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Interactive effects of belowground organic matter input, increased precipitation and clipping on soil carbon and nitrogen mineralization in a temperate steppe

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Abstract

Soil organic matter (SOM) inputs, increased precipitation and clipping (reducing belowground photosynthates allocation) are predicted to affect soil C and N cycling in temperate grassland ecosystems. However, the interactive effects between SOM inputs (or increased precipitation) and clipping on soil C and N mineralization in temperate steppes are still poorly understood. A field manipulation experiment was conducted to quantify the effects of SOM inputs, increased precipitation, clipping and their interactions on soil C and N mineralization in a temperate steppe of northeastern China from 2010 to 2011. The results showed that SOM inputs significantly increased soil C mineralization rate (CMR) and net N mineralization rate (NMR). Increased precipitation-induced enhancement of soil CMR essentially ceased after the first year, stimulation of soil NMR and NNR continued into the second year. However, clipping only marginally decreased soil CMR and NMR during the two years. There were significant synergistic interactions between SOM inputs (or increased precipitation) and clipping on soil CMR and NMR, as SOM inputs (or increased precipitation) showed greater effects on soil CMR and NMR under clipped plots than under unclipped plots, which could be explained by the relative shifts in soil microbial community structure because of bacterial biomass increases, and by the relative decreases in arbuscular mycorrhizal fungi biomass due to the reduction of belowground photosynthates allocation. These results highlight the importance of plants in mediating the responses of soil C and N mineralization to potentially increased SOM and precipitation by controlling belowground photosynthates allocation in the temperate steppe. Thus, the findings have important implications for improving prediction of C and N sequestration potential and its feedbacks to climate change in temperate steppe ecosystems.

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1 Introduction

About 75 % of organic carbon contained in terrestrial ecosystems and the majority of organic nitrogen is found in plant residues and soil organic matter (SOM) (Lal, 2008). Both soil organic carbon and nitrogen are mineralized to simple inorganic forms by a highly dynamic community of microbial decomposers (Paul, 2007; Manzoni and Porporato, 2009). Soil C and N mineralization processes provides most of the inorganic nutrient necessary for plant and microbial growth, and it further involves gross release of CO_2 to the atmosphere, and then feedback to climatic changes (Manzoni and Porporato, 2009; Xia et al., 2009). Therefore, soil C and N mineralization have important roles in the functioning of soil in nutrient cycling, structural formation and plant interactions (Wardle et al., 2004; Harris et al., 2009).

Climatic changes could impact both abiotic and biotic drivers (e.g., air temperature, precipitation regime, quantities and qualities of plant litter and soil microbial properties) in ecosystems and the responses of C and N mineralization to these changes (Wang et al., 2006; Jin et al., 2010; Ma et al., 2011). Anticipated global warming and increasing atmospheric CO_2 concentration are assumed to increase primary production in semiarid grassland ecosystems (IPCC, 2007), and consequently much more plant residue will be incorporated into soil organic matter (SOM). Soil C and N mineralization processes responses to SOM inputs have been well documented for forest, farmland and heath ecosystems (Rinnan et al., 2007, 2008; Busse et al., 2009; Feng et al., 2009; McIntyre et al., 2009). Previous studies suggest that SOM inputs not necessarily translate into increased C and N sequestration in energy-limited ecosystems, because microbial activity could be stimulated to such a degree that most or all of the added SOM is decomposed rapidly (Fontaine et al., 2004); however, a number of studies in long-term field experiments show that the amount of C sequestered is linearly related to SOM inputs (Jastrow et al., 2005). Therefore, whether SOM inputs result in the enhancement of soil C and N mineralization in temperate steppes remains controversial.

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Global climate models predict changing precipitation regimes in the future, with increasing precipitation at the mid-latitude regions (IPCC, 2007). Changing precipitation is anticipated to affect soil C and N mineralization processes, especially in arid and semi-arid ecosystems where water availability is a critical factor limiting plant and microorganism growth (Smith et al., 1997; Weltzin et al., 2003). Increased precipitation (or water addition) would stimulate rates of microbial respiration and nutrient mineralization in short- and long-term experimental conditions (Sparling et al., 1995; Xiao et al., 2007; Collins et al., 2008). However, this prediction still lacks mechanistic understanding of C and N mineralization processes responses to the increased precipitation region.

Previous studies have shown that plants controlled soil C and N cycling by producing litter of different quantities and qualities in water- and N-limited ecosystems (Zhang et al., 2005; Jin et al., 2011). However, recent evidence claim that plants may exert much greater effects on soil C and N cycling than previously estimated by controlling belowground photosynthates allocation (Chapman et al., 2006; Kaiser et al., 2011). Högberg and Read (2006) reported that approximately half of the soil respiration was derived from belowground photosynthates allocation. This large photosynthates input to the soil microbial community may in turn significantly affect soil C and N cycling. In temperate grasslands, land management practices such as heavily grazing and repeatedly harvesting during growing season probably significantly influences the direct impacts of SOM inputs and increased precipitation on soil C and N mineralization. For example, SOM inputs or increased precipitation might accelerate soil C and N mineralization processes, but these positive effects may be partly eliminated by above plant biomass removal due to the decreases in plant belowground photosynthates allocation and water fluxes (Zhang et al., 2005; Kaiser et al., 2011).

