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Effect of exogenous phosphorus addition on soil respiration in *Calamagrostis angustifolia* freshwater marshes of Northeast China

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ABSTRACT

Anthropogenic activities have increased phosphorus (P) inputs to wetland ecosystems. However, little is known about the effect of P enrichment on soil respiration in these ecosystems. To understand the effect of P enrichment on soil respiration, we conducted a field experiment in *Calamagrostis angustifolia*-dominated freshwater marshes, the Sanjiang Plain, Northeast China. We investigated soil respiration in the first growing season after P addition at four rates (0, 1.2, 4.8 and 9.6 g P m^{-2} $year^{-1}$). In addition, we also examined aboveground biomass, soil labile C fractions (dissolved organic C, DOC; microbial biomass C, MBC; easily oxidizable C, EOC) and enzyme activities (invertase, urease and acid phosphatase activities) following one year of P addition. P addition decreased soil respiration during the growing season. Dissolved organic C in soil pore water increased after P addition at both 5 and 15 cm depths. Moreover, increased P input generally inhibited soil MBC and enzyme activities, and had no effects on aboveground biomass and soil EOC. Our results suggest that, in the short-term, soil respiration declines under P enrichment in *C. angustifolia*-dominated freshwater marshes of Northeast China, and its extent varies with P addition levels.

1. Introduction

Wetlands cover only between 4% and 6% of the global surface area, but contain about 37% of global terrestrial carbon (C) (Watson et al., 2000; Roehm, 2005). A slight change in this huge C pool may have a substantial influence on global C cycling. Carbon storage in wetland ecosystems depends on the balance between plant primary production and decomposition (Mack et al., 2004). In recent decades, anthropogenic activities have increased phosphorus (P) inputs to wetland ecosystems, primarily due to the mining of P compounds for fertilizer (Falkowski et al., 2000). Phosphorus enrichment in wetland ecosystems may alter plant primary production and hence ecosystem C dynamics, because P is one of the most common limiting elements in these ecosystems (Vitousek et al., 2010).

Soil respiration is one of the main fluxes of C between soils and the atmosphere (Schlesinger and Andrews, 2000), and varies with soil substrate supply and plant net primary productivity (Raich and Schlesinger, 1992; Luo and Zhou, 2006). Previous studies have suggested that, increased P input to wetland ecosystems changed species composition (Macek and Rejmánková, 2007), increased plant primary production (Rejmánková, 2001; Daoust and Childers, 2004; Lund et al., 2009), altered plant nutrient use efficiency (Rejmánková, 2001; Li et al., 2010) and stimulated litter decomposition (Daoust and Childers, 2004; Rejmánková and Sirová, 2007). Therefore, P enrichment in wetlands might cause changes in soil respiration and ecosystem C cycling. However, there is little information on the response of soil respiration to P addition in wetland ecosystems. Considering that wetland soils hold much higher carbon density than other ecosystem types (Post et al., 1982), a better knowledge of the effect of P addition on soil respiration in this ecosystem is needed.

The Sanjiang Plain, the largest freshwater marshland in China, has experienced an intensive and extensive cultivation over the past 50 years (Zhao, 1999). More and more marshes have been drained for conversion to agricultural production. Subsequently, the undrained marshes in this region often have received increasingly exogenous P input from the adjacent agricultural lands, mainly due to the fertilization application during agricultural activities. The annual amount of P fertilizer applied in adjacent agricultural lands is approximately 60 kg P_2O_5 ha^{-1} once every cropping year in dry

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lands and 70 kg P_2O_5 ha^{-1} in paddy fields, respectively. In order to study the effect of increased P input on soil respiration and ecosystem C cycling, we conducted a field experiment in 2007 in *Calamagrostis angustifolia*-dominated freshwater marshes, the Sanjiang Plain, Northeast China. *C. angustifolia*-dominated freshwater marsh is a major component of wetlands in the Sanjiang Plain, which accounts about 31% for the wetland area in this region (Zhao, 1999). The objective of this paper was to examine the effect of one year of P addition on soil respiration in *C. angustifolia* freshwater marshes of Northeast China. For a better understanding of the effect of P addition on soil respiration in this ecosystem, we also examined the aboveground biomass, soil labile C fractions (dissolved organic C, DOC; microbial biomass C, MBC; easily oxidizable C, EOC) and enzyme activities following one year of P addition.

