. Total soil N (%) was analyzed using the micro-Kjeldahl method.

2.5. Calculations and statistical analysis

Soil organic C (SOC; g C m^{-2}) and N (SN; g N m^{-2}) were calculated on an area basis to a soil depth of 30 cm as described previously (He et al., 2011):

$$\mathrm{SOC} = \sum D_i \times S \times B_i \times \mathrm{OM}_i \times 10$$

$$\mathrm{SN} = \sum D_i \times S_i \times B_i \times \mathrm{TN}_i \times 10$$

where D_i, S, B_i, OM_i, and TN_i represent thickness of the soil layer (cm), cross-sectional area (m^2), bulk density (g cm^{-3}), organic C content (g kg^{-1}), and total N content (g kg^{-1}), respectively; i = 1, 2, and 3.

Similarly, C (g C m^{-2}) and N (g N m^{-2}) storage in soil fractions (sand, silt, and clay) were calculated as follows:

$$\mathrm{C}_{\mathrm{storage}}(\mathrm{fraction}_i) = \frac{\mathrm{C}_{\mathrm{con.}}(\mathrm{fraction}_i) \times F \times D \times S \times B}{100}$$

$$\mathrm{N}_{\mathrm{storage}}(\mathrm{fraction}_i) = \frac{\mathrm{N}_{\mathrm{con.}}(\mathrm{fraction}_i) \times F \times D \times S \times B}{100}$$

where $\mathrm{C}_{\mathrm{con.}}(\mathrm{fraction}_i)$ is the C content of the soil fraction (g kg^{-1}), $\mathrm{N}_{\mathrm{con.}}$ $(\mathrm{fraction}_i)$ is the N content of the soil fraction (%), and F is the fraction content in soil (g fraction kg^{-1} soil).

Commonly, the SOM in the sand fraction has a high C:N ratio and is considered labile, but SOM in silt plus clay is more processed and stable (Christensen, 2001). Therefore, we considered C and N storage in the sand fraction (particle size > 50 μm) as labile (i.e. labile C and labile N), and C and N storage in silt and clay (particle size < 50 μm) as recalcitrant (i.e. recalcitrant C and recalcitrant N).

We calculated the absolute change in soil C and N storage (ΔC, g C m^{-2}; ΔN, g N m^{-2}) based on CK sites to characterize changes in soil C and N storage that occurred through W and P treatments. A two-tailed *t*-test was performed to determine whether each mean change was significantly different from zero, and 95% confidence intervals were calculated as a measure of the minimal significant

