

The impacts of precipitation increase and nitrogen addition on soil respiration in a semiarid temperate steppe

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Abstract. Soil respiration, R_s , is strongly controlled by water availability in semiarid grasslands. However, how R_s is affected by precipitation change (either as rainfall or as snowfall) especially under increasing nitrogen (N) deposition has been uncertain. A manipulative experiment to investigate the responses of growing season R_s to changes in spring snowfall or summer rainfall with or without N addition was conducted in the semiarid temperate steppe of China during three hydrologically contrasting years. Our results showed that both spring snow addition and summer water addition significantly increased R_s by increasing soil moisture. The effect of spring snow addition only occurred in years with both relatively lower natural snowfall and later snowmelt time. Summer water addition showed a much stronger effect on R_s by increasing plant root growth and microbial activities, but the magnitude also largely depended on the possible legacy effect of previous year precipitation. Our results indicated that precipitation increase in the form of snowfall had weaker effects than that in the form of rainfall as the former only accounted for less than 30% of total precipitation. Compared with other ecosystem processes, R_s was less responsible for increase in N deposition as it did not increase root productivity and microbial activities in the soils. Our results provided field data constraints for modeling the ecosystem carbon balance under the future global change scenarios in semiarid grasslands.

Key words: Inner Mongolia; nitrogen addition; snow addition; soil respiration; temperate typical steppe; water addition.

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INTRODUCTION

Soil respiration, R_s , is considered one of the largest fluxes in global terrestrial carbon (C) cycle, and therefore, a small change in the magnitude of R_s could have a significant impact on the atmospheric CO₂ concentration (Schlesinger and Andrews 2000). As productivity in dryland areas, similar to many other terrestrial ecosystems, is

limited by the coupling availability of nitrogen (N) and water (Wang et al. 2015), changes in N deposition and precipitation regimes will inevitably affect R_s owing to changes in C supply for roots, microbial activities, and eventually ecosystem C cycle (Yan et al. 2010).

Nitrogen deposition has experienced significant increase since the Industrial Revolution (Galloway et al. 2008, Zhou et al. 2013) and will

continue to increase at least in the near future (Schlesinger 2009). Although N is one of the most important factors to limit terrestrial productivity (Xia and Wan 2008), R_s responses to N addition, however, are sometimes inconsistent due to different soil temperature and soil moisture conditions with studies having reported either positive, negative (Xu and Wan 2008, Yan et al. 2010), or no response (Ambus and Robertson 2006, Allison et al. 2008). In the more recent studies, it was reported that the effects of N addition on R_s highly depended on the amount of N added (Gao et al. 2014) and soil N content (Janssens et al. 2010). In areas where N is the limiting factor for plant growth, N addition might increase R_s , but in tropical and other regions with older severely weathered soils, where nitrogen may not be the most limiting nutrient, the opposite effect might occur (Janssens et al. 2010).

Several studies have suggested that increasing precipitation, generally coupled with increasing N deposition, can stimulate belowground productivity, resulting in more C supply to R_s especially in the dryland areas (Yan et al. 2010). With the expected changes in the amount and pattern of precipitation under current climate change scenario (Acharya et al. 2013), we may expect an increase in soil moisture and thus microbial metabolic activities (Liu et al. 2009, Chimner et al. 2010). Precipitation, however, may come in two different forms, rainfall and snowfall, with the majority of studies focusing on the effects of rainfall changes. Fewer studies have examined the effects of snowfall changes, yet areas with seasonal snow coverage account for more than 70% of global organic C stores (Brooks et al. 2011). Spring-time burst of dissolved soil organic C has been recorded as the snow melted, increasing R_s from microbial respiration (Scott-Denton et al. 2006). Increasing R_s from rhizospheric respiration, however, is expected to be delayed as warmer temperature is required for water to infiltrate and promote root growth, particularly when thick snow cover insulated the soil surface (Molotch et al. 2009). As the depth of snow and timing of snowfall determine the following growing season length and ecosystem C cycling (Brooks et al. 2011), small changes in snow depth and duration may have important biogeochemical effects in these areas.

Previous studies on snowfall changes in terrestrial ecosystem C fluxes were mainly performed

in high-latitude, high-altitude, or mid-elevation ecosystems (Sullivan 2010, Aanderud et al. 2013, Schindlbacher et al. 2014), but rarely in the dry temperate steppe (Qin et al. 2015) where C fluxes are very sensitive to fluctuation on the amount and timing of precipitation. Although snowfall accounts for only less than 10% of total annual precipitation, snowpack melt in this region provides considerable amount of water to plants in spring and the following summer (Chimner et al. 2010). With the expected increase in snowfall in the northern China region (Ma et al. 2011), the responses of R_s in these dry temperate steppes need to be examined, particularly with the varying responses of R_s to the combined change in water and N availability with different ecosystem types (Baldocchi et al. 2006).

To investigate the effects of increasing snowfall and summer rainfall and their interaction with increasing N deposition on R_s , we conducted a field manipulative experiment in a grazed and degraded semiarid steppe ecosystem of northern China during 2011–2013. We focused our study during the growing season when changes in R_s were greatest (Chen et al. 2014). We hypothesized that (1) spring snow addition would increase R_s by increasing soil moisture especially at the beginning of the growing seasons although the extent would be small as snow only accounted for ~8% of total precipitation in the study area; (2) the extent of additional summer water on R_s would be much greater than that by the snow since it corresponded with the peak of growing seasons; and (3) N addition, coupled with precipitation increase, would enhance R_s as both N availability and water availability are limiting for plant growth in this study area.