To examine the effects of the predicted changes in SOM inputs and precipitation regime on soil C and N mineralization under two management regimes (i.e. clipped and unclipped treatments), we conducted a field experiment in which we artificially manipulated organic matter inputs to topsoil (+60 %), added the precipitations by open-top

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iron boxes (+30 %) and simulated heavily grazing or harvest by clipping monthly in the temperate steppe of northeastern China. The specific questions addressed here were: (1) how do soil C and N mineralization respond to SOM inputs, increased precipitation and clipping during the two growing seasons; and (2) whether SOM inputs, increased precipitation and clipping interact to affect soil C and N mineralization in a temperate steppe.

2 Materials and methods

2.1 Study site and experimental design

This study was conducted at the Hulunber Grassland Ecosystem Observation and Research Station of Chinese Academy of Agriculture Sciences, which is located at Xiertala farm, the center of Hulunber steppe (49° 19′ N, 120° 02′ E, 628 m a.s.l), Inner Mongolia, China. Mean annual air temperature is −3 ~ −1 °C. The mean annual precipitation was approximately 350 mm for periods 1960–2000 (http://www.worldclim.com), 340.5 mm and 320.9 mm in 2010 and 2011 (http://www.climate.nm.cn), respectively. The soil in this area is classified as chestnut soil according to Chinese classification or kastanozems according to the FAO classification. Soil bulk density is 1.37 g cm^{-3} and pH is 7.7, with 5 % to 6 % organic matter in the surface layer. The native vegetation at the site was dominated by perennial grass *Stipa baicalensis*, other abundant plant species include *Leymus chinensis* (Trin.) Tzvel., *Artemisia frigida Willd.*, *Artemisia tanacetifolia Linn.* and *Serratula centauroides*. Total vegetation ground cover ranges from 60–75 %.

The experimental area with fairly uniform vegetation was selected in May 2010, and was divided into two sites. The one was natural site, and the other was clipping site. The distance between the two adjacent sites was 8 m. Forty-eight 2 m × 2 m plots were established in both sites with 24 plots in the natural site and the others in the clipped site. Every 24 plots were based on a complete randomized block factorial experimental

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design and exposed to ambient, soil organic matter (SOM) inputs, increased precipitation and combination of SOM inputs and increased precipitation. There were six replicates for each treatment.

Treatments of SOM input were addition of particulate organic matter to the surface soil layer (0–10 cm) at 720 g m^{-2}. Because the ecosystem total plant above- and belowground biomass was about 1200 g m^{-2} yr^{-1} (Ma et al., 2012), these SOM inputs correspond to increases in ecosystem biomass production of 60 %. The addition rate was designed to approximate projected increases in NPP of temperate steppes by 26–61 % under CO_2 concentration doubling (Gao and Yu, 1998). Senescent plant biomass was harvested from an adjacent field, air-dried and milled to 1–2 mm before use. The C and N contents, the C : N ratio, and the P and lignin contents of the SOM were 40.33 % (standard error (SE) = 2.64 %; n = 6), 0.32 % (SE = 0.03 %; n = 6), 144.6 (SE = 13.2 %; n = 6), 0.025 % (SE = 0.002 %; n = 6) and 20.41 % (SE = 1.24 %; n = 6), respectively. We expected to add the SOM to the upper soil layers without drastically damaging the root systems. For this purpose, we carefully used sharp forks to loosen the surface soil (10 cm), and a predetermined quantity of SOM was gradually and homogeneously added to the soil in the 0–10 cm layer. The soil pores were carefully filled with soil and gently compacted by hand. To create consistent soil disturbance across treatments, the plots with no SOM input were processed in the same manner as the plots that received SOM (Ma et al., 2012).

For increased precipitation treatment simulating a 30 % increase in growing season precipitation from 2010 to 2012, according climate models predict that annual precipitation will increase by 30–100 mm in this century in the steppe (Ni and Zhang, 2000). Two open-top iron boxes (length 85 cm × width 71.5 cm × height 15 cm) were set outside each increased precipitation plots (Fig. 1). The base area of each iron box was approximately 15 % of every plot (2 m × 2 m). The rubber water pipes (1.5 cm inner diameter) were connected to the boxes which could rapidly transfer the precipitations from the boxes to plots. Each pipe was an S-shaped distribution on the ground and many small

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holes were drilled along the pipe so that the rains uniformly flowed into the increased precipitation plots.

In the clipping site, plants were clipped thoroughly every month during the two growing seasons in order to interrupt the photosynthates flow from above canopy to belowground roots. In this water- and nutrient-limited temperate steppe (grass: forb biomass = 0.48), the recover and regrowth after clipping is very slowly, because most of forbs are eliminated after first clipping (Zhu et al., 2004). Moreover, removal of above ground biomass actually increase resource allocation to recover its aboveground photosynthetic organs, thus belowground allocation of photosynthates would significantly decrease (Atkinson, 1991). At the end of each growing season, the clipped plants were returned to their respective plots to maintain natural litter levels.

2.2 Soil samplings and microclimate measurements

Four soil cores (5 cm inner diameter, 10 cm length) were randomly collected from the topsoil (0–10 cm) of each plot in late June, mid-August and late September in 2010 and 2011. The four replicates were mixed to get one composite sample, and then each composite sample was sieved using a 2 mm sieve. Two subsamples of the sieved soil from each composite sample were obtained; one was kept in the refrigerator at 4 °C for routine analyses and the other at −70 °C, for phospholipid fatty acids (PLFAs) analysis.