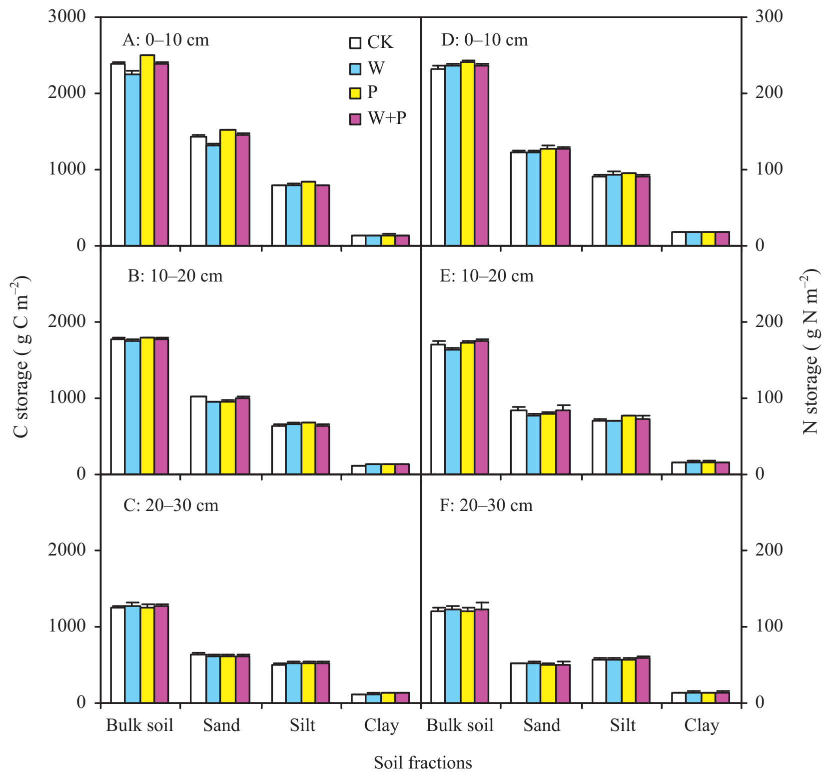

Fig. 1. Changes in carbon and nitrogen storage in the 0–30 cm soil layers. Experimental treatments were designed as control (CK), warming (W), increasing precipitation (P), and warming and increasing precipitation (W + P). Soil fractions observed included sand, particle size = 50–2000 μm; silt, particle size = 2–50 μm; and clay, particle size < 2 μm. Data were represented as means ± 1 S.D (n = 6).

difference (Zinn et al., 2005). Normality and homogeneity of variances were verified for all data using the Kolmogorov–Smirnov and Levene tests, respectively. Two-way analysis of the variance with a blocked nested design was used to determine the effects of W and P on soil C and N storage. Data were represented as mean ± 1 standard deviation (n = 6). All analyses were conducted using SPSS statistical software (ver. 11.0, SPSS, Chicago, IL, USA).

3. Results

3.1. Changes in soil C storage with experimental W and P

Results showed that W and P treatments had no significant effect on either soil bulk density or soil fractions (Table 1). However, in the W treatment, soil C storage decreased by 119.5 g C m^{-2} in the 0–10 cm soil layer (F = 113.25, P < 0.001) but increased 119.3 g C m^{-2}(F = 133.03, P < 0.001) in the P treatment (Fig. 1) while no significant interaction of W × P was observed (Table 2). Similarly, in the 10–20 cm soil layer, soil C storage decreased by 30.5 g C m^{-2} (F = 3.78, NS) in the W treatment and increased by 16.4 g C m^{-2} (F = 7.32, P < 0.05) in the treatment (Fig. 1). Finally, soil C storage increased by 20.7 g C m^{-2} and 10.3 g C m^{-2} in the W and P treatment, respectively, in the 20–30 cm soil layer; although, this increase was not significantly different from values found in the CK treatment. In summary, the influence of W and P on soil C storage occurred most substantially in the 0–10 cm soil layer and decreased with soil depth (Fig. 2 and Table 3).

3.2. Changes in soil N storage with experimental W and P

W did not have a significant impact on N storage in the 0–30 cm soil layer (Table 4). However, N storage increased by 9.0 g N m^{-2} in the 0–10 cm soil layer (F = 10.05, P < 0.01) and by 2.4 g N m^{-2} in the 10–20 cm soil layer, respectively (F = 16.75, P < 0.01) in the P treatment. No apparent interactive effects of W × P on soil N storage were observed. Like C storage, the effect of P on soil N storage occurred mainly in the 0–10 cm soil layer and decreased with soil depth.

3.3. Effect of experimental W and P on the C and N storage of soil fractions

The effects of W and P on soil C and N storage in soil particle-size fractions were mainly evident in sand (50–2000 μm) and silt (2–50 μm) and were minor in clay (<2 μm; Tables 2 and 3). In the sand fraction, soil C storage decreased with W (F = 53.41, P < 0.001) but increased with P (F = 31.55, P < 0.001); with evidence of W × P had interactive effects (F = 39.19, P < 0.001). In the silt fraction, P enhanced soil C storage (F = 8.15, P = 0.010), and W × P had significantly interactive effects (F = 19.09, P < 0.001; Table 4). Moreover, P and W × P significantly enhanced N storage in the sand and silt fractions.

