



Short Communication

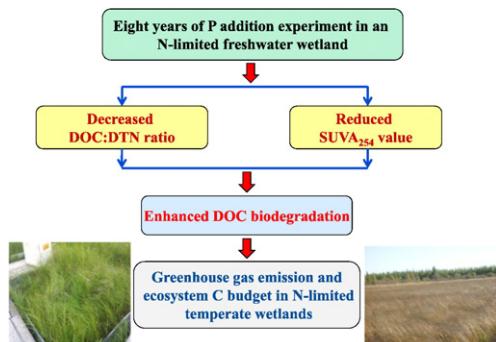
Long-term phosphorus addition enhances the biodegradability of dissolved organic carbon in a nitrogen-limited temperate freshwater wetland

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HIGHLIGHTS

GRAPHICAL ABSTRACT

- Long-term P addition enhanced DOC biodegradation in an N-limited temperate wetland.
- Long-term P addition decreased DOC:DTN ratio and SUVA₂₅₄ in this wetland.
- DOC biodegradation was negatively correlated with DOC:DTN ratio and SUVA₂₅₄, respectively.
- Enhanced DOC biodegradation would cause DOC loss from the waters in temperate wetlands.



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GRAPHICAL ABSTRACT

Phosphorus (P) enrichment is expected to strongly influence dissolved organic carbon (DOC) biodegradation. However, the relationship between P availability and DOC biodegradation is largely unknown in nitrogen (N)-limited ecosystems. Here, we investigated the changes in the ratio of DOC to dissolved total nitrogen (DTN), specific UV absorbance at 254 nm (SUVA₂₅₄), and DOC biodegradation in surface water and soil pore water (0–15 cm depth) following eight years of multi-level P addition (0, 1.2, 4.8, and 9.6 g P m⁻² year⁻¹) in an N-limited freshwater marsh in Northeast China. We found that P addition caused an increase in DOC biodegradation in surface water and soil pore water, irrespective of the P addition levels. Compared with the control treatment, the P addition rates of 1.2, 4.8, and 9.6 g P m⁻² year⁻¹ increased DOC biodegradation by 20.7%, 15.2%, and 14.5% in surface waters, and 11.3%, 9.4%, and 12.0% in soil pore waters, respectively. The DOC biodegradation was separately negatively correlated with the DOC:DTN ratio and SUVA₂₅₄, indicating that the positive effect of P addition on DOC biodegradation was caused by the elevated N concentration and the reduced DOC aromaticity. Our findings suggest that P enrichment enhances the biodegradability of DOC through increased N availability and altered DOC chemical composition, which would accelerate DOC loss from the waters and alter ecosystem C balance in N-limited temperate wetlands.

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1. Introduction

Dissolved organic carbon (DOC) is a complex mixture of soluble organic compounds with varying levels of degradability, and originates

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from plant litter and root exudates, soil organic matter, and microbial biomass (Zsolnay, 2003). In most terrestrial and aquatic ecosystems, DOC acts as the substrate for microbial activity and thus plays a critical role in modulating regional and global C cycles (Bouillon et al., 2003; Kalbitz et al., 2003). Microbial degradation of DOC not only regulates greenhouse gas production and ecosystem C balance, but also serves as a link between terrestrial and aquatic ecosystems (Marschner and Kalbitz, 2003). Generally, DOC biodegradation is controlled by the initial chemical composition (Fellman et al., 2009; Vonk et al., 2015) and the stoichiometric ratios between C and the availability of nutrients (i.e., nitrogen N and phosphorus P) (Wickland et al., 2012; Mao et al., 2017b).

In recent decades, the extensive use of fertilizers by farmlands, sewage discharge, and land use changes have substantially altered the global P cycle and resulted in P enrichment in most terrestrial and aquatic ecosystems, especially in wetland ecosystems (Filippelli, 2008). Because P is an essential nutrient for plant growth and other biological processes (Vitousek et al., 2010), P enrichment could alter nutrient availability, increase microbial biomass and activity, and change the molecular structure of DOC (Cleveland et al., 2002; Liu et al., 2014). Consequently, increased P availability generally increases the biodegradability of DOC in both terrestrial and aquatic ecosystems (Cleveland et al., 2002; Vadstein et al., 2003; Räsänen et al., 2014). Unfortunately, these studies have been conducted in P-limited ecosystems, and the effect of P enrichment on DOC biodegradation is still unclear in N-limited ecosystems. Since the cycles of C, N, and P are tightly coupled (Vitousek et al., 2010), P enrichment may substantially alter substrate quality and N availability and thus DOC biodegradation in N-limited ecosystems. To clarify the effect of P enrichment on greenhouse gas emissions and C balance on regional and global scales, knowledge regarding the effect of increased P availability on DOC biodegradation is urgently needed in N-limited ecosystems.

