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Effect of long-term phosphorus addition on the quantity and quality of dissolved organic carbon in a freshwater wetland of Northeast China

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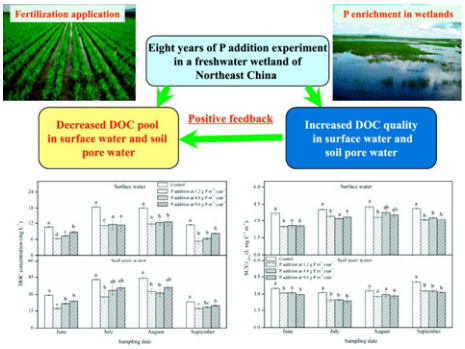
HIGHLIGHTS

- Effect of P enrichment on quantity and quality of DOC remains unclear.
- Eight years of P addition decreased DOC concentration in waters.
- Long-term P addition decreased $SUVA_{254}$ and C:C ratio, but increased E4:E6 ratio.
- Long-term P addition increased the quality of DOC in N-limited wetlands.
- Increased DOC quality after P enrichment would further cause DOC loss from waters.

GRAPHICAL ABSTRACT



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ABSTRACT

Understanding how P enrichment alters the quantity and quality of dissolved organic carbon (DOC) is important, because of their role in regulating the C cycle. Here, we established a four-level P addition experiment (0, 1.2, 4.8, and 9.6 g P m^{-2} $year^{-1}$) in a N-limited freshwater wetland in the Sanjiang Plain, Northeast China. The aim of this study was to examine the effects of eight years of P addition on DOC concentration, $SUVA_{254}$ (Abs^{254}/DOC concentration, indicating the aromaticity of DOC), C:C ratio (Abs^{400}/DOC concentration, indicating the proportion of colored humic substances in DOC), and E4:E6 ratio (Abs^{465}/Abs^{665}, indicating the molecular size of humic substances) in surface water and soil pore water (0–15 cm depth) during the growing season (June through September). Our results showed similar changing trends in concentration and optical properties of DOC following eight years of P addition in the both surface water and soil pore water across the sampling dates. Generally, P addition decreased DOC concentration, $SUVA_{254}$, and C:C ratio, and increased E4:E6 ratio, irrespective of P addition levels. These altered optical properties of DOC indicated that P addition decreased the molecular weight and aromaticity of DOC, and thus increased the quality of DOC. These results suggest P enrichment substantially reduces the quantity of DOC in N-limited temperate freshwater wetlands, and imply that increased DOC quality following P addition can further provide a positive feedback to decreased DOC pool.

1. Introduction

Dissolved organic carbon (DOC) is a complex mixture of soluble organic compounds that have a wide range of biogeochemical reactivity,

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and represents an substantial constituent of the organic carbon (C) pool in the terrestrial and aquatic ecosystems (McDowell, 2003; Kellerman et al., 2014; Williams et al., 2016). In these ecosystems, DOC acts as an important energy source for microbial growth, and thus plays a key role in regulating the C and nutrient cycles, and water quality (Zsolnay, 2003). Meanwhile, DOC serves as a key connection between terrestrial, inland water, and oceanic C cycle due to its transport from riverine upstream to downstream oceans (Battin et al., 2008; Williams et al., 2016). Therefore, the quantity and quality of DOC are tightly linked to biogeochemical cycles from regional to global scales.

Over the past century, anthropogenic activities such as fertilizer application, land use change, and sewage have drastically altered global phosphorus (P) cycle, leading to widespread P enrichment of terrestrial and aquatic ecosystems (Filippelli, 2008). Previous studies have found that increased P availability enhanced plant productivity (Rejmánková, 2001; Mao et al., 2015), increased soil microbial biomass and activity (Liu et al., 2014), and accelerated organic matter decomposition (Debusk and Reddy, 2005; Clivot et al., 2014). Since DOC generally originates from plant litter, soil humus, microbial biomass, and root exudation (Kalbitz et al., 2000; Moore, 2003), P enrichment has been observed to substantially influence the quantity of DOC (McLaughlin et al., 2000; Song et al., 2011; Pinsonneault et al., 2016a). However, these experimental results are highly variable probably due to the differences in the nutrient availability, hydrological regime, organic matter quality, and duration of the experimental treatments. As anthropogenic activities are intensified in response to escalating human population growth, P enrichment will understandably become more widespread (Filippelli, 2008; Williams et al., 2016). Clarifying the effect of P enrichment on DOC quantity from various ecosystem types is needed given the critical roles of DOC in regulating the biogeochemical cycles.