2. Material and methods

2.1. Site description

The field manipulative experiment was conducted at the Sanjiang Mire Wetland Experimental Station, Chinese Academy of Sciences (47°35′N, 133°31′E), the Sanjiang Plain, Northeast China. The climate of the study site belongs to the temperate continental monsoon of a seasonal frozen zone. Its mean annual temperature is 2.5 °C, precipitation is approximately 558 mm (more than 65% fall in July and August), and frost-free period is 125 days. The soil in the study site is mainly a typical meadow marsh soil, and the initial soil characteristics of experimental plots are presented in Table 1. Detailed information regarding the study site was described by Zhang et al. (2007).

2.2. Experimental design

During autumn 2006, *C. angustifolia*-dominated freshwater marshes (plant coverage is about 90%) were selected in the field experiment, and 12 plots of 1 m × 1 m were established. Plastic (PVC) frames (1 m × 1 m, 0.5 m depth) were installed to prevent horizontal movement and lateral loss of the added P, and each plot was separated by a 1-m buffer zone. Meanwhile, boardwalks giving access to the whole experimental area were installed to minimize further disturbance on the plots. Four P fertilization levels (CK, 0 g P m^{-2} $year^{-1}$; P1, 1.2 g P m^{-2} $year^{-1}$; P2, 4.8 g P m^{-2} $year^{-1}$; P3, 9.6 g P m^{-2} $year^{-1}$) were randomly assigned to the 12 plots, and each level had three replicate plots. Phosphorus was added as NaH_2PO_4. In order to simulate the exogenous P input in this region, P fertilizer was divided into nine equal doses and applied every two weeks during whole growing season of 2007 (from June to September). Fertilizer was weighed, mixed with 1 L surface marsh water, and applied to each plot. At the same time, CK treatment received 1 L water without P fertilizer. The surface marsh water used for P addition had the following properties: a dissolved organic C concentration of 40.79 mg L^{-1}; dissolved inorganic C concentration of 1.79 mg L^{-1}; total N concentration of 2.33 mg L^{-1}; total P concentration of 0.30 mg L^{-1}.

Table 1
Initial soil characteristics of experimental plots.

Dominated species	Depth cm	pH[a]	SOC mg g^{-1}	TN mg g^{-1}	TP mg g^{-1}
Calamagrostis angustifolia	0–10	5.33	167	6.8	1.5
	10–20	5.38	89	4.1	1.4

[a] Soil:water = 1:5.

2.3. Sample collection and analyses

2.3.1. Soil respiration

Soil respiration was measured using static dark chamber and gas chromatography techniques (Wang and Wang, 2003). The static chamber (length × width × height = 0.3 m × 0.3 m × 0.6 m) and its collar were made of stainless-steel. Before observation, the collar of chamber, one square box (without top and bottom, length × width × height = 0.3 m × 0.3 m × 0.2 m), was inserted directly into soil with 5 cm exposed above the soil surface. All green and standing-dead plants inside the box were clipped to ground level and litters were collected before gas sampling. A fan was fixed in the static chamber to keep the air mixed during gas sampling. The detailed information about the gas sampling by using static dark chamber see Song et al. (2006) and Song et al. (2009). Soil respiration was measured once a week following the first P fertilization application. Gas sampling usually occurred at 9:00 A.M. at local time because the preliminary experiments in this site suggested that the flux at 9:00 A.M. was almost equal to the daily mean flux (data not shown). Gas samples were collected with 60 mL plastic syringe at 0, 10, 20 and 30 min after the chamber closure. The gas samples were analyzed using a gas chromatogram (Agilent 4890D, Agilent Co., Santa Clara, CA, USA) within 12 h (Song et al., 2009).

Soil respiration rate is calculated by the following equation (Song et al., 2006, 2009):

$$F = \frac{M}{V_0}\frac{P}{P_0}\frac{T_0}{T}H\frac{dc}{dt}$$

Where M is the molecular mass of CO_2; P is the atmospheric pressure; V_0, P_0 and T_0 are the gas mole volume, air absolute temperature and atmospheric pressure under standard condition; H is the height of chamber during sampling; dc/dt is the slope of the linear regression for gas concentration gradient through time. Soil respiration rate was expressed as mg CO_2–C m^{-2} h^{-1}.