Temperate wetlands store large amounts of DOC in the soil and surface water, and are the main source of DOC inputs to rivers and oceans (Kayranli et al., 2010). In these ecosystems, plant growth and microbial activity are generally limited by N availability (Vitousek et al., 2010). Temperate wetlands are now experiencing increased P input as a result of intensified anthropogenic activities (Filippelli, 2008). To examine the effect of P enrichment on DOC biodegradation in these N-limited ecosystems, we established a long-term multi-level P addition experiment in a freshwater marsh in the Sanjiang Plain, Northeast China. Our previous studies observed that P addition to this wetland increased N and P concentrations in plants (Mao et al., 2015, 2016) and reduced the aromaticity of DOC (Mao et al., 2017a). Here, we investigated the changes in the stoichiometric ratios between DOC and nutrient concentrations and DOC biodegradation in surface water and soil pore water (0–15 cm depth) following eight years of P addition. We hypothesized that: (1) long-term P addition would cause an increase in the dissolved total N (DTN) concentration and a decrease in the DOC:DTN ratio in surface water and soil pore water due to the increased N concentration and decreased C:N ratio in plants (Mao et al., 2015, 2016), and (2) long-term P addition would increase the biodegradability of DOC in waters because of reduced DOC aromaticity (Mao et al., 2017a) and increased N availability (Song et al., 2013).

2. Materials and methods

2.1. Study site and experimental design

This study was conducted in a *Deyeuxia angustifolia*-dominated freshwater marsh at the Sanjiang Mire Wetland Experimental Station (47°35'N, 133°31'E; 56 m above sea level), which is located in the center of the Sanjiang Plain, Northeast China. The Sanjiang Plain is the low alluvial plain of the Heilongjiang, Songhua, and Wusuli Rivers, and includes the largest natural temperate freshwater wetlands in China. This region has a temperate humid and semi-humid monsoon climate,

The mean annual temperature (1990–2010) is 2.5 °C with monthly mean temperatures ranging from –20.4 °C in January to 21.6 °C in July. The mean annual precipitation is 566 mm with 80% distributed from May to September. The plant community is dominated by the perennial herbaceous species *D. angustifolia* and *Glyceria spiculosa*, which accounts for >90% of the aboveground plant biomass (Table S1). Soil (0–10 cm depth) organic C, total N, and total P concentrations are 167.0, 6.8, and 1.5 mg g^{−1}, respectively. In this freshwater wetland, plant productivity and microbial growth are limited by N availability (Song et al., 2013) rather than P availability (Song et al., 2011a). Since 1950s, approximately 80% of the freshwater wetlands in this region has been reclaimed because of the large-scale agricultural development in China (Wang et al., 2006). Consequently, the remaining wetlands have received increasing P loading via drainage and atmospheric deposition, and the annual P input is approximately 0.4 g P m^{−2} during the growing season (Mao et al., 2015).

Since 1995, a large amount of dry field has been extensively converted to paddy field in the Sanjiang Plain because rice growing can yield more economic incomes than maize and soybean (Wang et al., 2006). In this region, P fertilizer applied in the paddy field (about 5.16 g P m^{−2} year^{−1}) is higher than that in the dry field (about 2.37 g P m^{−2} year^{−1}). Moreover, the amount of P export from paddy field through artificial drainage and surface runoff is much greater than that from dry field (Zhu and Yan, 2010). Collectively, these would cause increasing P input to the freshwater wetlands. Thus, we experimentally increased the amounts of P loading by factors of 3, 12, and 24 to simulate future P enrichment in this wetland ecosystem. In this study, P was applied at four 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 the P addition experiment was initiated in May 2007. Four P addition levels were randomly arranged in 12 1 m × 1 m plots, and each P addition level was replicated three times. All of the plots were separated by 1-m-wide buffer strips, and were fenced with stainless steel frames (1 m × 1 m, 0.5 m in depth) to avoid the lateral loss of the added P. Meanwhile, the plots were surrounded by board walks to minimize site disturbance during sampling. Phosphorus fertilizer was added as NaH₂PO₄ solution, and was equally applied twice a month from May to September every year.

2.2. Sampling and measurement

In June 2014, surface water was collected with pre-rinsed polyethylene bottles (Wickland et al., 2012). Meanwhile, soil pore water at 0–15 cm depth was collected using slit PVC pipes (15 cm in length, 2.5 cm in diameter, and three replicate per plot) covered with filter gauze and attached to a stainless-steel capillary (2 mm in diameter) (Höll et al., 2009). The end of the capillary was closed using a three-way valve, and soil pore water was sampled with syringes. In each plot, approximately 250 mL of water was collected, transported to the laboratory on ice, and filtered using pre-combusted Whatman GF/F 0.70 µm filters, which are commonly used to determine DOC in the previous studies (Bouillon et al., 2003; Fellman et al., 2009; Vonk et al., 2015). The filtered water samples were stored in the dark at 4.0 °C until ready for analysis, which was initiated within 48 h. For all of the water samples, the DOC concentration was measured using a total organic carbon analyzer (TOC-5000, Shimadzu, Japan), and NH₄⁺-N and NO₃[−]-N concentrations were measured using the sodium salicylate-sodium hypochlorite method and the hydrazine reduction method on a spectrophotometer (UV-1750, Shimadzu Corporation, Japan), respectively. The DTN and dissolved total P (DTP) concentrations were measured using the hydrazine reduction method and the molybdenum blue method on a continuous-flow autoanalyzer (AA3, Seal Analytical, Germany) after peroxodisulfate oxidation, respectively (Ebina et al., 1983). The dissolved inorganic N (DIN) concentration was the sum of the NH₄⁺-N and NO₃[−]-N concentrations, and the dissolved organic N (DON) concentration was obtained from the difference between DTN

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