MATERIALS AND METHODS

Site description

The experiment was carried out in the Inner Mongolia Grassland Ecosystem Research Station, Institute of Botany, Chinese Academy of Sciences. The study ecosystem is classified as semiarid temperate steppe, dominated by C_3 grass species such as *Stipa grandis* and *Leymus chinensis*. The long-term mean temperature (1954–2013) ranged between -19.3° and 5.0°C (November–April) and between 12.3° and 21.1°C (May–September). The long-term mean annual precipitation (1982–2013)

Table 1. Precipitation from Novembers of previous year to Octobers in 2011, 2012, and 2013.

Precipitation (mm)	2011	2012	2013	Mean
Rainfall	199.8	426.8	242.7	307.5
Snowfall	31.9	37.9	77.6	27.1
Total	231.7	464.7	320.3	334.6

Notes: Mean values are from 1982 to 2013. Snowfall amount is described as water equivalent of snow.

is 333.3 mm (27.0 mm in the form of snowfall and 306.3 mm in the form of rainfall). The annual precipitation amount (from previous November to October) was greatest in 2012 (464.7 mm), with the majority of it as rainfall (426.8 mm). The largest snowfall was identified in 2013 (77.6 mm), almost double than the two preceding years (31.9 mm and 37.9 mm in 2011 and 2012, respectively). Of the 3 yr, 2011 was considered the driest year, with only 231.7 mm of precipitation, and 86% of it fell as rainfall (Table 1). The snowmelt time was on 16 March in 2011, 1 April in 2012, and 16 April in 2013 (Zhang et al. 2015).

Experimental design and treatments

We used 30 plots of 25 m² (5 × 5 m) with 1 m distance between the neighboring plots to create a randomized block design with two levels of N addition (0 and 10 g N) interactively with either two levels of spring snow addition (0 and 25 mm water equivalent of snow) or summer water addition (0 and 100 mm rainfall). The treatments, each with five replications, were coded as follows: no N and no water addition (control; N0W0), no N addition but with either spring snow addition (N0W1) or summer water addition (N0W2), N addition with no water addition (N1W0), N addition with spring snow addition (N1W1), and N addition with summer water addition (N1W2). Plots with spring snow addition were added with 25 mm water equivalent of snow (~37.5 cm thick of snowfall, equivalent to the long-term mean value of snowfall) at the beginning of March since 2010. Plots with summer water addition were treated with slow irrigation of 10 mm (1 m³/h water flow lasting for 15 min) water every week from 15 June since 2010, generating a total of 100 mm water each year (equivalent to 30% of the long-term mean value of rainfall). We used urea as N fertilizer because it was widely applied in crop lands and was easily available compared

with other forms of N fertilizers. At the beginning of July, urea (10 g N/m²) was added in the N addition plots since 2009. All treatments were conducted 2 yr earlier than the examination to observe possible legacy effects of water and N additions.

Soil respiration

In 2010, we inserted 30 polyvinyl chloride (PVC) collars (10 cm in diameter and 6 cm in height) into the soil (with about 3 cm left above the ground) and distanced by more than 1 m away from the plot edge. Soil respiration was measured by a portable soil CO₂ fluxes system (LI-8100; Li-Cor, Lincoln, Nebraska, USA) with its chamber placed on these PVC collars between 9:00 am and 11:00 am. Measurements were made three times every month in 2011 but once every week in 2012 and 2013 to better fit the summer water addition frequency we conducted. All measurements were taken from May to October on the third or fourth day each year after summer water addition. To avoid aboveground plant respiration, the green parts were clipped just before the measurement day. Upon measurements, the covering chamber (10 cm in diameter and 10 cm in height) was placed on the collar. Each measurement would generally take about 2 min.

Soil temperature and soil moisture

Soil temperature was determined simultaneously with R_s using thermocouple probe (LI 8100-201), which was connected to LI-8100 at 10 cm depth near each PVC collar. Similarly, soil moisture was determined at the same depth with a TDR-200 probe (Spectrum Technologies Inc., Plainfield, Illinois, USA).

Belowground biomass (BGB) and soil microbial biomass carbon (MBC)

Soil samples to 50 cm deep were collected using 7 cm diameter soil corer on 12 August 2011, 13 August 2012, and 10 August 2013, to collect root biomass. Samples were immediately washed, sieved (using 1.0-mm sieve), and oven-dried at 65°C, and the constant weight of these roots was seen as the total BGB.

Three cores of fresh soil were collected using 2.7 cm diameter soil corer at 0–10 cm deep and then mixed completely by plot on 23 July 2011, to measure MBC. After passing through a 2-mm

sieve, the soil samples were stored in the icebox and taken back to laboratory for further determination of soil MBC. The determination of MBC followed the procedures of fumigation–extraction method (Liu et al. 2009, Tan et al. 2013).

Temperature sensitivity of soil respiration

While soil respiration could have exponential relationship with soil temperature and linear relationship with soil moisture in the steppe and other ecosystems (Chen et al. 2015, Hinko-Najera et al. 2015), other studies demonstrated that the following exponential–quadratic function (Eq. 1) was the best regression model which could simulate the relationship of R_s with soil temperature and soil moisture (Saiz et al. 2007, Jiang et al. 2013):

$$R_s = ae^{bT_s}(C\theta v + d\theta v^2) \quad (1)$$

where R_s is soil respiration ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), T_s is the soil temperature ($^{\circ}\text{C}$) at the 10 cm depth, and θv (V/V%) was soil moisture at the 10 cm depth. The letters a , b , c , and d are fitted parameters and b is related to temperature sensitivity of R_s .

The sensitivity of R_s to temperature alone can be further determined using the Q_{10} value, which represents the increase rate of R_s with a 10°C change in temperature:

$$Q_{10} = e^{10b} \quad (2)$$

where b indicates the fitted parameter obtained from Eq. 1.