2.3 Soil microclimate and nutrient measurements

Soil temperature and moisture measurements were conducted one day after the rainfall events. Soil temperature at the depth of 10 cm was measured using a temperature probe connected to an infrared gas analyzer (Li-6400, Li-Cor, USA). Gravimetric soil moisture was measured by oven-drying samples at 105 °C for 24 h.

Concentrations of inorganic N (NH_4^+-N and NO_3^--N) in the filtered extracts were determined using a flow injection autoanalyzer (FIAstar 5000 Analyzer, Foss Tecator, Denmark). Soil organic C and total N contents were measured by the dichromate oxidation

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method (Nelson and Sommers, 1982) and Kjeldahl method (Bremner and Mulvaney, 1982). Soil extracts from the fresh samples were obtained by shaking soil samples with 60 mL 0.5 M K_2SO_4 for 30 min. The extracts were filtered through 0.45 µm filters and their extractable organic C analysed by dichromate digestion as described by Lovell et al. (1995). The data of C in the unfumigated soil extracts were used as soil dissolved organic C (DOC) (Liu et al., 2010). The soil light SOM and heavy SOM were determined using the density fractionation method (Sollins et al., 1984).

2.4 Soil microbial community

Phospholipid fatty acids (PLFAs) were extracted from 8.0 g (dry weight equivalent) soils using a procedure described by Bossio and Scow (1998). The separation and identification of extracted PLFAs were carried out according to the standard protocol of the Sherlock Microbial Identification System $V_{4.5}$ (MIDI) and a Gas Chromatograph (Agilent 6850, USA). Methyl nonadecanoate fatty acid (19: 0) was used as internal standard. Fatty acid nomenclature used in this study was as that defined by Bossio and Scow (1998). The fatty acids a13: 0, i14: 0, i15: 0, i16: 0, i17: 0 and a17: 0 were chosen to represent the gram-positive bacteria; 16: 1ω5c, 16: 1ω7c, 17: 1ω8c, 18: 1ω5c, 18: 1ω9t, 17: 0cy and 19: 0cy were chosen to represent the gram-negative bacteria (Frostegård et al., 1993, 1996); Three fatty acids (16: 1ω5c, 18: 2ω6, 9c and 18: 1ω9c) were chosen to represent the fungal group (Olsson et al., 1998; Ma et al., 2012). The PLFA 16: 1ω5 was used as an indicator of arbuscular mycorrhizal fungi (Olsson et al., 1999).

2.5 Soil C and N mineralization

Soil C mineralization rate, i.e. the microbial respiration, was estimated by determining CO_2 evolution over 2-wk incubation period. Respired CO_2 was then captured in 5.0 mL of 0.5 M NaOH contained in a beaker suspended inside each Mason jar. The NaOH

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solution was removed and titrated to determine the amount of CO_2 evolved (Hu and van Bruggen, 1997; Ma et al., 2012).

The in situ soil N mineralization method used was similar to that described by Raison et al. (1987). A pair PVC tubes (12 cm in length, 5 cm in diameter) were inserted vertically into a depth of 10 cm soil layer to incubate a soil volume in the absence of plant uptake. One soil core from each pair was taken as the unincubated sample to measure initial NH_4^+-N and NO_3^--N concentrations by a flow injection autoanalyzer (FIAstar 5000 Analyzer, Foss Tecator, Denmark). The other core was incubated in situ lasting an average of 30 d (minimum 27, maximum 32 (d) in capped tubes to prevent leaching with rainfall. Soil NH_4^+-N and NO_3^--N concentrations in the incubated samples were also analyzed after incubation. Net N mineralization and net nitrification rate were calculated as the changes in NH_4^+-N and NO_3^--N concentrations in the initial and incubated samples (Wang et al., 2006).

2.6 Statistical analysis

Monthly mean values used in this study were averaged from six replicates in the same month. Repeated measures ANOVAs were used to examine the temporal (inter- or intra-annual) variations and the effects of SOM inputs, increased precipitation and clipping on soil microclimate, soil DOC and inorganic N, soil microbial biomass, microbial community, and soil C and N mineralization. Between-subject effects were evaluated as SOM inputs, increased precipitation, clipping and their interactions, and within-subject effects were year (or measuring times within season) and its interactions with SOM inputs, increased precipitation or clipping. Stepwise multiple linear analyses were used to determine the relationships of soil C mineralization (or N mineralization) with control factors. Multiple comparisons were also performed to permit separation of effect means using the least significant difference test at a significance level of $P < 0.05$. Data management and statistical analyses were performed using SPSS 17.0 software (SPSS, Chicago, IL, USA).

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3 Results

3.1 Soil microclimate

Seasonal dynamics of both air temperature and precipitation showed one-peak patterns, which were higher in summer and lower in spring and autumn (Fig. 2a). No difference in mean annual or seasonal air temperature was detected between 2010 and 2011. Total precipitations in 2010 (340.5 mm) and 2011 (320.9 mm) were 2.7 % and 8.3 % lower than the long-term mean (350 mm), respectively.

Soil organic matter (SOM) inputs showed no effect on soil temperature and moisture (0–10 cm) during the two growing seasons (Fig. 2b, d). Increased precipitation elevated soil moisture by 12 % and 9 % ($P < 0.05$, Fig. 2d; one-way ANOVAs) in 2010 and 2011, respectively. Clipping increased top layer soil temperature by 0.6 °C and 0.8 °C ($P < 0.05$, Fig. 2c; one-way ANOVAs) in 2010 and 2011, respectively. SOM inputs interacted with increased precipitation to affect soil moisture ($P < 0.05$, Fig. 2d, Table 1; repeated measures ANOVAs), because increased precipitation treatments significantly increased soil moisture under ambient SOM conditions but had minor effect under SOM input plots.

