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ORIGINAL ARTICLE

Effect of nitrogen addition on soil organic carbon in freshwater marsh of Northeast China

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Abstract Increased nitrogen (N) input to ecosystems could alter soil organic carbon (C) dynamics, but the effect still remains uncertain. To better understand the effect of N addition on soil organic C in wetland ecosystems, a field experiment was conducted in a seasonally inundated freshwater marsh, the Sanjiang Plain, Northeast China. In this study, litter production, soil total organic C (TOC) concentration, microbial biomass C (MBC), organic C mineralization, metabolic quotient (qCO$_2$) and mineralization quotient (qmC) in 0–15 cm depth were investigated after four consecutive years of N addition at four rates (CK, 0 g N m^{-2} year^{-1}; low, 6 g N m^{-2} year^{-1}; moderate, 12 g N m^{-2} year^{-1}; high, 24 g N m^{-2} year^{-1}). Four-year N addition increased litter production, and decreased soil organic C mineralization. In addition, soil TOC concentration and MBC generally increased at low and moderate N addition levels, but declined at high N addition level, whereas soil qCO$_2$ and qmC showed a reverse trend. These results suggest that short-term N addition alters soil organic C dynamics in seasonally inundated freshwater marshes of Northeast China, and the effects vary with N fertilization rates.

Keywords Nitrogen enrichment · The Sanjiang Plain · Soil microbial biomass · Soil organic C mineralization · Wetlands

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Introduction

Nitrogen (N) is recognized as a key nutrient limiting net primary production in most terrestrial and aquatic ecosystems (Vitousek and Howarth 1991). In past decades, anthropogenic activities have altered global N cycle, and substantially increased the rate of N input into ecosystems at regional and global scales (Vitousek et al. 1997; Galloway et al. 2004), especially in Asia (Galloway et al. 2004). Increased N availability has the potential to enhance plant productivity and biomass accumulation (Vitousek and Howarth 1991), and hence affect ecosystem carbon (C) dynamic and global C cycle (Vitousek et al. 1997).

Increased N input could enhance net primary productivity, leading to increased vegetation C pool in most ecosystems (LeBauer and Treseder 2008; Xia and Wan 2008). However, the effect of N enrichment on soil organic C dynamics is inconsistent and remains remarkable uncertain (Neff et al. 2002; Lu et al. 2011). N fertilization caused an increase in soil organic C in some ecosystems (Turunen et al. 2004; Pregitzer et al. 2008) but a substantial decline in other ecosystems (Mack et al. 2004; Khan et al. 2007; Allison et al. 2010). In addition, some studies observed that increased N input to ecosystems had a negligible effect on soil organic C dynamics (Nadelhoffer et al. 1999; Zeng et al. 2010). Therefore, the response of soil organic C to increased N loading may vary with plant species composition and ecosystem types. Moreover, there is limited information on the effect of N addition on soil organic C in wetlands, although similar studies have been widely conducted in forests, grasslands and agricultural lands (Lu et al. 2011). Wetland ecosystems, especially those in mid-high northern latitudes, store a large amount of C in soils and thus play an important role in global C cycle (Post et al. 1982; Gorham 1991).

Considering that wetland soils hold much higher C density than other ecosystem types (Post et al. 1982), a better knowledge of the response of wetland soil organic C to N addition is needed to predict how changes in N availability influence soil organic C dynamics in terrestrial ecosystems.

The Sanjiang Plain, formerly the largest freshwater marsh region in China, has been extensively cultivated for agricultural production in the past 50 years (Huang et al. 2010). Due to fertilizer application during agricultural activities, the still uncultivated marshes in this region often receive increasingly exogenous N input (Zhang et al. 2007a, b). In the previous studies, N addition has promoted plant net primary productivity in freshwater marshes in the Sanjiang Plain, Northeast China (Zhang et al. 2007a; Liu and Song 2008). However, the effect of N enrichment on soil organic C is still unclear, given that soil organic C mainly depends on the balance between plant production and organic matter decomposition. Will N enrichment influence soil organic C in freshwater marsh ecosystems? To answer the question, this study investigated soil total organic C (TOC) concentration in a seasonally inundated freshwater marsh after four consecutive years of N addition. For a better knowledge of soil organic C dynamic under N enrichment, the present study also examined the effect of N addition on soil microbial biomass C (MBC), organic C mineralization, metabolic quotient (qCO_2) and mineralization quotient (qmC) in this freshwater marsh ecosystem.