Statistical analysis

We applied mixed-model analysis to detect the single or interactive effects of spring snow addition or summer water addition with N addition on T_s , θv , the growing season R_s , and soil moisture in May to early June before summer water manipulation started (SM, V/V%) in each year. In the linear mixed model, water addition and N addition were fixed factors, plots were specified random subject, and the measuring days were repeated variables to analyze dependent variables. Three-way ANOVA was used to analyze the effects of year (Y), water addition (W) either as spring snow or as summer water, N addition (N), and their interactions on R_s during 2011–2013. Two-way ANOVA was used to analyze the effects of water addition (W) either as spring snow or as summer water, N addition (N), and their interactions on BGB and Q_{10} in 2011–2013

and MBC in 2011. One-way ANOVA was applied to examine the effects of change in T_s , θv under water and N addition on Q_{10} . The relationships of R_s with BGB, T_s , θv in the three growing seasons, and MBC in 2011 were further explored using regression analysis. All data were analyzed with SPSS 17.0 for Windows (SPSS, Chicago, Illinois, USA).

RESULTS

Responses of soil temperature and moisture to water and N addition

Both spring snow addition and summer water addition showed significant effects either on soil temperature or on moisture, but the effects varied across different years. Averaged across growing seasons, spring snow addition showed no significant effects on soil temperature during the three study years (Fig. 1a, c, e, Table 2). However, it increased soil moisture significantly in 2012 (9.2%) and moderately in 2011 (by 6.4%) and 2013 (by 3.0%; Fig. 1b, d, f, Table 2). Besides, spring snow addition increased soil moisture more significantly in May and June in 2012 (22.8%) and 2013 (by 12.6%) before the rainy season starts. Summer water addition decreased soil temperature in 2011, 2012, and 2013 (by 3.1%, 2.6%, and 2.2%, respectively), but significantly increased soil moisture by 31.5%, 22.2%, and 31.0%, respectively, across the 3 yr (Fig. 1a–f, Table 2).

Similarly, N addition also showed notable effects on the soil temperature and moisture and they varied across the 3 yr. Nitrogen addition decreased soil temperature significantly by 1.4% in the spring snow addition treatment and 2.3% in the summer water addition treatment in 2013, respectively. Nitrogen addition and spring snow addition also interactively decreased soil temperature by 1.6% in 2011. N addition interacted with summer water addition, decreasing soil temperature significantly by 2.5% in 2012 and by 4.3% in 2013. Nitrogen addition decreased soil moisture in 2012 and 2013 in both the spring snow addition treatment (by 7.0% and 11.4%, respectively) and the summer water addition treatment (by 5.2% and 8.5%, respectively; Fig. 1d, f, Table 2). Nitrogen addition, however, only moderately increased soil moisture in May and June (by 2.9%) in the summer water addition treatment in 2012 (Table 2).