Regarding the absolute changes in C and N storage in soil fractions, W decreased C (t = –9.65, P < 0.001) and N storage (t = –3.48, P = 0.018) in labile SOM (particle size >50 μm) but increased C storage in recalcitrant SOM (particle size < 50 μm; t = 3.22, P = 0.023; Fig. 3 and Table 5). We observed a decreasing trend in soil C and N storage in the P treatment in labile SOM although this trend was insignificant, as well as a significantly increasing trend in C (t = 6.79, P = 0.001) and N storage (t = 4.98, P = 0.004) in recalcitrant SOM.

3.4. Effect of experimental W and P on the soil C:N ratios

W, P, and W + P had no significant effects on C:N ratios in bulk soil and soil fractions; however, the C:N ratios in soil with increased C and N were asynchronous among W, P, and W + P treatments

Table 1
Changes in soil bulk density and particle-size fractions.

	Treatments	Bulk density ($g\,cm^{-3}$)	Sand (50–2000 μm) ($g\,kg^{-1}$ soil)	Silt (2–50 μm) ($g\,kg^{-1}$ soil)	Clay (<2 μm) ($g\,kg^{-1}$ soil)
0–10 cm	CK[a]	1.29 ± 0.05[b,c]	774.00 ± 34.34	206.57 ± 32.81	19.43 ± 2.45
	W	1.30 ± 0.05	769.89 ± 19.13	211.58 ± 20.12	18.53 ± 1.87
	P	1.28 ± 0.04	773.02 ± 23.29	209.60 ± 23.26	17.38 ± 2.42
	W + P	1.29 ± 0.06	776.31 ± 24.14	204.38 ± 23.89	19.31 ± 2.52
10–20 cm	CK	1.35 ± 0.04	811.63 ± 23.09	171.34 ± 24.19	17.03 ± 2.48
	W	1.36 ± 0.04	812.63 ± 17.21	168.10 ± 19.32	19.26 ± 5.27
	P	1.35 ± 0.03	807.17 ± 16.63	173.26 ± 15.85	19.57 ± 3.78
	W + P	1.35 ± 0.03	819.89 ± 17.71	162.51 ± 15.37	17.60 ± 4.77
20–30 cm	CK	1.41 ± 0.03	832.03 ± 24.81	149.21 ± 25.95	18.26 ± 4.30
	W	1.40 ± 0.03	832.25 ± 18.92	148.72 ± 19.23	19.03 ± 3.01
	P	1.41 ± 0.03	826.16 ± 19.10	154.01 ± 19.92	19.83 ± 4.06
	W + P	1.40 ± 0.03	824.62 ± 9.29	155.66 ± 12.40	19.72 ± 4.51

[a] Experimental treatments were designed as control (CK), warming (W), precipitation (P), and warming plus precipitation (W + P).
[b] Data are represented as mean ± SD (n = 3 for bulk density, n = 6 for particle-size fractions).
[c] No significant differences were observed.

Table 2
F ratios of variance analysis on the effects of warming and increasing precipitation on soil carbon storage.

		Bulk soil	Sand (50–2000 μm)	Silt (2–50 μm)	Clay (<2 μm)	Silt + clay (<50 μm)
0–10 cm	W	113.25***	221.60***	21.45***	0.02NS	14.54***
	P	133.05***	320.13***	9.07**	0.92NS	7.74*
	W × P	0.21NS	28.57***	19.74***	0.03NS	15.53**
10–20 cm	W	3.78NS	6.14*	3.72NS	4.65*	7.29*
	P	7.32*	0.38NS	5.07*	4.48*	5.34*
	W × P	1.16NS	73.62***	17.60***	8.83*	0.43NS
20–30 cm	W	0.90NS	0.42NS	1.33NS	3.21NS	2.53NS
	P	0.06NS	5.72*	1.86NS	4.28*	3.84*
	W × P	0.21NS	1.17NS	2.51NS	1.43NS	3.61NS
0–30 cm	W	19.01***	53.41***	3.90NS	3.43NS	3.04NS
	P	31.55***	34.53***	8.15*	3.16NS	5.55*
	W × P	0.11NS	39.19***	19.09***	3.56NS	0.44NS

NS, $P > 0.05$.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

(Figs. 2 and 3). The ratios under W and P treatments were higher than those of grazing grasslands in the region (soil C:N = 10:1). However, C:N ratios under the W + P treatment were lower than those of grazing grassland soils.