Materials and methods

Study site

The study was conducted in a typical seasonally inundated freshwater marsh near the Sanjiang Experimental Station of Wetland Ecology (47°35′N, 133°31′E), which is located in the Sanjiang Plain, Heilongjiang Province, Northeast China. The station is administered by the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, and is one of the members of the Chinese Ecological Research Network. Freshwater marsh is the main wetland type in the study site, and *Carex lasiocapa* and *Deyeuxia angustifolia* are the dominant species in permanently inundated and seasonally inundated wetlands, respectively. The climate of the study site belongs to the temperate continental monsoon of a seasonal frozen zone, with a mean annual temperature of 2.5 °C, precipitation of 556 mm (about 50 % fall in July and August) and frost-free period of 125 days. The soils of study sites were marsh soil, meadow soil and peat soil, with high soil organic matter content and pH of 5–6.

Experimental design

During autumn 2003, 12 1 m × 1 m plots were established in a *D. angustifolia*-dominated seasonally inundated freshwater marsh. *D. angustifolia*-dominated freshwater marsh is one of the main wetland types in the Sanjiang Plain, and occupies approximately 31 % of the wetland area in this region (Zhao 1999). In the study plots, the ground was very flat, and the vegetation was relative uniform. The dominant species in the plots were *D.angustifolia* and *Glyceriaspiculosa*, which were perennial, cold-temperate mesophytes and accounted for about 90 % of the total aboveground biomass. The soil of the plots was developed from alluvial deposit, and was meadow soil according to the Chinese Classification, or Inceptisols according to the American Classification. Given that all the plots shared similar soil type, microtopography and vegetation, all plots were assumed to have similar soil properties prior to the establishment of this experiment. Before the experiment began, the initial soil TOC concentration, total nitrogen, pH, bulk density and C:N ratio at 0–15 cm layer in the study site were 71.3 mg g^{-1}, 6.4 mg g^{-1}, 5.73, 0.85 g cm^{-3} and 11.1, respectively.

In autumn 2003, each plot was separated by a 1-m wide buffer strip. Polyvinyl chloride frames (0.5 m depth) were installed to prevent horizontal movement and lateral diffusion of the added N. Board walks giving access to the whole experimental area were installed in 2004 to minimize disturbance on the plots. Four N fertilization levels (CK, 0 g N m^{-2} $year^{-1}$; low, 6 g N m^{-2} $year^{-1}$; moderate, 12 g N m^{-2} $year^{-1}$; high, 24 g N m^{-2} $year^{-1}$) were randomly assigned to the 12 plots, and each treatment had three replicate plots. From 2005 to 2008, N was added as ammonium nitrate (NH_4NO_3) during the growing season. In each year, N fertilizer was divided into nine equal doses and applied biweekly from May to September. Fertilizer was weighed, mixed with 1 L surface marsh water, and applied to each plots. At the same time, the CK plots received 1 L surface marsh water without N fertilizer. Low N addition level was approximately equal to the annual N input to wetlands through leaching and runoff during agricultural activities (5.8 g N m^{-2}) in this region, and moderate and high N addition levels were used to study the response of this ecosystem to N enrichment that may occur in the future (Zhang et al. 2007a).

Sample collection and analyses

At the end of the growing seasons (mid-September) of 2005, 2007 and 2008, litter was collected by using a 0.25 m^2 quadrat in each plot, oven-dried to a constant mass at approximately 65 °C for 48 h, and then weighed. After that, plant litter was returned to the corresponding plots.

Soil samples from 0 to 15 cm layer were collected in late July 2008. In each plot, five soil cores (2.5 cm in diameter) were randomly collected following removal of surface litter, and thoroughly mixed to homogenize a sample. After removing the plant roots, fauna and debris by hand, the soil was sieved through a 2-mm mesh screen and divided into two subsamples. One subsample was stored at 4 °C until analysis for soil MBC and organic C mineralization, and the second subsample was air-dried at room temperature around 20 °C, ground and then passed through a 0.25-mm sieve for measurement of soil TOC concentration.

Soil TOC concentration was determined using the $K_2Cr_2O_7$–H_2SO_4 wet oxidation method from Walkley and Black (Nelson and Sommers 1996).

Soil MBC was measured by the chloroform fumigation-extraction method of Vance et al. (1987). Briefly, three portions of fresh soil (equivalent to 20 g oven-dried soil) were weighed into 100 mL beakers and fumigated under dark at 25 °C for 24 h with ethanol-free chloroform. After fumigant removal, the soil was extracted with 50 mL 0.5 mol L^{-1} K_2SO_4 for 30 min. Three portions of unfumigated soil were extracted simultaneously when fumigation commenced. Organic C in the extracts was measured by the dichromate oxidation method. Soil MBC was calculated by dividing the difference in organic C concentration between the fumigated and unfumigated soils by a correction factor of 0.38 (Vance et al. 1987).