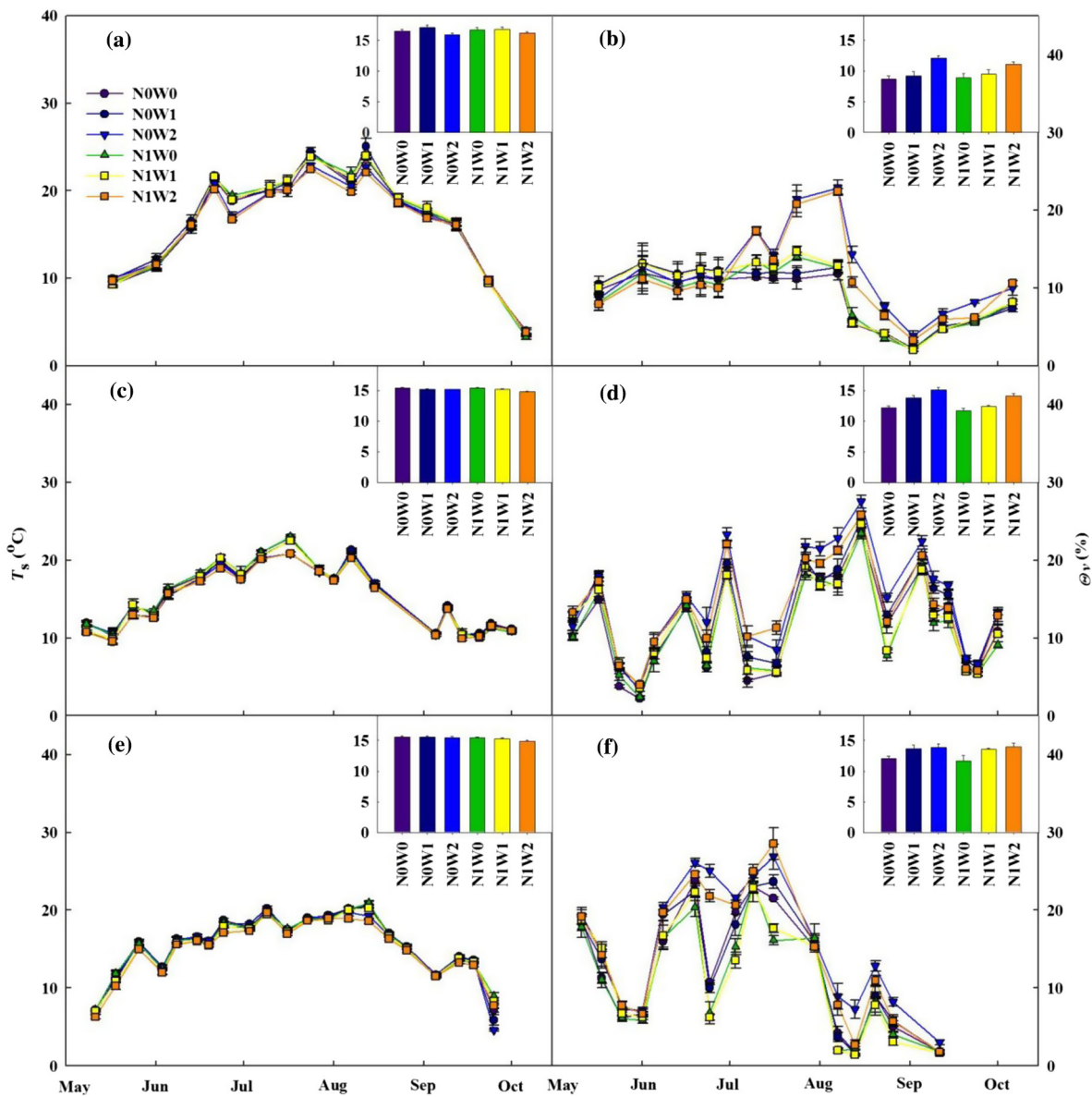


Fig. 1. Seasonal variations in soil temperature (T_s) in all the treatments of 2011 (a), 2012 (c), and 2013 (e) and soil moisture (θ_v , V/V%) in all the treatments of 2011 (b), 2012 (d), and 2013 (f) with inset figures indicating means through the whole growing seasons. Values represent mean \pm 1SE ($n = 5$). N0W0, the control; N0W1, no N addition but with spring snow addition; N0W2, no N addition but with summer water addition; N1W0, N addition with no water addition; N1W1, N addition with spring snow addition; N1W2, N addition with summer water addition.

Responses of soil respiration to water and N addition

There were substantial interannual variations in R_s with the spring snow and summer water addition across the three growing seasons (Table 3). When we analyzed the responses of R_s

to water and N addition separately by year, spring snow addition stimulated R_s by 10.9% in 2012 ($P < 0.001$), but had no significant effects in the other 2 yr (Table 2, Fig. 2a–c). In contrast, summer water addition enhanced R_s consistently in the 3 yr by 46.5% in 2011, 21.8% in 2012, and

Table 2. Results (*P*-values) of mixed-model analysis on the effects of water, N, and their interactions on soil water, temperature, and soil respiration in 2011, 2012, and 2013.

Year	Treatments	Spring snow addition treatment				Summer water addition treatment			
		T_s	θ_v	SM	R_s	T_s	θ_v	SM	R_s
2011	W	0.97	0.07	0.48	0.67	<0.001	<0.001	0.08	<0.001
	N	0.15	0.19	0.12	0.25	0.57	0.32	0.28	0.30
	W × N	0.07	0.94	0.86	0.38	0.14	0.43	0.54	0.59
2012	W	0.44	<0.001	0.001	<0.001	0.01	<0.001	<0.001	<0.001
	N	0.64	<0.001	0.29	0.68	0.26	0.01	0.10	0.83
	W × N	0.63	0.19	0.43	0.68	0.03	0.39	0.26	0.44
2013	W	0.39	0.08	<0.001	0.43	0.01	<0.001	0.002	<0.001
	N	0.004	0.001	0.71	0.001	0.001	0.05	0.74	0.002
	W × N	0.12	0.67	0.47	0.05	0.05	0.74	0.97	0.07

Note: W, Water addition, either as spring snow addition or as summer water addition; N, N addition; T_s , soil temperature (°C); θ_v , soil moisture (V/V%) across year; R_s , growing season soil respiration; SM, soil moisture in May and early June before summer water manipulation started (V/V%).

16.1% in 2013 (all $P < 0.001$, Fig. 2a–c). Nitrogen addition decreased R_s by 5.4% ($P = 0.001$) in the spring snow addition treatment and by 6.9% ($P = 0.002$) in the summer water addition treatment in 2013, while the effects were insignificant for either spring snow addition or summer water addition treatment in 2011 and 2012 (Table 2). There were positive interactive effects on R_s significantly in 2013 by 4.0% between spring snow addition and N addition and marginally by 8.0% between summer water addition and N addition (Table 2).

Soil respiration changed quadratically with total annual precipitation (natural + manipulative) in both spring snow addition and summer water addition treatments regardless of N addition (Fig. 3). It first increased and peaked at

around 360 and 440 mm for spring snow and summer water addition, respectively, but then decreased as precipitation increased (Fig. 3).

Effects of water and N addition on BGB and soil MBC

The effects of increase in both water and N supply on BGB were also year specific. Spring snow addition showed increasing effects on BGB only in 2012 (by 25.1%, $P = 0.05$), while the effects were insignificant in other 2 yr. Summer water addition increased BGB by 22.5% in 2011 ($P = 0.05$), 18.4% ($P = 0.04$) in 2012, and 16.7% ($P = 0.04$) in 2013 and marginally stimulated MBC ($P = 0.07$) by 35.1% in 2011 (Fig. 4, Table 4). Nitrogen addition had no significant effect on BGB across the 3 yr and on MBC in 2011 (Table 4). There were no significant interactive effects between water addition and N addition on BGB and soil MBC.

Relationships of soil respiration with soil temperature, moisture, and BGB

Across the 3 yr, growing season R_s exponentially increased with soil temperature for both spring snow (Fig. 5a) and summer water addition (Fig. 5c), and linearly increased with soil moisture for both spring snow (Fig. 5b) and summer water addition (Fig. 5d) regardless of N addition. There was no difference in sensitivity with soil moisture across the three growing seasons (Fig. 5b, d). Spring snow addition increased the R_s temperature sensitivity (Q_{10}) by 12.3% in 2012 and 4.8% in 2013, but had no effect on it in

Table 3. Results (*F* and *P*-values) of three-way ANOVA on the effects of year, water, N, and their interactions on soil respiration during 2011–2013.