3.2 Treatment effects on control factors over soil C and N mineralization

SOM inputs significantly increased soil dissolved organic C (DOC) by 12.8 % and 9.1 % ($P < 0.01$, Fig. 3a), total PLFAs by 22.8 % and 28.1 % ($P < 0.001$, Fig. 4a), fungal PLFAs by 24.5 % and 29.5 % ($P < 0.001$, Fig. 4c), gram-negative bacterial PLFAs by 48.2 % and 52.2 % ($P < 0.001$, Fig. 4e), arbuscular mycorrhizal fungal (AMF) PLFAs by 16.2 % and 24.2 % ($P < 0.001$, Fig. 4k) in 2010 and 2011, and slightly increased soil light organic matter by 9.5 % after two years ($P < 0.1$, Fig. 3e). However, SOM inputs showed no effect on soil inorganic N (IN), soil heavy organic matter (SHOM), gram-positive bacterial PLFAs, or the ratio of fungal to bacterial PLFAs ($F : B$) in the two years.

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Increased precipitation significantly reduced soil IN by 8.3 % ($P < 0.05$) and 20 % ($P < 0.01$, Fig. 3c) in 2010 and 2011, respectively. In addition, increased precipitation only increased soil $F : B$ by 23.1 % and 22.8 % ($P < 0.001$, Fig. 4i) in August and September 2011.

Clipping significantly reduced soil DOC by 15.3 % and 20 % ($P < 0.001$, Fig. 3b), fungal PLFAs by 14.5 % and 16.6 % ($P < 0.001$, Fig. 4d), F: B by 11.7 % and 15 % ($P < 0.01$, Fig. 4j), AMF by 31.8 % and 36.2 % ($P < 0.001$, Fig. 4l) in 2010 and 2011, and decreased soil light organic matter by 19 % after two years ($P < 0.05$, Fig. 3f). Moreover, clipping increased soil IN by 9.8 % and 7.7 % in the two years ($P < 0.05$, Fig. 3d). However, no main effect of clipping on soil total PLFAs, gram-negative bacterial PLFAs, and gram-positive bacterial PLFAs were detected across the two years.

There were significant interactive effects between clipping and SOM inputs (or increased precipitation) on soil IN, fungal PLFAs, gram-negative and gram-positive bacterial PLFAs, $F : B$, and AMF ($P < 0.05$, Figs. 3d, 4d, f, h, j, and l, Table 1; repeated measures ANOVAs). Moreover, SOM inputs interacted with increased precipitation to affect soil fungal PLFAs, gram-negative bacterial PLFAs, and $F : B$ ($P < 0.05$, Figs. 4c, e, and 3l, Table 1).

3.3 Control factors over soil C and N mineralization at the temporal and spatial scales

In general, soil C mineralization rate (CMR), net N mineralization rate (NMR) and net nitrification (NNR) showed pronounced seasonal variations with the higher values in summer and lower values in spring and autumn during the two growing seasons (Fig. 5a–f). Across the two growing seasons, stepwise multiple regression analysis of soil CMR (NMR or NNR) with control factors indicated the combination of soil temperature (partial $r^2 = 0.54$ in CMR, partial $r^2 = 0.47$ in NMR, partial $r^2 = 0.53$ in NNR; $P < 0.001$) and soil moisture (partial $r^2 = 0.32$ in CMR, partial $r^2 = 0.35$ in NMR, partial $r^2 = 0.30$ in NNR; $P < 0.001$) explained 86 %, 82 % and 83 % of the seasonal variations of soil CMR, NMR and NNR. These results suggest that all the concurrent seasonal

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variations of soil temperature and moisture contributed to the temporal fluctuations of soil C and N mineralization.

Across the 48 plots, stepwise multiple regression analyses demonstrated that 67.2 % of the spatial variation in soil CMR could be explained by seasonal mean soil total PLFAs, soil moisture, $F : B$ and AMF in 2010 (Table 2). In 2011, soil total PLFAs, $F : B$, soil moisture, AMF and IN were responsible for 87 % of the spatial variation in CMR. Sixty-five percent of the spatial variation in soil NMR could be explained by seasonal mean soil moisture, total PLFAs, $F : B$ and AMF in 2010. In 2011, soil moisture, total PLFAs and $F : B$ together accounted for 61 % in NMR (Table 2). Dissimilar to soil CMR and NMR, soil moisture alone contributed to 16.4 % of the spatial variation in soil NNR in 2010. In 2011, 33 % of the spatial variability in NNR could be attributable to the combination of soil moisture, total PLFAs and soil temperature (Table 2). These results suggest that abiotic (soil temperature and moisture, and IN) and biotic (soil microbial community structure, microbial biomass and AMF biomass) drivers played important roles in regulating spatial variations in soil C and N mineralization in the temperate steppe.