Soil organic C mineralization was measured by the method described in Goberna et al. (2006). Field-moist soil equivalent to 15 g oven-dried soil was placed in 600 mL glass jars and aerobic incubated for 27 days at 25 °C and 60 % water-holding capacity. Additionally, three jars without soil were considered as blanks. After 1, 3, 8, 14, 20 and 27 days of incubation, the evolved CO_2 was measured by extracting 10 mL gas from the jars by using a syringe and injecting it into a gas chromatograph (Agilent 4890D, Agilent CO., Santa Clara, CA, USA). And then, the jars were left open for 20 min to allow the air in the flasks to be replenished. In order to maintain at 60 % water-holding capacity throughout the incubation, soil moisture content was checked by weighing the flask every week and adjusted by adding distilled water when necessary. Soil organic C mineralization was calculated as the difference between the CO_2 produced from the soil and that from the blank, and expressed as μg CO_2–C g^{-1} soil. Soil qCO_2, respiration rate per unit of MBC, was calculated by dividing the mean values of soil organic C mineralization during the entire incubation period by the corresponding MBC. Soil qmC was calculated as the portion of TOC that was mineralized during the entire incubation period (CO_2–$C_{(27D)}$/TOC) (Pinzari et al. 1999).

Statistical analyses

Data were statistically analyzed using procedures of SPSS (v. 13.0), and the accepted significance level was $\alpha = 0.05$. Data were tested for normality using the Kolmogorov–Smirnov test, and all data were conformed to a normal distribution (data not shown). Fisher's least significant difference analysis was used to determine significant difference in litter production, soil TOC, MBC, organic C mineralization, qCO_2, qmC and MBC/TOC among all treatments. In addition, the effects of N addition on soil TOC, MBC, MBC/TOC were assessed by calculating the N-induced changes (%) = $(k_N - k_C)/k_C \times 100$, where k_N is the soil properties in the N addition plots, k_C is the soil properties in the CK plots.

Results

In 2005 and 2007, only high N addition treatments had greater litter production than the CK treatments (Fig. 1). However, low, moderate and high N addition treatments had higher litter production than the CK treatments in 2008 (Fig. 1). On average, N addition generally increased litter production (Fig. 1). Moreover, low and moderate N addition treatments had lower litter production than the high N addition treatment (Fig. 1).

Four-year N addition significantly altered soil TOC concentration, MBC and MBC/TOC, and the effects varied with fertilization rates (Fig. 2). Soil TOC concentration increased by 14 and 10 % at low and moderate N addition levels, respectively, and declined by 24 % at high N

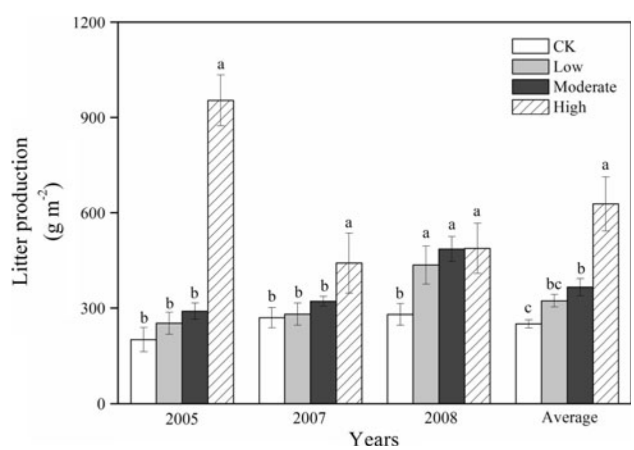


Fig. 1 Effect of N addition on litter production in freshwater marshes of Northeast China. Values are means and *errors bars* represent standard deviation ($n = 3$). Means with different *lowercase letters* are significantly different at $P < 0.05$. CK, 0 g N m^{-2} $year^{-1}$; Low, 6 g N m^{-2} $year^{-1}$; Moderate, 12 g N m^{-2} $year^{-1}$; High, 24 g N m^{-2} $year^{-1}$

addition level (Fig. 2). In addition, low N addition caused a 17 % increase in soil MBC, while high N addition caused a 39 % reduction (Fig. 2). However, soil MBC/TOC decreased only at high N addition level, and did not change at low and moderate N addition levels (Fig. 2).