Treatments	df	Spring snow addition		Summer water addition	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
W	1	1.15	0.29	70.18	<0.001
N	1	1.10	0.30	1.85	0.18
Y	2	77.89	<0.001	49.05	<0.001
W × N	1	0.70	0.41	0.30	0.59
W × Y	2	1.57	0.22	2.85	0.07
N × Y	2	0.38	0.69	0.72	0.49
W × N × Y	2	0.22	0.80	0.36	0.70

Note: Y, year; W, water addition, either as spring snow addition or as summer water addition; N, N addition; R_s , soil respiration.

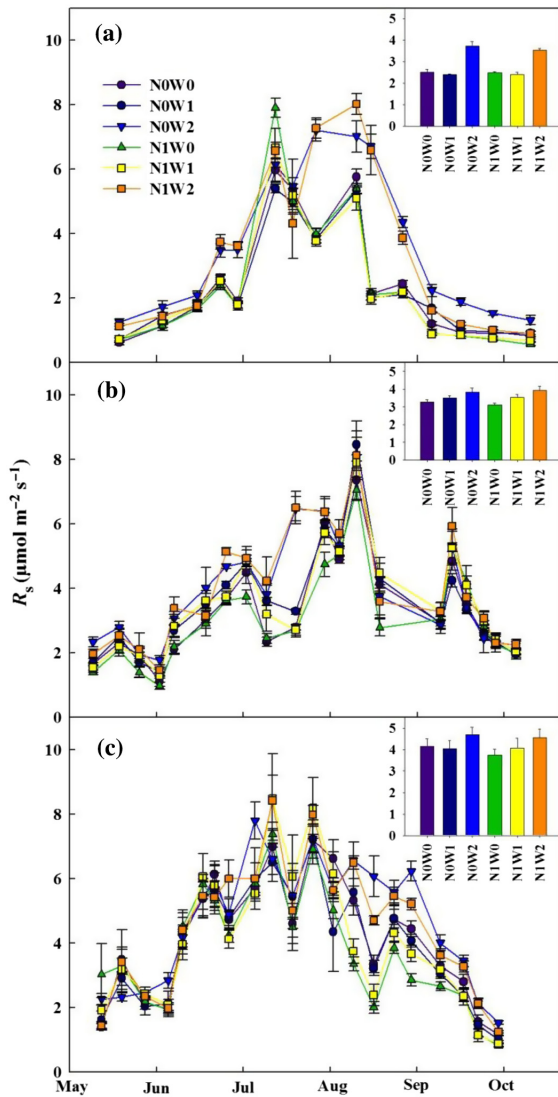


Fig. 2. Seasonal dynamics of soil respiration (R_s , mean \pm SE) with inset figures of means (mean \pm SE) during growing seasons in 2011 (a), 2012 (b), and 2013 (c). See Fig. 1 for explanation of treatment and Table 2 for statistical analysis results.

2011. Summer water addition elevated Q_{10} by 16.3% in 2011, 40.9% in 2012, and 11.9% in 2013 ($P < 0.01$ for all years). There were no significant interactive effects on Q_{10} between spring snow addition or summer water addition and N addition (Tables 4 and 5).

Soil respiration increased linearly with BGB both in spring snow addition and in summer water addition treatments with or without N addition

across the three study years (Fig. 6a). Similar trend also occurred with MBC in spring snow ($r^2 = 0.18$, $P = 0.06$) and summer water treatment ($r^2 = 0.23$, $P = 0.03$) regardless of N addition (Fig. 6b).

DISCUSSION

Effects of spring snow and summer water addition on soil respiration

Changes in both snowfall and rainfall can alter R_s during growing season by modifying both abiotic (soil temperature and soil moisture content) and biotic (belowground plant growth and microbial activities) conditions. Increased snowfall during winter time may release higher amount of water gradually to the soil as it starts to melt, and its effects on R_s can extend to the growing season. The effect of spring snow addition on growing season R_s , however, was only significant when (1) the background snowfall was relatively low (as in 2012) and (2) it did not melt early (as in 2011). Spring drought is common in this region as seasonal rainfall generally will not start until June (Yan et al. 2010, Zheng et al. 2011). Consequently, our results showed that spring snow addition only increased the growing season R_s in 2012, but not in the other 2 yr as either the snowmelt time was too early in 2011 (March 16) or the annual snowfall was relatively too high in 2013 (77.6 mm) compared with the amount of snow added. Our results

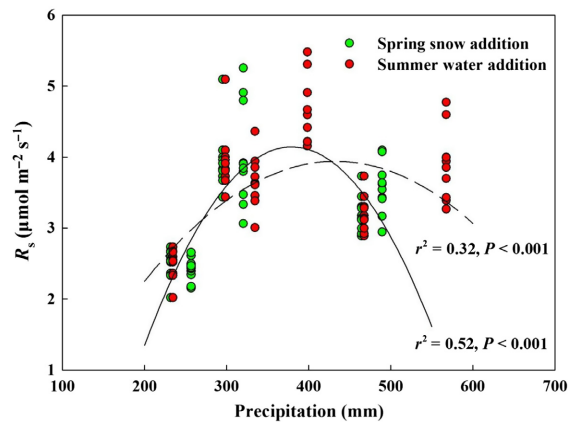


Fig. 3. Relationships of soil respiration (R_s) with precipitation (natural + manipulative) as spring snow addition (green circles and solid lines) and summer water addition (red circles and dashed lines) regardless of nitrogen addition in 2011, 2012, and 2013.