Dissolved organic C is a heterogeneous pool of different organic compounds with varying biodegradability, and thus DOC quality is primarily controlled by its chemical composition (Kalbitz et al., 2003). In general, different fractions of DOC have intrinsic spectroscopic properties (Kalbitz et al., 2003; Weishaar et al., 2003). Consequently, a wide range of absorbances is used to indicate the chemical characteristics of DOC (Weishaar et al., 2003; Wallage et al., 2006; Fong and Mohamed, 2007). For example, specific UV absorbance at 254 nm ($SUVA_{254}$) is a useful parameter for estimating the aromaticity of DOC (Weishaar et al., 2003); the ratio of the absorbance at 400 nm to DOC concentration (C:C ratio) can indicate the proportion of colored humic substances to uncolored non-humic substances in DOC (Wallage et al., 2006); and the ratio of the absorbance at 465 nm to that at 665 nm (E4:E6 ratio) is related to the molecular size of humic substances in the DOC, and has been suggested as an index of the proportion of fulvic acid to humic acid (Thurman, 1985; Fong and Mohamed, 2007). Phosphorus enrichment is believed to change DOC optical properties by altering the chemical composition of plant-derived organic matter (Mao et al., 2015; Pinsonneault et al., 2016b) and microbial activity (Liu et al., 2014; Pinsonneault et al., 2016a). Unfortunately, little information is available on the effect of P enrichment on optical characteristics of DOC in terrestrial and aquatic ecosystems.

In order to understand how P enrichment affects the quantity and quality of DOC, we established a long-term multi-level P addition experiment (initiated since 2007) in a nitrogen (N)-limited freshwater wetland of northeastern China. This P addition experiment consisted of four levels of P addition treatments, 0 P added (CK), 1.2 (P1), 4.8 (P2), and 9.6 g P m^{-2} $year^{-1}$ (P3). Previous studies have found that six years of P addition stimulated plant growth and increased plant N and P concentrations (Mao et al., 2015, 2016). Here, we investigated the changes in DOC concentration, $SUVA_{254}$, C:C ratio, and E4:E6 ratio in surface water and soil pore water (0–15 cm depth) following eight years of P addition. We tested the following hypotheses: (1) long-term P addition would increase DOC concentration in the surface water and soil pore water due to the enhanced plant biomass (Mao et al., 2015); and (2) long-term P addition would change the optical characteristics of DOC because of altered plant nutrient concentrations (Mao et al., 2015, 2016).

2. Materials and methods

2.1. Study site and experiment design

This study was performed in a herbaceous-dominated freshwater marsh at the Sanjiang Mire Wetland Experimental Station (47°35′N, 133°31′E; 56 m above sea level) located in the center of the Sanjiang Plain, Northeast China. The Sanjiang Plain includes the largest natural freshwater wetlands in China, and *D. angustifolia*-dominated wetland is the main wetland type in this region (Zhao, 1999). Mean annual temperature and precipitation of the study site are 2.5 °C and 566 mm, respectively. The soil is a marsh soil according to the Soil Classification of China, which corresponds to Fluvisols in the FAO Soil Classification. Soil organic C concentration, total N concentration, total P concentration, and pH (with soil:water ratio 1:5) in the 0–10 cm depth are 167.0 mg g^{-1}, 6.8 mg g^{-1}, 1.5 mg g^{-1}, and 5.33, respectively (Song et al., 2011). The plant community was dominated by *D. angustifolia* and *Glyceria spiculosa*, together accounting for >90% of the total aboveground biomass. Other species include *Humulus japonicas*, *Equisetum arvense*, *Lycopus lucidus*, *Hypericum kouytchense*, and *Stachys baicalensis*. This freshwater wetland is regularly flooded from May to October. In this wetland, plant growth and microbial activity are generally limited by N availability (Song et al., 2011; Mao et al., 2015). In the recent decades, wetland ecosystems have been experienced increasingly nutrient loadings via atmospheric deposition and surface runoff mainly due to the fertilizer application in the adjacent agricultural lands (Mao et al., 2015). Annual N and P inputs are estimated to about 1.5 g N m^{-2} and 0.4 g P m^{-2}, respectively.