4. Discussions

We found that experimental warming and increased precipitation have individually impact on soil C storage in the Inner

Table 3
Absolute change in soil carbon and nitrogen storage under warming and increasing precipitation.

		Bulk soil		Sand (50–2000 μm)		Silt (2–50 μm)		Clay (<2 μm)	
		ΔC ($g\,m^{-2}$)	ΔN ($g\,m^{-2}$)	ΔC ($g\,m^{-2}$)	ΔN ($g\,m^{-2}$)	ΔC ($g\,m^{-2}$)	ΔN ($g\,m^{-2}$)	ΔC ($g\,m^{-2}$)	ΔN ($g\,m^{-2}$)
0–10 cm	W	−119.5 ± 58.6**,[a]	4.0 ± 4.5NS	−118.6 ± 23.9***	1.0 ± 2.2NS	−1.1 ± 19.4NS	2.6 ± 3.9NS	0.2 ± 4.3 NS	0.4 ± 0.6 NS
	P	119.3 ± 24.5***	9.0 ± 5.8*	73.5 ± 6.8 ***	6.3 ± 5.0*	43.8 ± 18.6**	2.9 ± 3.5NS	1.9 ± 8.0 NS	−0.2 ± 0.7NS
	W + P	9.6 ± 18.9NS	4.0 ± 3.8*	17.6 ± 12.1*	5.0 ± 3.8*	−9.5 ± 15.3NS	−0.8 ± 2.0NS	1.5 ± 3.4 NS	−0.3 ± 0.7 NS
10–20 cm	W	−30.5 ± 29.8NS	−7.7 ± 5.7*	−66.1 ± 11.9***	−7.9 ± 4.2**	15.8 ± 22.7NS	−1.4 ± 1.8NS	19.8 ± 4.5***	1.6 ± 1.0*
	P	16.4 ± 13.4*	2.4 ± 3.3NS	−47.6 ± 12.7***	−4.5 ± 3.6*	44.9 ± 11.3***	5.1 ± 1.7**	19.2 ± 4.6***	1.8 ± 0.4***
	W + P	7.7 ± 16.6NS	3.5 ± 5.1NS	−11.1 ± 25.4NS	0.6 ± 4.5 NS	2.3 ± 12.9NS	1.9 ± 6.0NS	16.6 ± 3.3***	1.0 ± 1.0NS
20–30 cm	W	20.7 ± 63.9NS	1.7 ± 6.2NS	−15.7 ± 35.8NS	−0.6 ± 5.2NS	22.9 ± 26.5NS	1.0 ± 3.1NS	13.5 ± 4.0***	1.3 ± 1.1*
	P	10.3 ± 40.9NS	−0.9 ± 2.3NS	−31.6 ± 24.8*	−2.0 ± 2.8NS	24.7 ± 25.5NS	0.3 ± 1.8NS	17.1 ± 7.5**	0.8 ± 1.0NS
	W + P	17.4 ± 41.1NS	1.5 ± 10.4NS	−27.7 ± 31.2NS	−1.7 ± 5.5NS	21.1 ± 25.0NS	1.6 ± 4.9NS	24.0 ± 5.3***	1.7 ± 0.7**
0–30 cm	W	−129.3 ± 54.3*	−1.9 ± 9.5NS	−200.4 ± 50.9***	−7.5 ± 5.3*	37.6 ± 50.4NS	2.2 ± 7.4NS	33.6 ± 4.6***	3.4 ± 1.1**
	P	145.9 ± 58.1**	10.5 ± 8.0*	−5.7 ± 37.4NS	−0.2 ± 6.4NS	113.4 ± 49.5**	8.3 ± 5.2*	38.2 ± 5.7***	2.4 ± 1.5**
	W + P	34.7 ± 49.4NS	9.0 ± 10.6NS	−21.2 ± 39.3NS	3.9 ± 6.5NS	13.8 ± 30.5NS	2.7 ± 7.3NS	42.0 ± 4.8***	2.4 ± 1.2**