N addition significantly decreased soil organic C mineralization during the entire incubation periods, albeit there were no significant differences in soil organic C mineralization among low, moderate and high N addition levels (Fig. 3). In addition, soil qCO_2 and qmC decreased initially with increased N addition level, and subsequently increased (Fig. 4). Soil qCO_2 and qmC in both low and moderate N addition treatments were significantly lower than those in the CK and high N addition treatments, respectively (Fig. 4).

Discussion

In the present study, both low and moderate N addition levels increased soil TOC concentration, whereas high N addition level decreased soil TOC concentration. Indeed, previous studies also found varying responses of soil TOC to N fertilization, including positive (Turunen et al. 2004; Pregitzer et al. 2008), negative (Mack et al. 2004; Khan et al. 2007; Allison et al. 2010), and negligible (Nadelhoffer et al. 1999; Zeng et al. 2010). Nevertheless, the results of the present study clearly demonstrate that the effect of N addition on soil organic C varies with fertilization rates, and suggest that N enrichment alters surface soil organic C in natural wetlands in the Sanjiang Plain, and the effect varies with N loading rates.

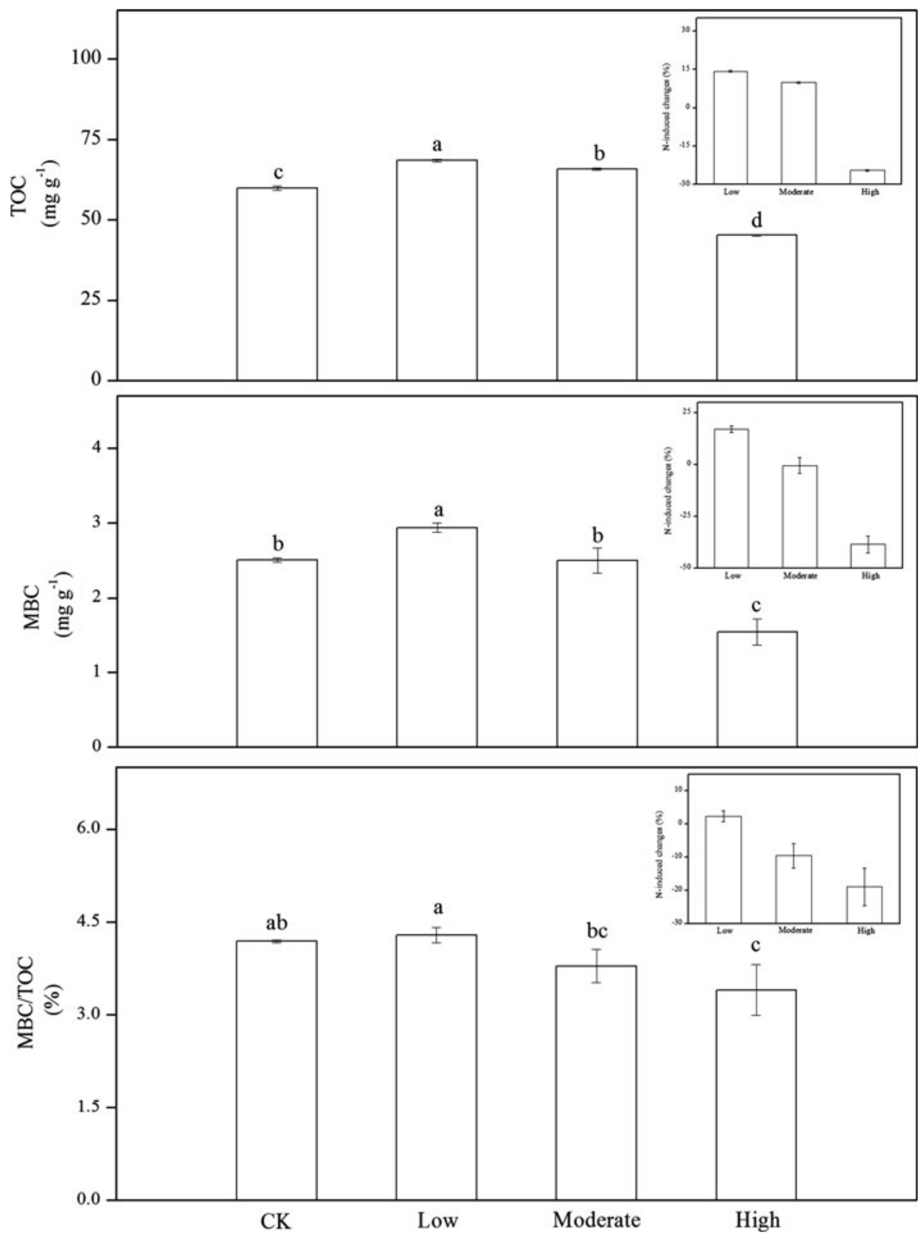


Fig. 2 Effects of N addition on soil total organic carbon (TOC), microbial biomass carbon (MBC) and MBC/TOC in freshwater marshes of Northeast China. Values are means and *errors bars* represent standard deviation ($n = 3$). Means with different lowercase letters are significantly different at $P < 0.05$. CK, 0 g N m^{-2} year^{-1}; Low, 6 g N m^{-2} year^{-1}; Moderate, 12 g N m^{-2} year^{-1}; High, 24 g N m^{-2} year^{-1}