2.3.2. Aboveground biomass

In each plot, aboveground biomass was harvested by using a 0.25 m^2 quadrat in the end of growing season (mid-September) of 2007. Plant biomass was over-dried at 80 °C until constant weight, and weighed. Aboveground biomass was calculated using the following equation: aboveground biomass = B/0.25, where B is the biomass obtained from the 0.25 m^2 quadrat. After that, aboveground biomass was divided into two subsamples. One subsample was returned to the corresponding plot, and another subsample was milled for total N and P concentrations determination. Total N concentration was measured by indophenol-blue spectrophotometry after the plant materials were wet digested with concentrated H_2SO_4 and H_2O_2, and total P concentration was by phosphomolybdenum-yellow spectrophotometry (Lu, 2000).

2.3.3. Dissolved organic C in soil pore water

In the mid-September of 2007 (after the whole fertilizer application), soil pore water was taken from the water tubes in situ at two soil depths (5 and 15 cm below ground) (Zhang et al., 2007). Stainless steel tubes (3.0 mm outer diameter, 2.0 mm inner diameter, and with four lateral ports at the bottom), equipped with silicon tube, a three-way valve and a silicon septum were installed prior to flux measurement both at 5 cm and 15 cm depths. Pore water was collected using syringes, injected into plastic bottles, and stored in a cooling box (about 4 °C) in the field. Soil pore water samples were immediately transported to the experimental station, filtered through a 0.45 μm Whatman filter, and frozen in refrigerator until analysis for DOC (Ghani et al., 2003). Soil DOC in pore water was determined using a total

organic C analyzer (TOC V_{CPH}, Shimadzu Corp., Tokyo, Japan), and expressed as mg L^{-1}.

2.3.4. Soil sampling and analyses

After sampling soil pore water, 5 soil cores (2.5 cm in diameter) were randomly collected at 0–20 cm layer in each plot, and mixed thoroughly to homogenize a sample. Following removal of plant roots, fauna and debris, the soil was sieved (<2 mm) and divided into two subsamples. One subsample was stored at 4 °C until analysis for determination of soil MBC. The second subsample was air-dried at room temperature around 20 °C for analyses of soil EOC and enzyme activities. In the present study, we did not measure soil total organic C and N concentrations, as we did not expect the changes in these chemical properties to be detectable in such a short period. Actually, Wang et al. (2008) found that, in subtropical plantation forest ecosystems, soil total organic C concentration showed no significant change after one year of P fertilizer application. Soil MBC was determined by the chloroform fumigation–extraction method (Vance et al., 1987). Fresh soil (20-g oven-dried soil) was fumigated for 24 h in the dark at 20 °C with ethanol-free chloroform, extracted with 80 mL 0.5 mol L^{-1} K_2SO_4 for 30 min, and then filtered. Unfumigated samples were kept in the dark at 20 °C during the fumigation and then extracted as described above. Organic C concentration in fumigated and unfumigated extracts was determined by the potassium dichromate oxidation–redox titration method (Vance et al., 1987). Soil MBC was calculated by dividing the difference in extractable organic C between fumigated and unfumigated soils with a conversion factor of 0.38 (Vance et al., 1987), and expressed as μg g^{-1}.

Soil EOC was determined by the 333 mmol L^{-1} $KMnO_4$ oxidation method as described by Blair et al. (1995), and expressed as μg g^{-1}. Soil invertase, urease and acid phosphatase activities were assayed by the method of Guan (1986). Invertase activity was determined by using sucrose as the substrate, and expressed as μg glucose g^{-1} h^{-1}. Urease activity was determined by using urea as the substrate, and expressed as μg NH_3–N g^{-1} h^{-1}. Acid phosphatase activity was determined by using p-nitrophenyl phosphate (pNP) as the substrate, and expressed as μg pNP g^{-1} h^{-1}.

2.4. Statistical analyses

Statistical analysis was conducted with SPSS 11.5 package, and the accepted significance level was $\alpha = 0.05$. Fisher's least significant difference (LSD) method was used to determine significant differences in soil respiration, aboveground biomass, labile C fractions and enzyme activities among all treatments.