In order to simulate future P enrichment in this N-limited wetland, we experimentally increased the annual P inputs by factors of 3, 12, and 24. Thus, the experiment consisted of four P addition 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} $year^{-1}$; and P3, 9.6 g P m^{-2} $year^{-1}$), and started in 2007. This fertilization experiment included twelve 1 m × 1 m plots, and each P addition level replicated three times in a random design. Each plot was separated by a 1-m-wide buffer strips, and was fenced with stainless steel frames (100 cm × 100 cm × 50 cm, 30 cm in soil depth) to prevent the lateral loss of P fertilizer. Board walks were installed for minimizing site disturbance during sampling. Each year, P was added 10 times from May to September as NaH_2PO_4 solution, and the CK plots received the same amount of distilled water.

2.2. Water sampling and analysis

From June to September in 2014, surface water was collected monthly in plastic bottles, and soil pore water at 0–15 cm depth was collected monthly by sippers (Höll et al., 2009). Meanwhile, surface water depth in the plots was measured using a stainless steel rule (50 cm length). In each plot, about 300 mL of water was collected in brown bottles to prevent photodegradation, transported to the laboratory in a cool box, and filtered within 8 h through 0.45 μm membrane filters. After filtration, water samples were split into two parts: (1) 80 mL was stored at −18 °C prior to measurement of DOC concentration, and (2) 80 mL was immediately used to determine the specific UV absorbance. For all water samples, DOC concentration was measured using a total organic carbon analyzer (TOC-5000, Shimadzu, Japan), and the absorbance values at 254, 400, 465, and 665 nm were measured on a UV-7504 spectrophotometer (Shanghai Xinmao Instrument, China) using quartz cells with 1 cm path length. For each water sample, $SUVA_{254}$ was calculated as the absorbance at 254 nm divided by the DOC concentration (L mg C^{-1} m^{-1}) (Weishaar et al., 2003), C:C ratio was determined by dividing the absorbance value at 400 nm by the corresponding DOC concentration (Wallage et al., 2006), and E4:E6 ratio

was obtained by dividing the absorbance value at 465 nm by that at 665 nm (Fong and Mohamed, 2007). In the study site, Fe^{3+} and NO_3^--N concentrations in waters were lower than 0.5 mg L^{-1} and 1.0 mg L^{-1}, respectively (Supplement materials). Moreover, Fe^{2+} had negligible effects on optical properties of DOC in waters (Poulin et al., 2014). Therefore, we assumed that, for the determination of $SUVA_{254}$ in water samples, the potential interferences by iron and nitrate were limited (Weishaar et al., 2003; Poulin et al., 2014).

2.3. Statistical analyses

Before statistical analyses, all data were test for normality using the Levene's test. The data that did not follow a normal distribution were natural log transformed. Repeated measures analysis of variance (ANOVA) was used to assess the effects of P addition level, sampling date, and their interactions on DOC concentration and optical properties. At each sampling date, one-way ANOVA with the Turkey's HSD post hoc test was used to examine the significant difference in DOC parameters among four P addition treatments. These analyses were carried out with the SPSS statistical package (v. 13.0) for Windows, and a p value < 0.05 was accepted as statistically significant.

3. Results

Surface water depth in the plots significantly varied with sampling date, and ranged from 9.7 cm to 15.7 cm (Table 1). However, at each sampling date, there was no significant difference in surface water depth among the four treatments of P addition.