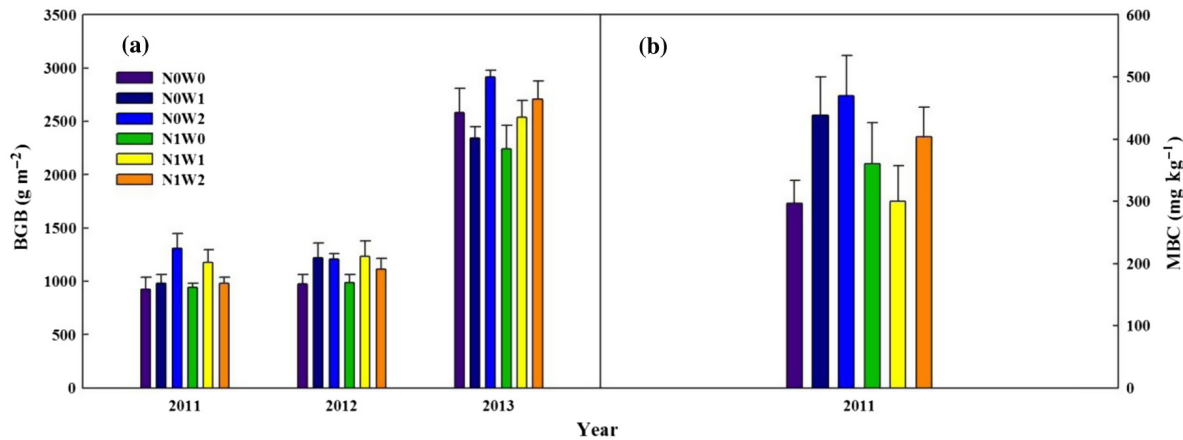


Fig. 4. Results of mean total belowground biomass to 50 cm depth (BGB, g/m^2) in 2011, 2012, and 2013 (a) and soil microbial biomass carbon (MBC, mg/kg) in 2011 (b). See Fig. 1 for explanation of treatment.

indicated that the increase in R_s with spring snow addition was caused by the change in soil moisture as spring snow addition increased soil moisture in 2012 (Fig. 1), and further stimulated BGB (Fig. 4). Although we did not determine MBC in the other 2 yr, previous studies showed that soil microbial activities could be stimulated by increase in soil water content, which had important effect on respiration process especially in the semiarid areas (Lipson et al. 2009, Harding et al. 2011).

Summer water addition showed consistent positive effects on R_s during the studied growing seasons, which largely corroborated with results from previous studies in similar (Chen et al. 2013) or different ecosystems (Lai et al. 2013, Qi et al. 2014), consistent with the responses of ecosystem respiration (Zhang et al. 2015). We found that summer water addition enhanced plant BGB in the three growing seasons, indicating more supply

of C substrate for R_s (McCulley et al. 2007, Ren et al. 2015) and consequently soil microbial activities as indicated by the increase in MBC in 2011 (Table 4). Our results, however, indicated that the increasing effect of summer water addition on R_s was larger when annual rainfall was relatively low (as in 2011; Fig. 2a), but smaller when annual rainfall was relatively high (as in 2012; Fig. 2b). Nevertheless, the extent of R_s increase was low in 2013 despite the low annual rainfall compared with that in 2012. We considered that the relatively high precipitation in 2012 might have strong legacy effect on R_s in 2013 as indicated by the soil moisture, which showed that average soil moisture in the growing season was significantly higher in 2012 and 2013 than in 2011 (Appendix S1: Table S1), consistent with some previous studies (Sala et al. 2012, Reichmann et al. 2013, Monger et al. 2015). Besides, the higher precipitation in 2012 or the legacy effects of higher precipitation in 2013 also

Table 4. Results (P -values) of two-way ANOVA on the effects of water, N, and their interactions on belowground biomass and Q_{10} in 2011–2013 and microbial biomass carbon in 2011.

Form of water addition	Treatments	BGB			MBC	Q_{10}		
		2011	2012	2013	2011	2011	2012	2013
Spring snow addition	W	0.16	0.05	0.88	0.48	0.34	0.02	<0.001
	N	0.28	0.93	0.69	0.52	0.80	0.16	0.55
	W × N	0.36	0.99	0.16	0.09	0.72	0.09	0.72
Summer water addition	W	0.05	0.04	0.04	0.07	0.02	0.001	0.02
	N	0.13	0.64	0.15	0.99	0.37	0.87	0.93
	W × N	0.10	0.53	0.72	0.26	0.73	0.62	0.91

Note: W, water addition, either as spring snow addition or as summer water addition; N, N addition; BGB, belowground biomass; MBC, microbial biomass carbon.