3. Results

Soil respiration showed a significant decline in the first year of P fertilizer application (Fig. 1). Soil respiration of P1, P2 and P3 treatments were significant lower than that of CK treatment (Fig. 1). In addition, soil respiration in P2 treatment was significant greater than that in P3 treatment (Fig. 1).

Phosphorus addition did not significantly change aboveground biomass (Table 2). However, total N concentration of aboveground biomass showed an initial increase with P addition level, and then declined (Table 2). In addition, total P concentration of aboveground biomass significantly increased with P addition level, whereas N/P ratio showed a reverse trend (Table 2).

Dissolved organic C in soil pore water increased significantly with P addition level at both 5 and 15 cm depths (Fig. 2). In 5 cm depth, DOC in P1, P2 and P3 treatments were significant greater than that in CK treatment (Fig. 2). In addition, DOC of P3 treatment at 15 cm depth was significant higher than that of CK treatment

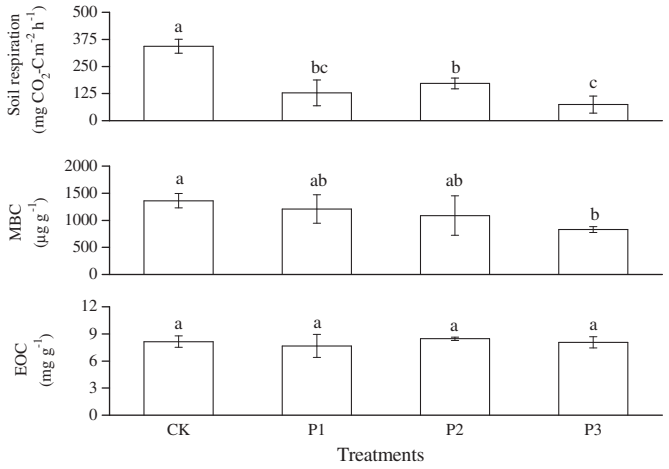

Fig. 1. Effect of P addition on soil respiration, microbial biomass C (MBC) and easily oxidizable C (EOC) in *Calamagrostis angustifolia*-dominated freshwater marshes of Northeast China. Values are means and errors bars represent standard deviation ($n = 3$). Means with different lowercase letters are significantly different at $P < 0.05$. CK, 0 g P m^{-2} $year^{-1}$; P1, 1.2 g P m^{-2} $year^{-1}$; P2, 4.8 g P m^{-2} $year^{-1}$; P3, 9.6 g P m^{-2} $year^{-1}$.

(Fig. 2). Soil MBC declined significantly after P addition (Fig. 1). Soil MBC of P3 treatment was significant lower than that of CK treatment (Fig. 1). However, P addition had no significant effect on soil EOC (Fig. 1).

P addition generally inhibited soil enzyme activities (Table 3). Soil invertase activity in both P1 and P3 treatments were significant lower than that in CK treatment (Table 3). In addition, soil urease and acid phosphatase activities of CK treatment were significant higher than those of P3 treatment (Table 3).

4. Discussions

In the present study, we only measured soil respiration in the first growing season after P fertilizer application, and the aim was to evaluate the short-term effect of P addition on soil respiration in *C. angustifolia* freshwater marshes of Northeast China. Therefore, we did not discuss the seasonal pattern of soil respiration and its correlation with soil temperature or moisture.

The mean respiration rate of CK plot was 343.1 mg CO_2–C m^{-2} h^{-1} during the whole growing season. This is higher than the data reported in the wetland during the June–August period at Harvard Forest (174.1 mg CO_2–C m^{-2} h^{-1}, Phillips et al., 2010), and is in the adjacent temperate forest ecosystem of Northeast China during the complete growing season (120.4 mg CO_2–C m^{-2} h^{-1}, Wang et al., 2006). This may be due to the differences in soil conditions, vegetation type and climate (Raich and Schlesinger, 1992; Raich and Tufekcioglu, 2000; Luo and Zhou, 2006). Our

Table 2
Effect of P addition on aboveground biomass in *Calamagrostis angustifolia*-dominated freshwater marshes.