NS, $P > 0.05$.
[a] Data were deduced from the differences in values in treated and CK plots, and were represented as means ± 1 S.D. (n = 6).
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Table 4
F ratios of variance analysis on the effects of warming and increased precipitation on soil nitrogen storage.

		Bulk soil	Sand (50–2000 μm)	Silt (2–50 μm)	Clay (<2 μm)	Silt + clay (<50 μm)
0–10 cm	W	0.11NS	0.01NS	0.36NS	0.95NS	2.87NS
	P	10.05^{**}	23.85^{***}	0.06NS	5.34^{*}	0.51NS
	W × P	10.20^{**}	1.14NS	11.86^{**}	1.17NS	0.15NS
10–20 cm	W	3.98NS	0.95NS	5.06^{*}	1.74NS	2.87NS
	P	16.75^{*}	1.87NS	16.75^{**}	3.91NS	15.11^{**}
	W × P	6.92^{*}	19.62^{***}	0.74NS	15.26^{**}	2.35NS
20–30 cm	W	0.72NS	0.02NS	1.01NS	3.80NS	0.02NS
	P	0.06NS	1.10NS	0.14NS	4.41^{*}	0.64NS
	W × P	0.02NS	0.09NS	0.01NS	0.85NS	3.16NS
0–30 cm	W	0.26NS	0.43NS	1.30NS	3.33NS	5.59^{*}
	P	9.71^{**}	4.50^{*}	8.52^{**}	3.14NS	10.66^{**}
	W × P	0.02NS	4.84^{*}	6.73^{*}	16.18^{**}	0.00NS

NS, $P > 0.05$.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Mongolian grasslands. W had negative effects on soil C sequestration, which is consistent with previous research on soil respiration and photosynthetic rate in this ecosystem (Liu et al., 2009; Niu et al., 2008). A plausible explanation is that W caused greater respiratory C losses relative to photosynthetic C gains, thus leading to a negative impact on net ecosystem exchange in the semiarid temperate steppe.

We also found that increased in precipitation significantly increased soil C storage in this grassland ecosystem. Precipitation plays a predominant role in regulating productivity and soil respiration which then drives the C cycle and ultimately determines how much C is sequestered in grassland soil in arid regions (Jones and