3.4 Main and interactive effects on soil C and N mineralization

During the two years, SOM inputs increased CMR by 15.9 % and 15.1 % ($P < 0.001$, Fig. 5a), NMR by 7.3 % and 9.2 % ($P < 0.05$, Fig. 5c) in 2010 and 2011, whereas had no effect on soil NNR (Fig. 5e). Increased precipitation only increased CMR by 10 % ($P < 0.05$, Fig. 5a) in 2010, but it increased soil NMR by 9.3 % and 12.6 % ($P < 0.05$, Fig. 5c), NNR by 14 % and 11.7 % ($P < 0.01$, Fig. 5e) in the two years, respectively. Clipping slightly decreased soil CMR by 6.6 % and 6 % ($P < 0.1$, Fig. 5b), NMR by 6.1 % and 5.9 % ($P < 0.1$, Fig. 5d) in 2010 and 2011, but showed no effect on soil NNR. SOM inputs (or increased precipitation) interacted with clipping to affect soil CMR and NMR, as SOM inputs (or increased precipitation) showed greater effects on soil CMR and NMR under clipped plots than under unclipped plots ($P < 0.05$, Fig. 5b, d, and f, Table 1; repeated measures ANOVAs). In addition, significant interactive effects

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between SOM inputs and increased precipitation on the soil CMR, NMR and NNR, in that the increases in soil CMR (NMR or NNR) were significantly smaller than would be expected if the two factors acted additively during the two growing seasons ($P < 0.01$, Fig. 5a, c, and e, Table 1).

4 Discussion

In generally, soil organic matter (SOM) inputs caused sustained increases in soil C mineralization rate (CMR) and net N mineralization rate (NMR) as expected, but did not affect net nitrification rate (NNR) during the two growing seasons (Fig. 5a, c, and e). The rapid increase in soil organic matter decomposition was possibly because SOM inputs increase soil fertility and supply greater energy for soil biota, and consequently stimulate significant increase in active microbial activity. These are supported by observations in other ecosystems (Carter et al., 2003; Tu et al., 2006; Xiao et al., 2007). The findings indicate that the energy limitation of soil microorganisms in the temperate steppe and may have some long-term implications to soil C and N sequestration. The greater SOM inputs to soils only slightly increased light SOM fraction rather than heavy SOM fraction after the two years (Fig. 3e and g) suggest that mineral soils in the temperate steppe have a limited capacity to accumulate mineral component of the soil (Six et al., 2002; Stewart et al., 2007; Chung et al., 2008).

There have been many reports showed the positive responses of soil C and N mineralization to water addition (or increased precipitation) in water-limited temperate ecosystems in short- and long-term field experiments (Wang et al., 2006; Xiao et al., 2007; Kim et al., 2011). In contrast, we found increased precipitation only increased soil CMR in the first year, but not increased it in the second year (Fig. 5a). Some seasons probably have lead to the decreases in the water sensitivity of soil CMR as the increased precipitation progresses. Stepwise multiple regression analyses demonstrated that soil CMR was positively related to inorganic N content, and negatively related to soil $F:B$ at spatial scale in 2011. On one hand, the relative lower soil in-

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organic N levels following increased precipitation (Fig. 3c) may gradually contribute to N limitation for soil microbial activity due to the uptake of plant (Kaye and Hart, 1997; Hu et al., 2001). On the other hand, the increases in soil $F:B$ in the second year (Fig. 4k) under increased precipitation treatments probably alter the decomposition process from a faster bacterial-based channel to a slower fungal-based channel (Jastrow et al., 1998; Zhang et al., 2005; Ma et al., 2012). Fungal-dominated soils have slow C turnover rates because fungi incorporate more C into biomass than bacteria. Some studies documented that the fungal storage of C from plant litter was 26 times greater than the corresponding bacterial storage of C (Suberkropp and Weyers, 1996, Six et al., 2002). Additionally, fungal hyphae have long been recognized to enmesh soil microaggregates into macroaggregates (Jastrow et al., 1998; Bossuyt et al., 2001), which could facilitate soil organic matter pool protection and stabilization in the long-term (Bailey et al., 2002).

It is interesting in our study, although the water-induced enhancement of soil CMR essentially ceased after the first year, stimulation of soil NMR and NNR continued into the second year (Fig. 5c and e). A possible explanation for this result is likely because of the changes in plant quality. We found that the decreases in plant C : N under increased precipitation treatments in the same study (Ma et al., 2012) could contribute to a large quantity of lower C : N ratio residues incorporating into soil organic matter, and then increasing N release during decomposition process (Aber et al., 1998; Boggs et al., 2000).

SOM inputs (or increased precipitation) and land cover change may potentially trigger complex interactive influences on ecosystem functioning. However, contrary to our expectation, there were significant synergistic interactions (amplifying effects) between clipping and SOM inputs (or increased precipitation), because SOM inputs (or increased precipitation) exerted more effects on soil CMR and NMR under clipped plots than unclipped plots. Several mechanisms may contribute to the synergistic effects. Firstly, in the clipped plots, SOM inputs could supply more available energy and nutri-

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ent to soil microorganisms without plant uptake, and subsequently could exert larger priming effects on soil CMR and NMR in the temperate steppe.

Secondly, SOM inputs induced alteration in the microbial community structure under clipped plots may partially contribute to the observed synergistic effects. In this study, the negative linear correlations between soil CMR (or NMR) and $F:B$ across the two growing seasons support above findings. The relative lower soil $F:B$ mainly due to strong increase in bacterial biomass, especially gram-negative bacteria (Fig. 4h), may accelerate soil CMR and NMR. The main component of bacterial membranes is phospholipids, while two component of fungal cell walls are the polymers of melanin and of chitin. The phospholipids are energy-rich, readily-decomposable substrates, whereas the polymers are much more resistant to degradation. Therefore, bacteria tend to store less of the C and N they metabolize (Bailey et al., 2002). Consequently, the relative lower soil $F:B$ likely alter the decomposition process from a slower fungal-based channel to a faster bacterial-based channel (Zhang et al., 2005).