For both surface water and soil pore water, P addition and sampling date independently produced significant effects on DOC concentration and $SUVA_{254}$ (Table 2). DOC concentration in waters was high in July and August, but low in June and September (Fig. 1). The treatments P1, P2, and P3 generally had lower DOC concentration and $SUVA_{254}$ than the treatment CK in both surface water and soil pore water at each sampling date (Figs. 1 and 2). In most cases, there were no significant differences in DOC concentration and $SUVA_{254}$ among the treatments P1, P2, and P3 across the sampling dates (Figs. 1 and 2).

In both surface water and soil pore water, P addition caused a significant decline in C:C ratio, but a significant increase in E4:E6 ratio (Table 2). At each sampling date, the treatment CK generally had greater C:C ratio, and lower E4:E6 ratio than the treatments P1, P2, and P3 (Fig. 3). Only in June and August, C:C ratio of surface water in treatments P2 and P3 was significantly higher than that in treatment P1. In addition, there was no significant difference in E4:E6 ratio of soil pore water among treatments P1, P2, and P3 across the sampling period (Fig. 3).

4. Discussion

Contrary to the first hypothesis, eight years of P addition decreased DOC concentration in both surface water and soil pore water across the sampling dates, although there was an increase in aboveground plant biomass (Mao et al., 2015, 2016). Pinsonneault et al. (2016a) also observed a decline in extractable organic C following 14 years of combined P and potassium additions in an ombrotrophic bog. However, McLaughlin et al. (2000) found that four years of P addition increased DOC concentration in the soil solution in a forested wetland in the Upper Great Lakes Region of the US. In the same fertilization experiment, Song et al. (2011) also observed that DOC concentration in soil pore water at 15 cm depth increased following one year of P addition. Moreover, the effects of P addition on species composition, plant productivity, biomass allocation, and plant nutrient concentration were time-dependent in temperate wetlands (Güsewell et al., 2003; van der Hoek et al., 2004). Thus, these inconsistent change trends of DOC concentration in temperate wetlands were possibly caused by the different duration of P addition experiments. In this P addition experiment, the differential responses of DOC in the short term and long term probably resulted from the differences in plant C:N:P stoichiometry and associated microbial physiological responses (Midgley and Phillips, 2016). One year of P addition might exacerbate the N limitation of microbial growth and decrease C-degrading enzyme activity, leading to an increase in DOC concentration in soil pore water (Song et al., 2011). Nevertheless, these results highlight that long-term fertilization experiments are needed to accurately assess how changed P availability affects DOC dynamics in wetland ecosystems. Considering that wetland ecosystems are important sources of DOC to fluvial networks (Wallage et al., 2006), the reduction in DOC quantity induced by long-term P addition would have the potential to substantially alter biogeochemical cycles in aquatic ecosystems.

In wetland ecosystems, the amount of DOC in waters depends on the balance between inputs and outputs via mineralization and leaching (Kalbitz et al., 2003). Given the increased DOC inputs through enhanced amount of organic matter returns, increased microbial growth, and accelerated organic matter decomposition following long-term P addition (Song et al., 2011; Mao et al., 2015, 2016), the negative effect of eight years of P addition on DOC concentration may be related to the following mechanisms in this wetland. First, long-term P addition could make DOC become more accessible to microbial attack through decreased molecular size and aromaticity of DOC (Liu et al., 2014). Second, P addition could increase nutrient returns through enhanced quantity and quality of organic matter (Mao et al., 2015), and thus, alleviated the nutrient limitation of microbial activities and stimulated microbial decomposition of DOC. Third, P addition would increase the amount of DOC leaching because of the displacement of DOC from binding sites by phosphate (Scott et al., 2015).

According to the second hypothesis, $SUVA_{254}$ and C:C ratio generally decreased, and E4:E6 ratio increased following eight years of P addition at each sampling date. Lower $SUVA_{254}$ value and C:C ratio indicate a lower DOC aromaticity and a smaller proportion of colored humic substances in the DOC, respectively (Weishaar et al., 2003; Wallage et al., 2006), and a greater E4:E6 ratio indicates a higher molecular weight of humic substances and a lower ratio of humic acid to fulvic ratio in the DOC (Thurman, 1985; Fong and Mohamed, 2007). Generally, the E4:E6 ratio for humic acid was <5.0, and humic acid had higher molecular weight and lower E4:E6 ratio than fulvic acid (Fong and Mohamed,

Table 1
Surface water depth of the plots.