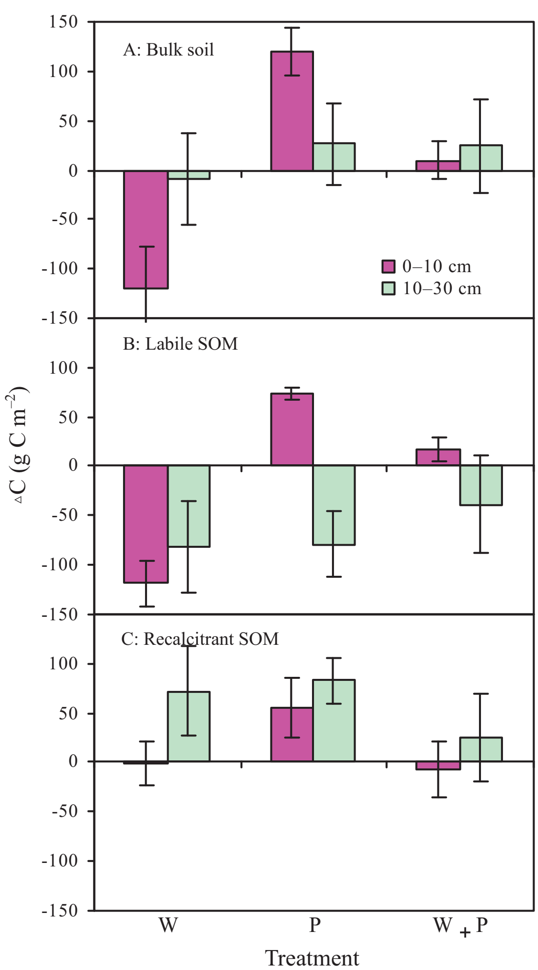

Fig. 2. Response in soil carbon storage to warming and increased precipitation in the 0–30 cm soil layer. Data were deduced from the differences in values in treated and control sites, and were represented as means ± 1 S.D. ($n = 6$). C stored in the particle size > 50 μm fraction was considered labile whereas C stored in the particle size < 50 μm fraction was considered recalcitrant.

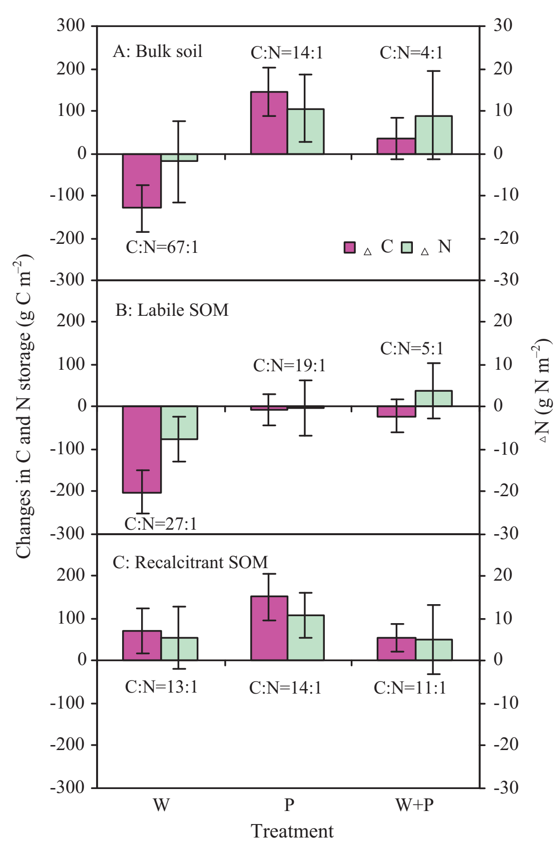

Fig. 3. Effects of warming and increased precipitation on the carbon and nitrogen storage in bulk soil, labile soil organic carbon, and recalcitrant SOC in the 0–30 cm soil layer. Data were deduced from the differences in values in treated and CK sites and were represented as means ± 1 S.D. ($n = 6$). C stored in the particle size > 50 μm fraction was considered labile, whereas C stored in the particle size < 50 μm fraction was considered recalcitrant.

Table 5
Absolute change in soil carbon and nitrogen storage in the silt plus clay fractions.

		Silt + clay	
		ΔC (g m^{-2})	ΔN (g m^{-2})
0–10 cm	W	−0.9 ± 21.7NS[a]	3.0 ± 3.9NS
	P	45.7 ± 24.6**	2.7 ± 3.3NS
	W + P	−8.0 ± 16.3NS	−1.0 ± 1.7NS
10–20 cm	W	35.6 ± 23.8NS	0.2 ± 1.9*
	P	64.0 ± 12.9***	6.9 ± 1.6***
	W + P	18.8 ± 13.0NS	2.9 ± 7.0*
20–30 cm	W	36.5 ± 30.0*	2.4 ± 3.4NS
	P	41.9 ± 31.2*	1.1 ± 2.2NS
	W + P	45.1 ± 27.2**	3.2 ± 5.5NS
0–30 cm	W	71.2 ± 54.2*	5.6 ± 7.3NS
	P	151.6 ± 54.7**	10.7 ± 5.3**
	W + P	55.9 ± 32.9***	5.1 ± 8.2NS