Thirdly, the synergistic effect could have been partly attributed to the interactive effect between clipping and SOM inputs on soil arbuscular mycorrhizal fungi (AMF). Being a major component of soil microbial biomass (a second subset of fungi), AMF, also plays a distinct and unique role in soil C and N sequestration (Rillig et al., 2001; Kaiser et al., 2011). This group, which symbiotically colonizes plant roots, forms associations with 80 % of plant species (Smith and Read, 1997). Plants allocate an estimated 10–20 % of net photosynthate to AMF. AMF hyphae produce glomalin, a recalcitrant glycoproteinaceous substance highly directly correlated with soil aggregate stability (Jastrow and Miller, 1997; Steinberg and Rillig, 2003). Additionally, AMF hyphae together with fine roots create a “sticky-string bag” that enmeshes and entangles soil particles, helping to stabilize macroaggregates (Miller and Jastrow, 2000). Here we presented evidence that SOM inputs significantly increased soil AMF biomass under unclipped plots but showed modest effect under clipped plots (Fig. 4c and d). Therefore, the relative lower AMF biomass likely contributes to a higher SOM decomposition in this study.

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Increased precipitation interacted with clipping also causing synergistic effects on soil CMR and NMR (Fig. 5b and d), as the increases in CMR and NMR were significantly larger than would be expected if the two factors acted additively during the two growing seasons. The most probable explanation for the synergistic effect is also attributed to the shift in soil microbial community structure. In this experiment, increased precipitation enhanced soil $F : B$ under unclipped plots after the two growing seasons, whereas it significantly decreased soil $F : B$ under clipped plots. The relative lower soil $F : B$ due to the rapid increase in soil bacterial biomass (Fig. 4l) may contribute to the higher soil CMR and NMR.

In addition, there were significant antagonistic effects between SOM inputs and increased precipitation on soil CMR, NMR and NNR ($P < 0.01$, Table 1), in that the increases in CMR, NMR and NNR were significantly smaller than would be expected if the two factors acted additively during the two growing seasons. This is likely because the strongly positive response of aboveground plant biomass, especially dominant species (*Stipa baicalensis* and *Leymus chinensis*), to combined SOM inputs and increased precipitation (Ma et al., 2012) may lead to more evapotranspiration by local plants and the rapid decline in soil moisture, which could partly suppress the positive precipitation effects on soil C and N mineralization. During the two years, SOM inputs interacted with increased precipitation to affect soil moisture ($P < 0.05$, Table 1, Fig. 2d); the increased precipitation treatments significantly increased soil moisture under ambient SOM conditions but had minor effect under SOM input plots.

These findings highlight the multifactor effects would be more complex than simple combinations of single-factor responses. Considering unprecedented climatic change associated with human activity (Schmidt et al., 2011), multifactor experiments are needed to fully understand the impacts of climatic change and human activity on terrestrial ecosystems structure and function.

Taken together, our results indicate that soil organic matter inputs, increased precipitation, clipping, and their interactions significantly affected soil C and N mineralization in the temperate steppe. These results suggest the importance of plants in mediating

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the responses of soil C and N mineralization to potentially enhanced SOM and precipitation by controlling belowground photosynthates allocation in the temperate steppe. The findings have important long-term implications for constructing mechanistic models of C and N cycling under the influence of climatic change and human activity, and for improving prediction of C and N sequestration potential and its feedbacks to climate change in temperate steppe ecosystems.

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Table 1. Results (significance values) of repeated measures ANOVAs on the effects of soil organic matter inputs (S), increased precipitation (P) and clipping (C), year (Y), and their interactions on soil temperature (ST) and moisture (SM), soil dissolved organic C (DOC) and inorganic N (IN), soil total PLFAs (TP), soil fungal PLFAs (F), gram-negative and gram-positive bacterial PLFAs (G^-, G^+), the ratio of fungal to bacterial PLFAs ($F:B$), arbuscular mycorrhizal fungi PLFAs (AMF), soil C mineralization rate (CMR), net N mineralization rate (NMR) and net nitrification rate (NNR). *, **, and *** represent significant at $P < 0.05$, 0.01, and 0.001, respectively; ns represents not significant at level of $P = 0.05$.

	ST	SM	DOC	IN	TP	F	G^-	G^+	$F:B$	AMF	CMR	NMR	NNR
S	ns	**	***	**	***	***	***	ns	***	***	***	**	ns
P	ns	***	***	***	ns	***	*	***	*	**	***	***	***
C	***	ns	***	**	ns	***	ns	ns	***	***	***	**	ns
$S \times P$	ns	**	ns	ns	ns	*	ns	ns	*	**	**	**	**
$S \times C$	ns	ns	ns	*	ns	**	***	ns	**	***	***	*	ns
$P \times C$	ns	ns	ns	*	ns	**	*	**	***	ns	***	*	ns
$S \times P \times C$	ns	ns	ns	ns	ns	ns	ns	*	*	ns	**	ns	ns
Y	***	***	***	***	ns	***	**	*	*	ns	ns	ns	ns
$Y \times S$	ns	ns	ns	*	ns	ns	*	ns	**	*	ns	ns	ns
$Y \times P$	ns	ns	ns	**	ns	ns	*	**	*	ns	ns	ns	ns
$Y \times C$	ns	ns	ns	*	ns	ns	ns	ns	ns	**	*	ns	ns
$Y \times S \times P$	ns	ns	ns	ns	ns	ns	ns	*	*	ns	ns	ns	ns
$Y \times S \times C$	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns
$Y \times P \times C$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns
$Y \times S \times P \times C$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

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Table 2. Results of stepwise multiple regression analyses. Dependent variables: seasonal mean soil C mineralization rate (CMR), soil net N mineralization rate (NMR) and soil net nitrification rate (NNR); Independent variable: seasonal mean total PLFAs, the ratio of soil fungal to bacterial PLFAs ($F:B$), arbuscular mycorrhizal fungi (AMF), soil temperature (ST) and moisture (SM), soil inorganic N (IN). Negative values of parameter estimate refer negative relationships between the examined dependent variable and the independent variables.