Treatments	Surface water depth (cm)			
	June	July	August	September
CK	11.7 (0.5)	15.7 (0.5)	11.2 (0.3)	10.0 (0.4)
P1	11.5 (0.6)	15.4 (0.6)	11.2 (0.5)	9.7 (0.4)
P2	11.6 (0.5)	15.2 (0.6)	11.6 (0.4)	10.1 (0.4)
P3	11.5 (0.2)	15.5 (04.)	11.0 (0.5)	10.2 (0.3)

Data in the parenthesis are the standard errors of means.

Table 2
Results (F-values) of repeated measures ANOVAs on the effects of P addition level (P), sampling date (D), and their interactions on dissolved organic matter characteristics.

	DOC	$SUVA_{254}$	C:C ratio	E4:E6 ratio
Surface water				
P	**38.1*****	**52.1*****	**49.8*****	**21.0*****
D	**31.9*****	**21.1*****	**6.0***	**17.1****
P × D	0.8	0.9	1.1	1.2
Soil pore water				
P	**13.0****	**16.0****	**54.2*****	**17.5****
D	**90.9*****	**36.8*****	**80.9*****	1.6
P × D	1.3	0.8	1.8	1.5

Statistically significant results were shown in bold.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

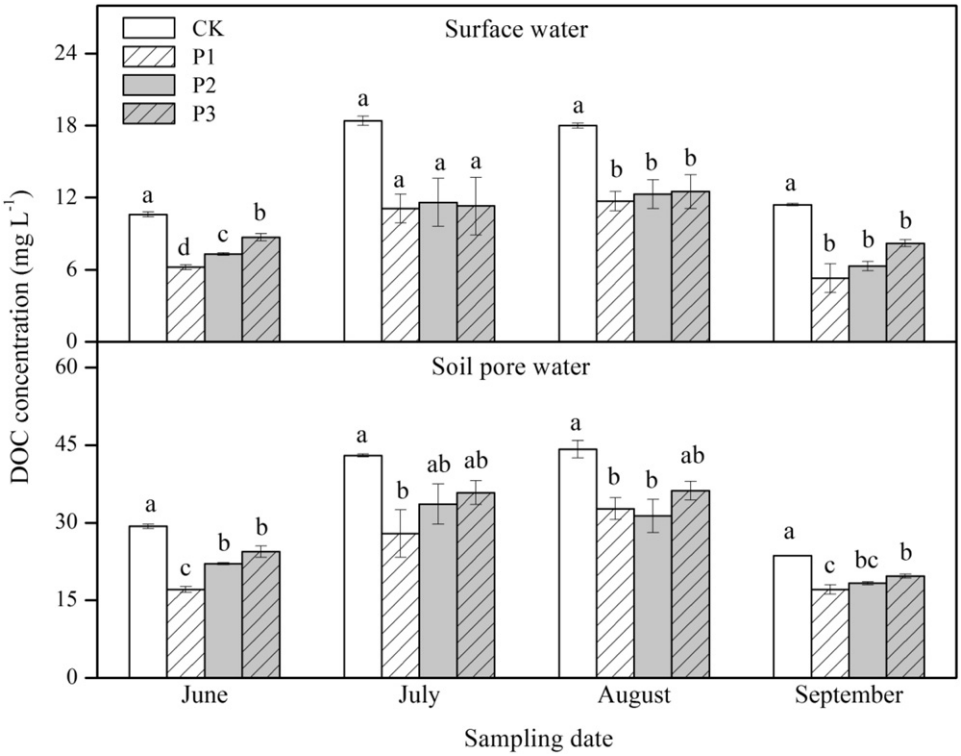

Fig. 1. Effect of P addition on dissolved organic carbon (DOC) concentration in waters. Data are mean value and error bars are standard error ($n = 3$). Different letters indicate significant differences ($P < 0.05$) among four P addition treatments.