NS, $P > 0.05$.
[a] Data were deduced from the differences in values in treated and CK plots, and were represented as means ± 1 S.D. ($n = 6$).
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Donnelly, 2004; Liu et al., 2009). Some studies have demonstrated that P significantly increased aboveground and belowground productivity (Bai et al., 2010), enhanced soil respiration (Liu et al., 2009), and increased soil C storage. Using a process-based ecosystem model, Shen et al. (2009) found that P, individually or in combination with W, improved soil C sequestration in drylands. Overall, W depressed while P improved soil C sequestration in the semiarid Inner Mongolian grasslands.

The impacts of W and P on soil C storage varied in the different soil fractions with the strongest impacts occurring in sand (50–2000 μm) and silt (2–50 μm). Short-term changes in soil C storage are mediated through a small but highly bio-reactive labile pool, but long-term C storage is often determined by the long-lived recalcitrant fraction. Our results showed that W significantly decreased labile C and increased recalcitrant C in the Mongolian grassland ecosystem. However, in a study of the Great Plains in OK, USA, Belay-Tedla et al. (2009) found that W significantly increased labile C fractions and soil microbial biomass C. A major difference in mean annual temperature and precipitation between the Great Plains and the continental steppe is likely the main reason for these contrary trends.

The indirect negative effects of warming-induced water stress on soil C storage are stronger than the direct positive effects of elevated temperature. In arid Inner Mongolian grasslands, W decreased soil moisture and, consequently, significantly reduced soil microbial respiration and microbial biomass (Liu et al., 2009). Pendall et al. (2011) have reported that W increased particulate organic matter under C_3-dominated vegetation and suggested that C_3–C_4 vegetation responses to future climate conditions will strongly influence SOM storage in temperate grasslands. Feng et al. (2008) concluded that future warming could alter the composition of SOM at the molecular level, accelerating lignin degradation and increasing leaf-cuticle-derived C sequestration.

We determined that the responses of the soil labile and recalcitrant fractions to W and P varied under warming and changes in precipitation regimes. Several studies have demonstrated that the labile pool is sensitive to alterations in soil moisture, temperature, and plant community structure (Christensen, 2001; Olk and Gregorich, 2006). Using a controlled incubation experiment and reanalysis of publicly published data, Conant et al. (2008) have suggested that temperature sensitivity of SOM decomposition increases with decreasing SOM liability and that future losses of litter and soil C from recalcitrant SOM may be even greater than those previously supposed. Together, the responses of the labile and recalcitrant fractions to W and P remain understudied in long-term experiments, and the diversity in responses in different regions represents a substantial source of uncertainty in predicting grassland ecosystem feedbacks to climate change.

Clear differences in C sequestration occurred in the topsoil and subsoil in the W and P experimental plots. The fresh C supply controls the stability of organic C in deep soil layers (Fontaine et al., 2007). By controlling oxygen concentration, soil structure, and energetic and nutritional status, Salomé et al. (2010) demonstrated that C dynamics in topsoil and subsoil are controlled through different mechanisms under incubation conditions, and the spatial separation of decomposer and substrate levels appears to play a more important role in the subsoil. In fact, an increase in recalcitrant SOM in subsoil is a likely scenario. Topsoil and subsoil have different pedological, environmental, and physicochemical features, which together affect the mechanisms and biological factors impacted by future climate change. We recommend further research into the various responses of topsoil and subsoil to better understand global C dynamics under future perturbations and climate changes.