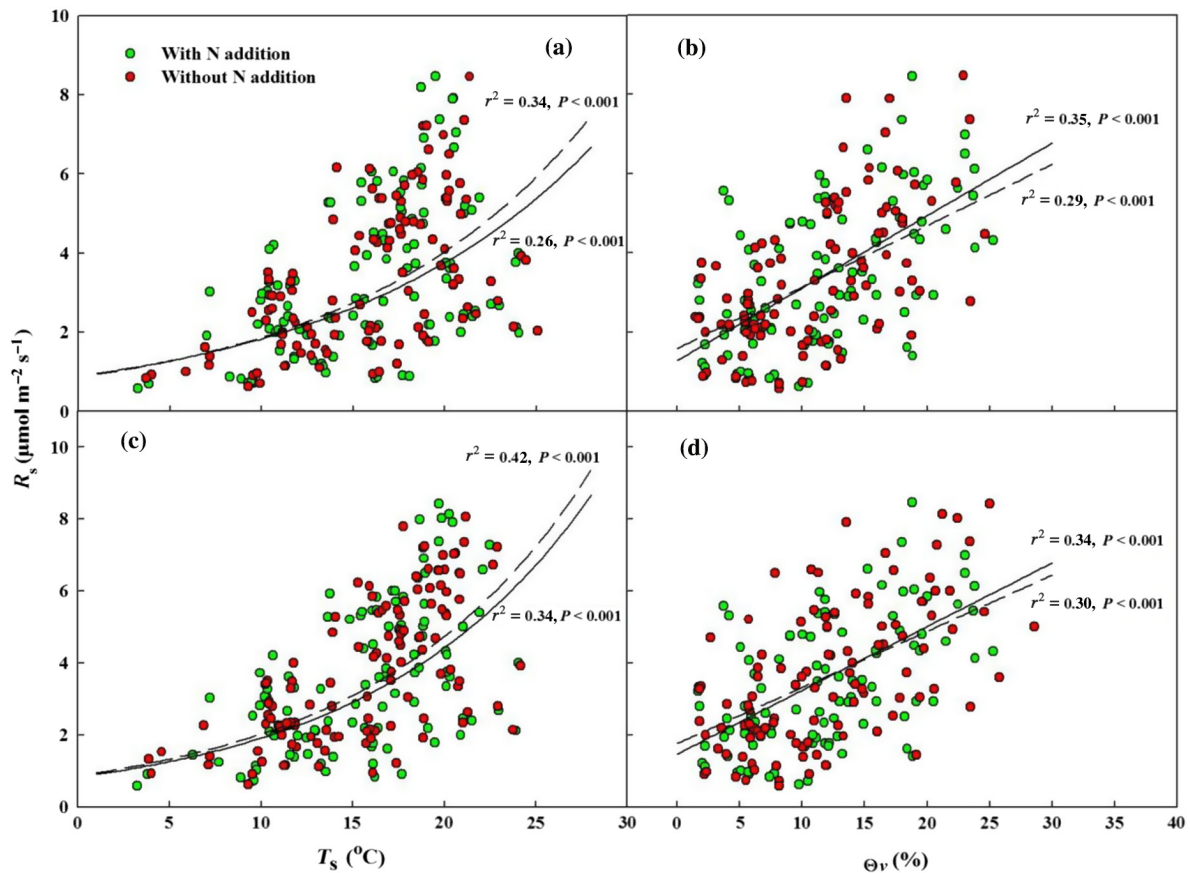


Fig. 5. Relationships of soil respiration (R_s) with soil temperature (T_s) in spring snow addition plots (a) and summer water addition plots (c), and soil moisture (θ_v , V/V%) in spring snow addition plots (b) and summer water addition plots (d) with (green circles and solid lines) or without nitrogen addition (red circles and dashed lines) in 2011, 2012, and 2013.

lowered soil temperature (Appendix S1: Table S1), which induced strong negative effects and offset the positive effects of higher soil moisture on R_s .

We thus concluded that precipitation increase in both snowfall and rainfall can increase R_s via improving soil moisture in the semiarid grasslands although such effect was year specific. Spring snow increase noticeably enhanced R_s , while summer water increase had more important positive effects on R_s until soil moisture becomes too high. However, the difference in the effect on R_s between the spring snowfall increase and summer rainfall increase may be induced by the different increasing amount in spring snow and summer water. Summer water increase also corresponds with high temperature in the growing seasons, influencing R_s to a greater extent than snow.

Nitrogen addition effects on soil respiration

We found no significant responses of R_s to N addition in the first two study years, but a small negative effect in the third year (Table 2). While N addition generally stimulated R_s by (1) increasing relative growth of plant roots and their autotrophic respiration and (2) improving litter quality and decomposition rates of soil organic matter (Fog 1988, Liu et al. 2010), N addition could also inhibit R_s by decreasing microbial activities (Bowden et al. 2004) due to reduction in root exudation (Treseder 2008) and heterotrophic respiration (Janssens et al. 2010). Nitrogen addition also reduced the production of litter-degrading enzymes by decomposing microbes (Fog 1988, Allison et al. 2008), potentially lowering decomposition rates. Indeed, a

Table 5. The sensitivity (Q_{10}) of soil respiration to soil temperature depending on the regression equations of $R_s = ae^{bT_s} (c\theta v + d\theta v^2)$ with soil moisture (θv , V/V %) in 2011, 2012, and 2013.

Treatments	2011	2012	2013
N0W0	1.80 ± 0.14^c	1.72 ± 0.04^c	1.98 ± 0.07^b
N0W1	1.94 ± 0.12^{bc}	2.17 ± 0.11^a	2.79 ± 0.12^a
N0W2	2.56 ± 0.25^{ab}	2.17 ± 0.05^a	2.45 ± 0.20^{ab}
N1W0	2.14 ± 0.35^{abc}	1.75 ± 0.14^c	1.94 ± 0.28^b
N1W1	2.44 ± 0.19^{abc}	1.83 ± 0.10^{bc}	2.63 ± 0.09^a
N1W2	2.71 ± 0.27^a	2.11 ± 0.11^{ab}	2.45 ± 0.16^{ab}

Notes: N0W0, the control; N0W1, only spring snow addition; N0W2, only summer water addition; N1W0, only N addition; N1W1, N addition with spring snow addition; N1W2, N addition with summer water addition; R_s , soil respiration; T_s , soil temperature ($^{\circ}\text{C}$); θv , soil moisture (V/V%). Different superscript letters in a column indicate significant difference ($P < 0.05$) between treatments.

previous study indicated that N addition might have no significant impacts on litter decomposition rates because the positive effect from litter quality was offset by the negative effect on microbial activities (Liu et al. 2010).

Our results showed the BGB had no significant responses to N addition across the 3 yr in our studied system, indicating that there was no additional supply of C for R_s possibly because of the positive effect of N on the community coverage (Appendix S1: Table S2). Nitrogen addition decreased soil temperature and moisture as a

result of the shading effect and increased plant transpiration with increasing aboveground growth. The results were consistent with our findings that the additional N had no significant effects on MBC although we have only 1 yr data, which might explain the insignificant effects of N addition at least in 2011 on plant belowground growth and soil microbial activities. The same results occurred in previous studies which showed that N addition had inhibitive effects on soil microbial respiration (Janssens et al. 2010, Ramirez et al. 2010, Yan et al. 2010, Bae et al. 2015). However, in our earlier publication (Zhang et al. 2015), N addition has trigger effects on ecosystem C exchange which shift from negative to positive with increase in precipitation. We speculated that the effects of N addition on soil respiration were generally weak and trivial as R_s was impacted more by soil moisture and temperature in this steppe ecosystem (Fig. 5), while ecosystem C exchange is determined more by those biological factors.