Treatments	Aboveground biomass g m^{-2}	TN mg g^{-1}	TP mg g^{-1}	N/P ratio
CK	179.8(55.0)a	5.01(0.59)b	0.91(0.04)c	5.49(0.41)a
P1	214.5(80.6)a	5.26(0.23)ab	1.17(0.01)b	4.49(0.16)b
P2	269.0(50.2)a	5.83(0.10)a	1.67(0.11)a	3.50(0.17)c
P3	286.5(93.2)a	4.68(0.28)b	1.75(0.06)a	2.67(0.07)d

Data represent means and values in parentheses are standard deviation ($n = 3$). Means with different lowercase letters in the same column are significantly different at $P < 0.05$. CK, 0 g P m^{-2} $year^{-1}$; P1, 1.2 g P m^{-2} $year^{-1}$; P2, 4.8 g P m^{-2} $year^{-1}$; P3, 9.6 g P m^{-2} $year^{-1}$.

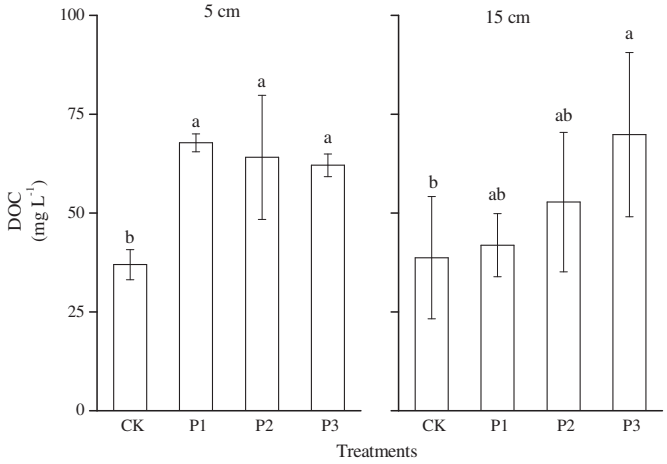

Fig. 2. Effect of P addition on dissolved organic C (DOC) concentration in soil pore water in *Calamagrostis angustifolia*-dominated freshwater marshes of Northeast China. Values are means and errors bars represent standard deviation ($n = 3$). Means with different lowercase letters are significantly different at $P < 0.05$. CK, 0 g P m^{-2} $year^{-1}$; P1, 1.2 g P m^{-2} $year^{-1}$; P2, 4.8 g P m^{-2} $year^{-1}$; P3, 9.6 g P m^{-2} $year^{-1}$.

results also showed that P addition reduced soil respiration (Fig. 1), which was consistent with those found in both a subarctic heath (Christensen et al., 1998) and a high arctic semi desert (Illeris et al., 2003). The decrease in soil respiration under P addition treatments may be related to the following mechanisms. First, heterotrophic respiration from the microbial community may be declined following P addition. Our results showed that P addition did not increase aboveground biomass (Table 2), and reduced soil microbial biomass (Fig. 1) and enzyme activities (Table 3) in *C. angustifolia* freshwater marshes. Thirukkumaran and Parkinson (2000) also observed that P addition inhibited soil microbial activity and suppressed soil basal respiration in a lodgepole pine (*Pinus contorta* Loud. Var. *latifolia* Engelm) forest stand located in southwestern Alberta, Canada. Second, P addition may reduce root biomass and hence decrease root respiration. Generally, P addition stimulated root growth and increased plant root biomass in wetlands (Lissner et al., 2003; Macek and Rejmánková, 2007). However, Daoust and Childers (2004) found that belowground biomass decreased after the first year of P fertilizer application in wet prairie community, and then remained unchanged after the second year. In our study, one year of P addition may also cause an initial decline in root biomass in *C. angustifolia* freshwater marshes. However, the direct loss of C as DOC represents a significant part of organic C outputs and plays an important role in ecosystem C budget in wetlands (Freeman et al., 2001; Rastetter et al., 2005). In the present study, we observed that, P addition resulted in an increase in DOC in soil pore water at both 5 and 15 cm depths (Fig. 2), and may increase the C output as DOC in freshwater marshes. Therefore, in the short-term, the net effect of P addition on soil C balance in our study remains unclear. Although increased P input could reduce soil respiration in *C. angustifolia* freshwater marshes of Northeast China, the loss of C as DOC leaching may have increased. Further studies on multiple aspects of soil C cycling are needed to examine the effect of P enrichment on soil C budget in long-term P addition experiments.