The limiting effect of N availability on soil C sequestration will be intensified in the continental steppe under current climate change projections. N deficiency is common (Yuan et al., 2006) and water availability is the prime factor regulating productivity and decomposition in the region (Bai et al., 2010; Liu et al., 2009). Lü et al. (2011) reported that N addition can increase soil C storage in Inner Mongolian grasslands. Far higher C:N ratios in the soil with increased C and N under the W + P treatment, however, showed that increasing soil C sequestration causes higher N demands because terrestrial ecosystem C gain and soil C decomposition are both controlled by stable stoichiometric regulation (Anderson et al., 2004; Hessen et al., 2004; Taylor and Townsend, 2010). Moreover, some models, by increasing the supply of one potentially limiting element (N), demonstrated that N will affect the distribution, cycling, and retention of other nutrients in most terrestrial ecosystems (Hungate et al., 2003; Perring et al., 2008). In our study, loss of N was far lower than that of C under W, implying that most of the N emitted by decomposition was reused by plant and microbe. Increased efficiency in N use via a shift in species composition rather than in plant N uptake is a key mechanism underlying the warming stimulation of plant growth (Yuan et al., 2006; Niu et al., 2010). Given that limitations in productivity resulting from insufficient N availability are widespread in both unmanaged and managed vegetation, soil N supply is probably an important constraint on global terrestrial responses to climate change (Reich et al., 2006). Our findings highlight the impact of N input on soil C sequestration based on C:N stoichiometry in soil with increasing C and N under future climate scenarios.

Several possible mechanisms could explain the diverse effects of W and P on soil C storage reported herein. The indirect effects of W on soil moisture and nutrient supply may alter belowground C turnover processes in complicated directions. In the water-limited steppe, decreasing soil water availability caused by increased evapotranspiration under W suppresses plant growth, root production, microbial activities, and soil respiration (Bai et al., 2010; Liu et al., 2009). In ecosystems with higher soil moisture, W enhanced soil respiration and decomposition rates (Allison et al., 2010; Luo et al., 2008; Pendall et al., 2004; Wan et al., 2007). In addition, P can significantly enhance aboveground and belowground productivity and increase SOM input. P stimulated gross ecosystem productivity more than soil respiration, although productivity and soil respiration increased at the same time (Bai et al., 2010; Liu et al., 2009; Wan et al., 2009). W + P also enhanced new SOM input through higher productivity while stimulated soil respiration, which partly offset the increasing SOM input (Wang et al., 2006; Liu et al., 2009, 2010). Finally, the differing effects of W and P treatments on C and N storage in bulk soil and soil fractions as well as in topsoil and subsoil are a consequence of the direct input of labile SOM, which was elevated in topsoil, and higher rapid decomposition of labile SOM increased recalcitrant SOM in subsoil.

In addition, wet sites generally had smaller relative changes in net primary productivity, respiration, runoff, and transpiration but larger absolute changes in net ecosystem productivity compared to those in dry sites in response to W and P (Luo et al., 2008). The control over and consequences of these processes are still unclear at the ecosystem scale. Therefore, we speculated that the effects of W, P, and W + P in the continental steppe and Great Plain of United States represent not only the strongest effect on soil C sequestration but also the main controlling factors and underlying mechanisms. As reported by Chapin et al. (2009), the responses and direction of belowground C flux and its partitions to climate change are diverse in different terrestrial ecosystems, which appear to be critical to soil C dynamics, and require further research.

5. Conclusions

The results from this six-year experiment demonstrated first that the response of soil C sequestration to climate variability (W and P) is more complex than previously thought. Soil C storage in Inner Mongolian grasslands varied under W and P at different scales (bulk soil, labile vs. recalcitrant SOM, and topsoil vs. subsoil). Therefore, additional research on the responses of soil fractions and of soil layers is warranted to better understand global C dynamics under climate changes. In addition, we found that increased precipitation is a much stronger driving factor than warming in arid and semiarid regions. Moreover, C:N ratios in soil with increasing or decreasing C and N were asynchronous between W and P treatments. The much higher C:N ratios in soil with increasing C and N under the W + P treatment suggested that increasing soil C sequestration results as a function of higher N demands. Our study, which considered the manipulation and stability of soil C storage, provided direct evidence for changes in C sequestration or loss in Inner Mongolian grasslands under changing regimes in temperature and precipitation in the future.

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