Soil TOC depends on the balance between organic C input and output (Mack et al. 2004). In the present study, low and moderate N addition increased plant productivity, leading to increased organic matter inputs to soils, and hence increased soil TOC concentration (Zak et al. 1994). Moreover, N addition may enhance soil organic matter quality (low C/N ratio) (Song et al. 2011) and improve soil microbial utilization efficiency (low qCO_2 and qmC) (Fig. 4), and thus decreased soil organic C mineralization (Fig. 3). In addition, the decrease in soil TOC concentration under high N addition level may be related to the following mechanisms. First, high N addition rates may exceed the N-demand of plants, and lead to N-saturation in freshwater marshes (McNulty et al. 1996). Actually, Liu and Song (2008) found no significant difference in aboveground biomass among low, moderate and high N addition treatments. Second, according to the resource optimization hypothesis, increased N availability generally alters plant allocation between shoots and roots, and decreases root biomass (Ågren and Franklin 2003). High N addition level may decrease root biomass and reduce belowground C input to the soil in this freshwater marsh. Third, high N addition level may increase soil dissolved organic C leaching (Treseder 2008). Since soil dissolved organic C is very critical in controlling C accumulation in soils (Bolan et al. 2011), increased dissolved organic C outputs would cause a decline in soil TOC.

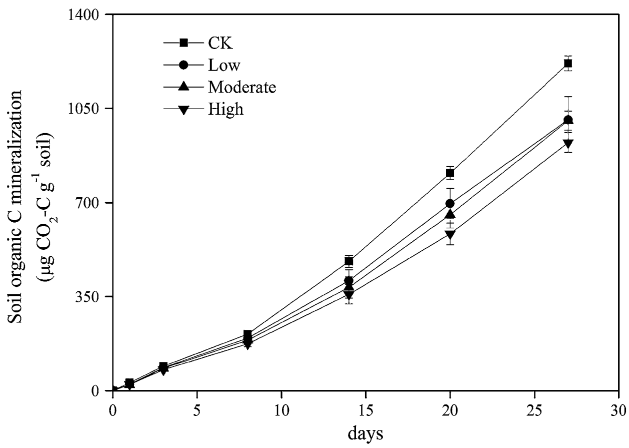
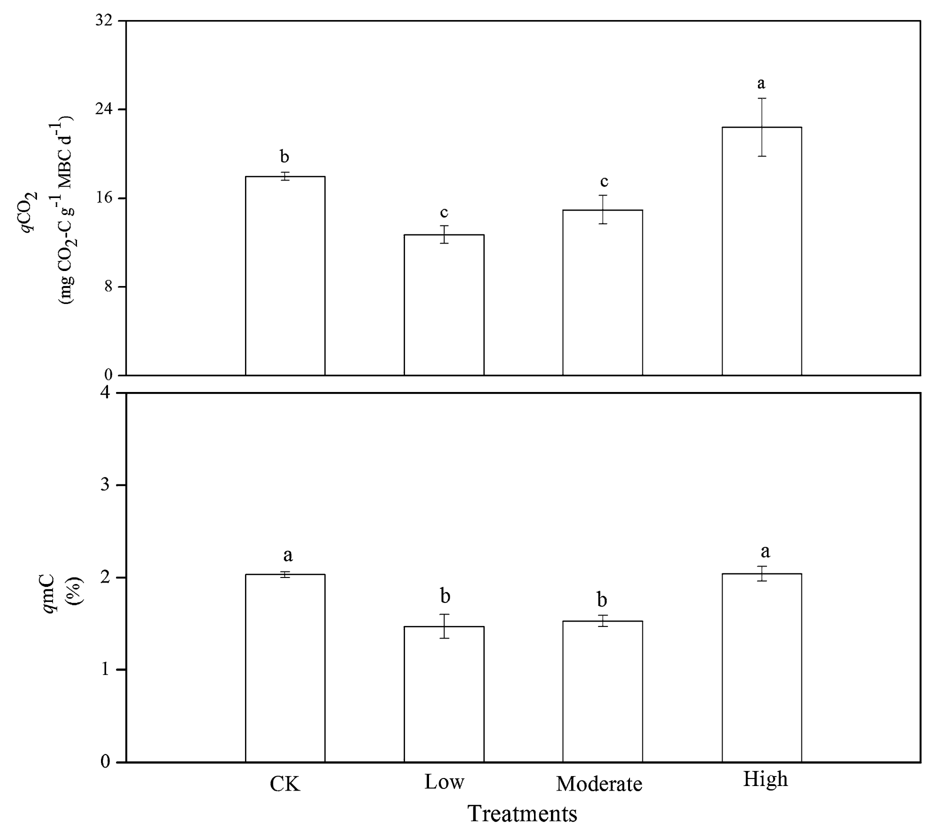