2007). These results indicated that long-term P addition decreased both amount and molecular weight of humic substances in the DOC, and reduced the aromaticity of DOC in this wetland ecosystem. Liu et al. (2014) also found that, under laboratory conditions, increased P availability drove the transition of soil-derived DOC composition to much more labile components with a simpler molecular structure and a

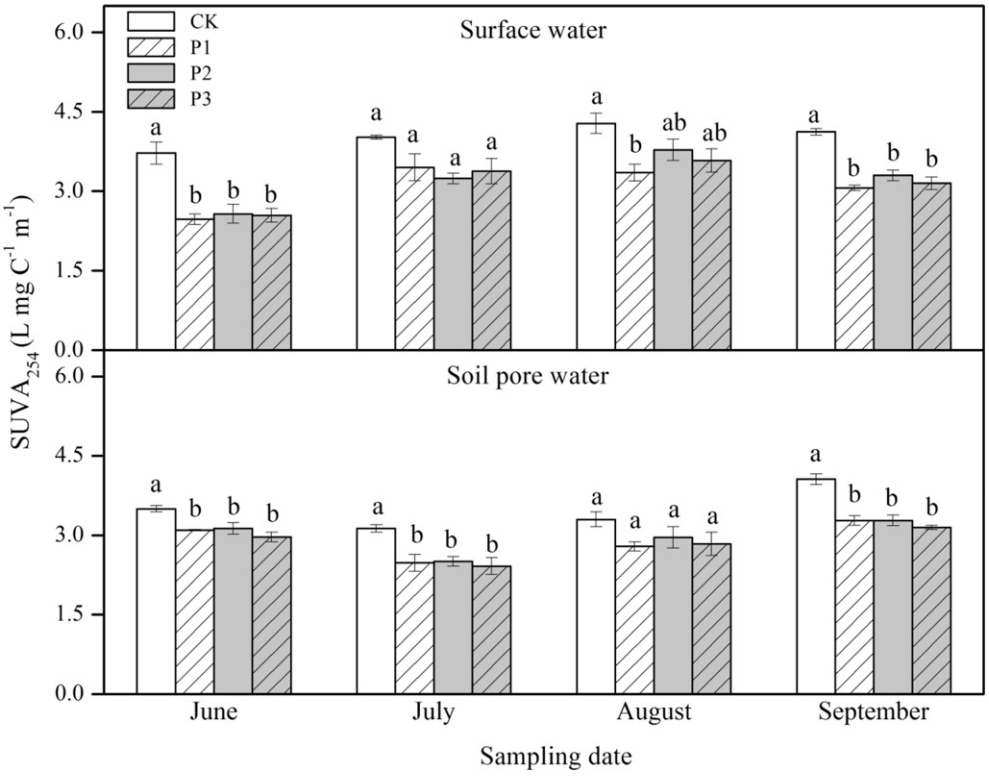

Fig. 2. Effect of P addition on $SUVA_{254}$ in waters. Data are mean value and error bars are standard error ($n = 3$). Different letters indicate significant differences ($P < 0.05$) among four P addition treatments at each sampling date.