Adjustment of soil respiration by water and N addition

The sensitivity of R_s to temperature (Q_{10}) is considered as an important feedback determinant of C cycle to climate in terrestrial ecosystem (Luo et al. 2001, Mahecha et al. 2010, Zhao et al. 2016). With our results showing that the increase in

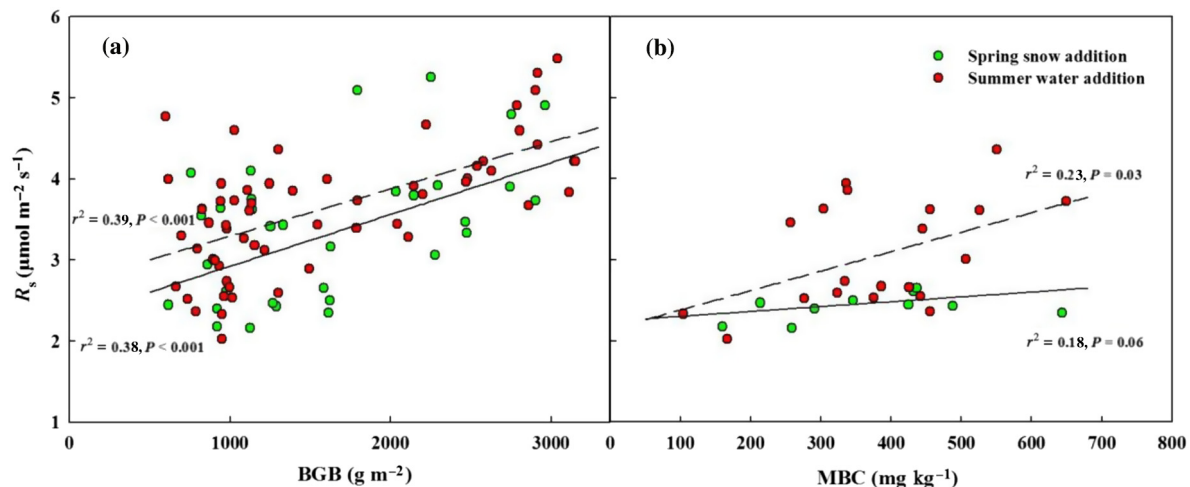


Fig. 6. Seasonal mean soil respiration (R_s) on total belowground biomass (BGB, a) during 2011–2013 and soil microbial biomass carbon (MBC, b) in 2011 in spring snow addition plots (green circles and solid lines) and summer water addition plots (red circles and dashed lines).

spring snowfall and summer rainfall would raise Q_{10} value of growing season R_s , soil respiration under warming would be stimulated by water addition. We supported the findings that the acclimatization of R_s to warming will be tremendously adjusted not only by temperature (Luo et al. 2001), but also by precipitation (Harper et al. 2005, Yan et al. 2010). Across the three study years, we found that growing season R_s had a quadratic relationship with total annual precipitation (natural + manipulative) when the data were pooled with both with and without N addition (Fig. 3). This finding was different from other studies such as in (1) tallgrass prairie where R_s increased at early precipitation, but then leveled off at higher precipitation (Liu et al. 2002), and (2) forested ecosystem where R_s changed linearly with precipitation (Raich and Schlesinger 1992, Tu et al. 2013). While possible occurrence of limited soil aeration at high precipitation might account for the decrease in R_s after reaching a threshold value (Luo and Zhou 2006), we suggested that two abiotic factors, that is, soil moisture (θv) and soil temperature (T_s), primarily controlled the change in R_s with precipitation in this study. The increase in θv could be responsible for the early increase in R_s but then the decrease in T_s reduced faster the R_s as soil temperature exponentially correlated with R_s , while soil water correlated with R_s linearly. This mechanism was supported by our data which showed that T_s was negatively but θv was positively correlated with precipitation change (Appendix S1: Fig. S1a, b).

While water addition increased plant growth and/or microbial activities, and correspondingly the soil respiration, N addition only showed a small decreasing effect on R_s , possibly because of the non-significant effects of N on plant BGB growth during the study years and accumulative negative effect on microbial activities as in 2011. In contrast to our third hypothesis, we did not find much coupling effects on R_s between N addition and water increase. This finding was completely different from the strongly coupling effects on ecosystem C exchange in that the effect of N addition was highly dependent on precipitation amount (Zhang et al. 2015). This study indicated that soil respiration could be more inert to future increase in N deposition, but more sensitive to precipitation change in both snowfall and rainfall in this temperate steppe ecosystem.

CONCLUSIONS

Our study suggests that spring snow addition had increasing effects on the growing season R_s , but the effect highly depended on the amount of background snowfall and snowmelt time. Summer water addition has significantly increasing effects on R_s in all the 3 yr ascribing to the increase in plant growth and/or microbial activities. The extent, however, may decline with increasing background precipitation and change with possible legacy effect of previous year precipitation. Nitrogen addition showed a trivial or decreasing effect on R_s only in the third year possibly because of non-significant effects on plant belowground growth and a possibly accumulative negative effect on microbial activities. The change in the growing season R_s with precipitation in this steppe grassland was controlled more by the change in precipitation and consequent changes in soil moisture and soil temperature, but less by the increasing N deposition. Overall, precipitation change in the form of snowfall had weaker effects than that in the form of rainfall on R_s because the snowfall only accounted for a small portion of the precipitation after all in this steppe area in Inner Mongolia. Our results thus provided an important contribution for modeling ecosystem C balance under the current and future global change scenarios.

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