	Variable entered	Parameter estimate	Partial r^2	Probability
2010				
CMR	Total PLFAs	0.865	0.345	0.000
	SM	1.944	0.194	0.000
	$F:B$	−78.098	0.073	0.002
	AMF	−0.601	0.060	0.003
NMR	SM	0.042	0.272	0.000
	Total PLFAs	0.002	0.156	0.000
	$F:B$	−1.828	0.124	0.000
	AMF	−0.083	0.102	0.001
NNR	SM	0.039	0.164	0.004
2011				
CMR	Total PLFAs	1.013	0.370	0.000
	$F:B$	−52.839	0.245	0.000
	SM	3.938	0.134	0.000
	AMF	−0.103	0.068	0.007
	IN	0.085	0.053	0.046
NMR	SM	0.126	0.258	0.000
	Total PLFAs	0.002	0.243	0.000
	$F:B$	−0.817	0.109	0.001
NNR	SM	0.074	0.164	0.004
	Total PLFAs	0.007	0.095	0.020
	ST	0.038	0.069	0.040

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Fig. 1. The picture of increased precipitation treatments in a temperate steppe of northeastern China.

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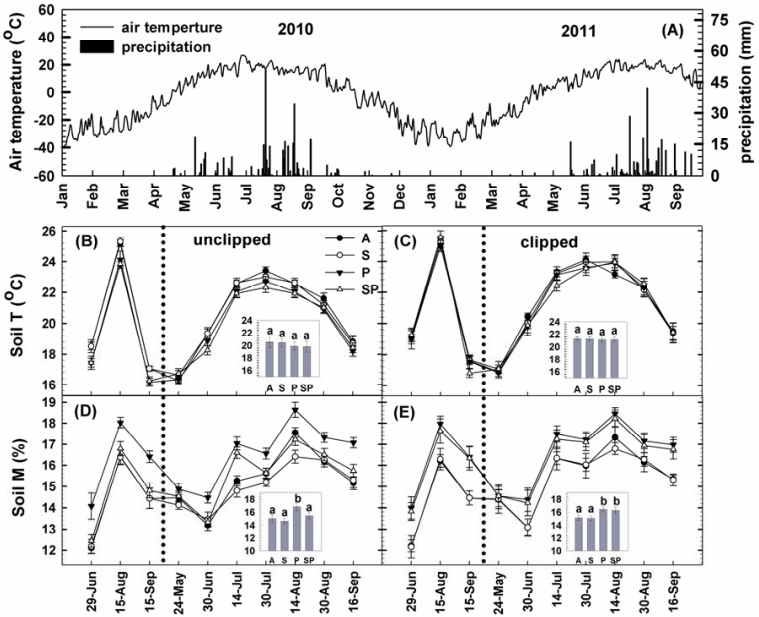

Fig. 2. Daily mean air temperature and daily precipitation in 2010 and 2011 (data are from the eddy tower adjacent to the experimental site). Seasonal variations of soil temperature (T) and soil moisture (M) at 10 cm depth in response to soil organic matter inputs (+60 %), increased precipitation (+30 %) and clipping during the two growing seasons in the temperate steppe of northeastern China. Insets represent the two seasonal mean values of soil temperature and moisture. Vertical bars indicate standard errors of means (n = 6). Difference lowercase letters indicate statistically significant differences ($P < 0.05$). A = ambient condition, S = soil organic matter inputs, P = increased precipitation, SP = combined soil organic matter inputs and increased precipitation.

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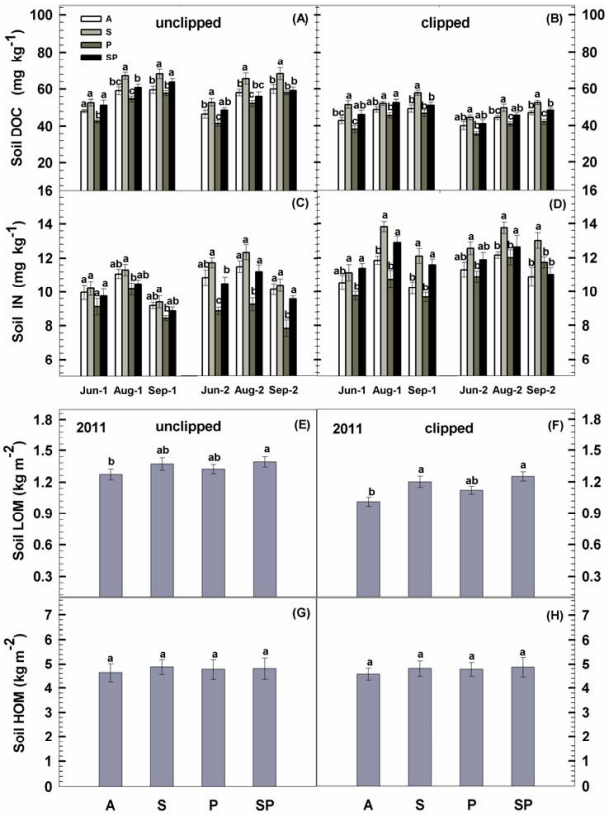

Fig. 3. Responses of soil dissolved organic C (DOC), inorganic N (IN), soil light organic matter (LOM) and heavy organic matter (HOM) to soil organic matter inputs (+60 %), increased precipitation (+30 %) and clipping in the temperate steppe of northeastern China. Vertical bars indicate standard errors of means (n = 6). Difference lowercase letters indicate statistically significant differences ($P < 0.05$). See Fig. 2 for abbreviations.