Table 3
Effect of P addition on soil enzyme activities in *Calamagrostis angustifolia*-dominated freshwater marshes.

Treatments	Invertase activity μg glucose g^{-1} h^{-1}	Urease activity μg NH_3–N g^{-1} h^{-1}	Acid phosphatase activity μg pNP g^{-1} h^{-1}
CK	162.5(17.9)a	12.3(0.4)ab	217.7(16.7)a
P1	119.6(27.9)b	13.9(1.5)a	195.3(20.7)a
P2	135.4(2.5)ab	10.5(1.6)bc	199.8(1.7)a
P3	123.3(13.3)b	9.3(1.0)c	165.5(3.3)b

Data represent means and values in parentheses are standard deviation ($n = 3$). Means with different lowercase letters in the same column are significantly different at $P < 0.05$. CK, 0 g P m^{-2} $year^{-1}$; P1, 1.2 g P m^{-2} $year^{-1}$; P2, 4.8 g P m^{-2} $year^{-1}$; P3, 9.6 g P m^{-2} $year^{-1}$.

In our study, soil respiration varied with P addition levels (Fig. 1). Soil microbial biomass and activity are generally limited by substrate C or/and N availability (Vance and Chapin, 2001). Compared to P1 and P2 treatments, P3 treatment decreased aboveground biomass N concentration (Table 2), although there was no significant difference in aboveground biomass among three P addition treatments. Soil microbes might be limited by substrate N availability under P3 treatment. Therefore, high P addition rate decreased soil microbial biomass and activity (Fig. 1 and Table 3), and resulted in a relative low soil respiration rate in *C. angustifolia* freshwater marshes (Fig. 1).

In our study, P fertilizer application did not stimulate plant growth and increase aboveground biomass (Table 2). Daoust and Childers (2004) also observed that net annual primary production did not change after the first year of P fertilizer application in wet prairie community, and then increased after the second year. This may be explained by that the vegetation in our plots adapted to the current soil P condition and responded slowly to the P addition (Macek and Rejmánková, 2007). In addition, P addition increased total P concentration of aboveground biomass and decreased N/P ratio (Table 2). Since vegetation N/P ratio would be used to indicate the nature of nutrient limitation (Koerselman and Meuleman, 1996), reduced N/P ratio after P addition implied that, in the future, increased P input may highlight the importance of N limitation to primary production in *C. angustifolia* freshwater marshes of Northeast China. However, in this region, the natural wetlands also received more exogenous N input due to the N fertilization application in the adjacent agricultural lands (Zhang et al., 2007). Therefore, the variations of plant primary production and its N/P ratio induced by nutrient enrichment during the agricultural activities still remain unclear in this ecosystem. Further study is needed to examine the combined effect of N and P additions on plant production in freshwater marshes in the Sanjiang Plain, Northeast China.

In *C. angustifolia* freshwater marshes, P addition caused a decline in soil MBC, invertase, urease and acid phosphatase activities, and had no effect on soil EOC. Previous similar studies in forest ecosystems also found that P addition inhibited soil microbial biomass (Thirukkumaran and Parkinson, 2000) and enzyme activities (Wang et al., 2008; Olander and Vitousek, 2000). The decrease in soil MBC and enzyme activities may be resulted from the reduced root biomass and thus organic matter input to soils (Wang et al., 2008).

5. Conclusions

In conclusion, exogenous P addition decreased soil respiration during the first growing season in *C. angustifolia* freshwater marshes of Northeast China. In addition, one year of P enrichment decreased soil MBC and enzyme activities, increased DOC concentration in soil pore water, and did not affect aboveground biomass and easily oxidizable C. Although soil respiration declined under P enrichment, the net result of P addition on soil C balance was still unclear. Increased P inputs to *C. angustifolia* freshwater marshes increased DOC in soil pore water and may enhance the loss of C as DOC leaching. To resolve the effect of P enrichment on soil C budget in freshwater marshes, further studies on multiple aspects of soil C cycling are needed in long-term P addition experiments.

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