Fig. 3 Effect of N addition on soil organic C mineralization in freshwater marshes of Northeast China. Values are means and *errors bars* represent standard deviation ($n = 3$). CK, 0 g N m^{-2} $year^{-1}$; Low, 6 g N m^{-2} $year^{-1}$; Moderate, 12 g N m^{-2} $year^{-1}$; High, 24 g N m^{-2} $year^{-1}$

In this study, N addition caused a change in soil MBC in freshwater marsh ecosystems, and the effect varied with N addition levels. However, Treseder (2008) reviewed the effect of N enrichment on soil microbial biomass and found that soil microbial biomass declined 15 % on average under N addition. Compared to CK treatment, a greater soil MBC under low N addition level may be due to the

Fig. 4 Effects of N addition on soil metabolic quotient (qCO_2) and mineralization quotient (qmC) in freshwater marshes of Northeast China. Values are means and *errors bars* represent standard deviation ($n = 3$). Means with different *lowercase letters* are significantly different at $P < 0.05$. CK, 0 g N m^{-2} $year^{-1}$; Low, 6 g N m^{-2} $year^{-1}$; Moderate, 12 g N m^{-2} $year^{-1}$; High, 24 g N m^{-2} $year^{-1}$



increased litter production and thus soil organic C input (Zak et al. 1994). However, greater N loads may lead to the soil acidification by nitrification (Li et al. 2010), N toxicity (Treseder 2008) and reduced belowground C sources (Bowden et al. 2004; Mo et al. 2008). Therefore, soil MBC did not change at moderate N addition level, and even declined at high N addition level, although there were increases in plant litter production. Moreover, the decrease of soil MBC/TOC with increased N addition rates suggests that, increased N inputs declines soil C availability in wetland ecosystems.

N addition decreased soil organic C mineralization, and substantially altered soil qCO_2 and qmC in freshwater marshes. Treseder (2008) also observed that N addition could decrease soil CO_2 emission due to reduced microbial biomass. Interestingly, N addition decreased soil organic C mineralization at low and moderate N addition levels, although there was no reduction in soil MBC. At low and moderate N addition rates, decreased soil organic C mineralization may be caused by the increased soil microbial C-utilization efficiency (low qCO_2 and qmC) (Mao et al. 2010), resulting from the enhanced quality of soil organic matter input (low C/N ratio) (Song et al. 2011). At high N addition rate, reduced soil microbial biomass may be responsible for the decline in soil organic C mineralization (Treseder 2008). In addition, an increase in soil qCO_2 may be explained by the increased stress induced by high N addition and decreased C use efficiency for growth by soil microorganisms (Fisk and Fahey 2001).

In conclusion, N addition over 4 years have altered litter production, soil TOC concentration, MBC, organic C mineralization, qCO_2 and qmC in freshwater marshes of Northeast China. Both low and moderate N addition increased soil TOC concentration and MBC, and decreased soil qCO_2 and qmC, whereas high N addition had reverse effects on soil TOC, MBC and qCO_2. Moreover, N addition increased litter production, and decreased soil organic C mineralization, irrespective of N fertilization rates. These results suggest that short-term N addition alters soil organic C dynamics in wetland ecosystems of Northeast China, and the effects vary with N addition rates.

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