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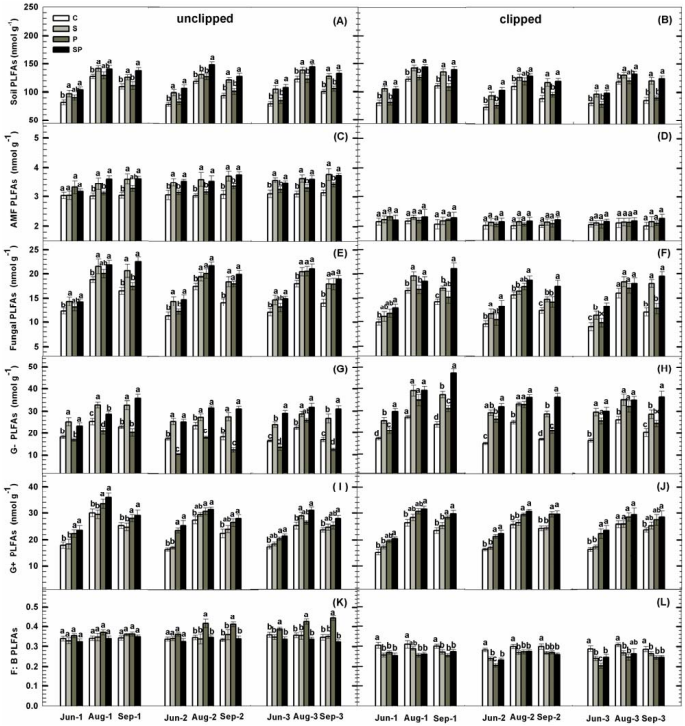

Fig. 4. The soil total phospholipid fatty acids (PLFAs), fungal PLFAs, gram-negative bacterial (G^-) PLFAs, gram-positive bacterial (G^+) PLFAs, the ratio of fungal to bacterial PLFAs ($F:B$) and arbuscular mycorrhizal fungi PLFAs (AMF) as influenced by soil organic matter inputs (+60 %), increased precipitation (+30 %) and clipping in the temperate steppe of northeastern China. Values show the monthly means in 2010 (1 June, 1 August, 1 September) and 2011 (2 June, 2 August, 2 September). Vertical bars indicate standard errors of means ($n = 6$). Difference lowercase letters indicate statistically significant differences ($P < 0.05$). See Fig. 2 for abbreviations.

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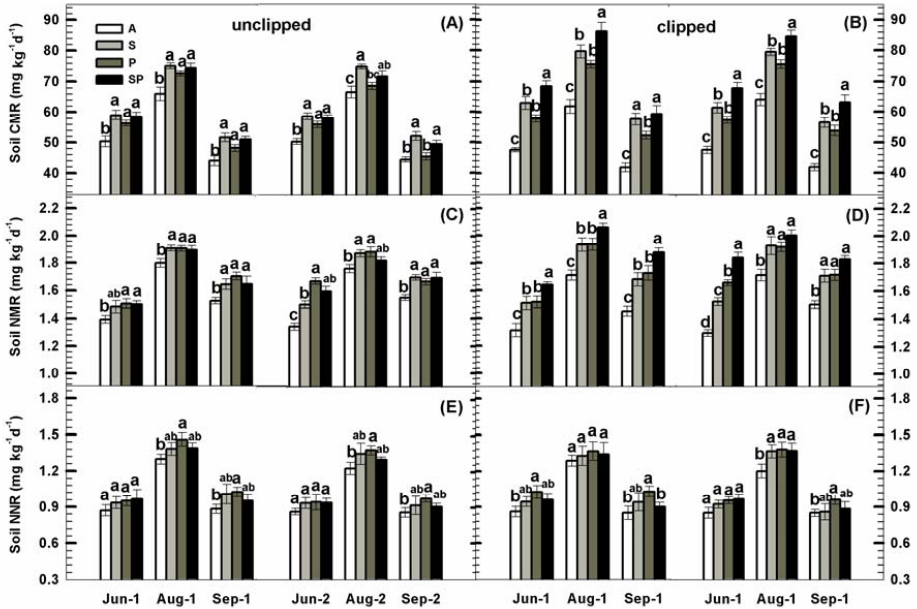

Fig. 5. Seasonal dynamics of soil C mineralization rate (CMR), net N mineralization rate (NMR) and net nitrification rate (NNR) under soil organic matter inputs (+60 %), increased precipitation (+30 %) and clipping in the temperate steppe of northeastern China. Values show the monthly means in 2010 (1 June, 1 August, 1 September) and 2011 (2 June, 2 August, 2 September). Vertical bars indicate standard errors of means ($n = 6$). Difference lowercase letters indicate statistically significant differences ($P < 0.05$). See Fig. 2 for abbreviations.

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