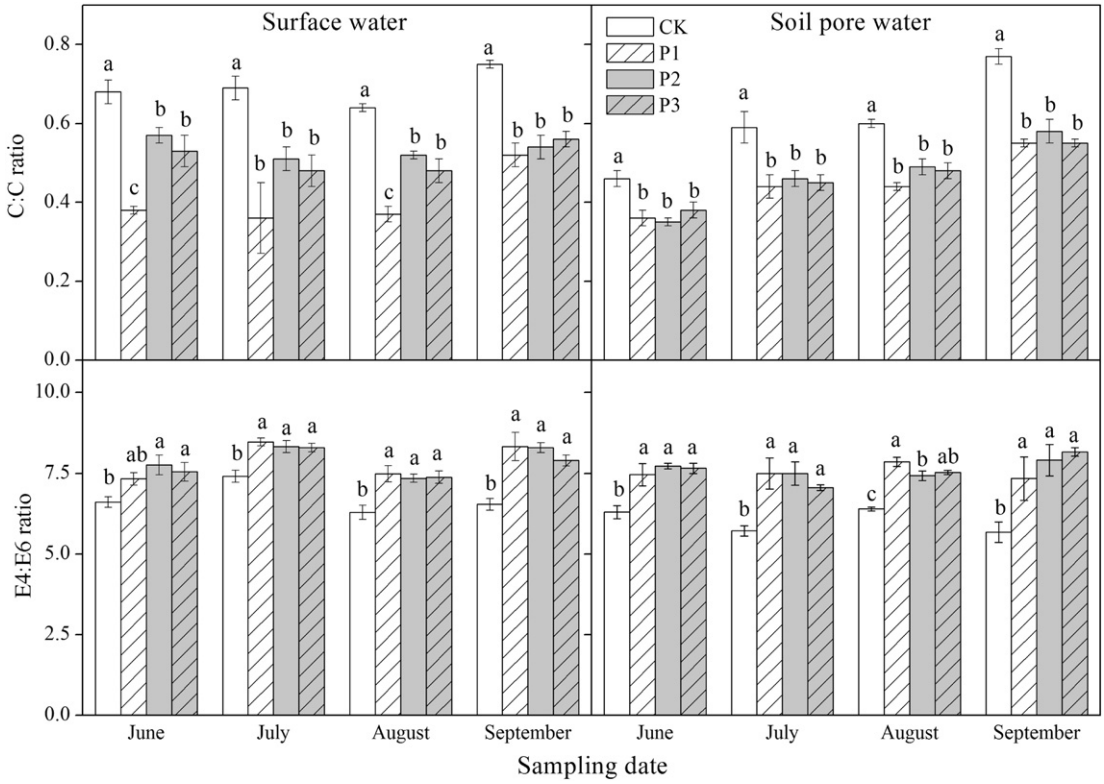

Fig. 3. Effect of P addition on C:C and E4:E6 ratios in waters. Data are mean value and error bars are standard error ($n = 3$). Different letters indicate significant differences ($P < 0.05$) among four P addition treatments at each sampling date.

lower degree of aromatic polycondensation in agricultural riparian wetlands. Considering that DOC biodegradation is negatively correlated to the aromaticity of DOC (Fellman et al., 2008), our results suggest that long-term P addition could weaken the chemical recalcitrance of DOC, thereby accelerating C cycles in temperate freshwater wetlands.

The chemical composition of DOC is often influenced by the vegetation type, soil nutrient availability, and microbial activity (Martin et al., 1998; Liu et al., 2014). In this wetland, long-term P addition increased the dominance of *G. spiculosa* over *D. angustifolia* and enhanced plant nutrient concentrations (Mao et al., 2015, 2016), which would enhance nutrient availability and alleviate the nutrient limitation of microbial growth. This might increase microbial decomposition of higher molecular groups and destruction of cementing, easily mineralizable compounds, and thus reduce the formation of high molecular weight humic substances and decrease the aromaticity of DOC (Martin et al., 1998; Liu et al., 2014). Meanwhile, DOC in surface water and soil pore water may be derived from the newly decomposing organic matter, and thus had high immature fulvic acids, since long-term P addition decreased the chemical recalcitrance of DOC and made DOC more accessible to microbes (Wallage et al., 2006). Therefore, DOC in the P addition treatments generally had lower $SUVA_{254}$ and C:C ratio, and higher E4:E6 ratio than that in the control treatment.

In summary, DOC concentration in both surface water and soil pore water significantly declined following eight years of P addition in a temperate freshwater wetland in the Sanjiang Plain, Northeast China. In addition, long-term P addition generally caused reductions in $SUVA_{254}$ and C:C ratio, and an increase in E4:E6 ratio, irrespective of P addition levels. These results imply that long-term P addition not only decreases the quantity of DOC, but also reduces the aromaticity and humification of DOC in this N-limited temperate wetland. Given the widespread occurrence of increasing P loading due to the intensified anthropogenic activities (Filippelli, 2008), these findings presented here imply that enhanced DOC quality induced by P enrichment would further cause organic C loss from waters in temperate freshwater wetland ecosystems.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.02.084.

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