Phosphorus Fluxes at the Sediment-Water Interface in Subtropical Wetlands Subjected to Experimental Warming: A Microcosm Study

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A B S T R A C T

Global warming is increasingly challenging for wetland ecological function. A temperature controlled microcosm system was developed to simulate climate change scenarios of an ambient temperature (control) and an elevated temperature (+5 ℃). The effects and associated mechanisms of warming on phosphorus (P) fluxes at the sediment-water interface of six subtropical wetlands were investigated. The results indicated that P fluxes were generally enhanced under the experimental warming as measured by higher P concentrations in the porewater and overlying water as well as higher benthic P fluxes. The release of P from sediment to porewater occurred more strongly and quickly in response to experimental warming compared to the subsequent upward transfer into overlying water. The average accumulative benthic P output from the tested wetlands under the experimental warming was greater by 12.9 μg cm^{-2} y^{-1} (28%) for total P and 8.26 μg cm^{-2} y^{-1} (25%) for dissolved reactive P, compared to the ambient scenarios. Under warming the redistribution of P fractions in sediments occurred with greater NH_4Cl-P and lower BD-P (extracted by a bicarbonate buffered dithionite solution) accompanied by greater NaOH-P. The higher temperature enhanced total phospholipid fatty acids. A shift in the microbial community was also observed with a relative dominance of fungi (a 4.7% increase) and a relative decline (by 18%) in bacterial abundance, leading to the higher secretion of phosphatase. Comparing between wetlands, the potential P fluxes in the nutrient-enriched wetlands were less impacted by warming than the other wetland types investigated. Thus wetlands characterized by low or medium concentrations of P in sediments were more susceptible to warming compared to P-rich wetlands.

Keywords: Phosphorus Transfer, Global Warming, Flux, Wetland, Microcosm

1. Introduction

Global air temperatures are predicted to increase by 1.8 to 4.0 ℃ over this century (IPCC, 2007). Many ecosystems are particularly vulnerable to elevated temperatures induced by global climate change (Scheffran and Battaglini, 2011). Understanding of nutrient dynamics and matter fluxes within wetland ecosystems are considered as major knowledge gaps in predicting climate change impacts (Paul et al., 2006).

As one of the most productive and biologically diverse ecosystems, wetlands are well known for their carbon storage functions and the capacity to remove nutrients from regional waterbodies, both of which are critical for phosphorus (P) retention at the sediment-water interface (Zedler and Kercher, 2005; Verhoeven et al., 2006). Protection and restoration of wetlands is now a priority in many subtropical areas including large parts of China. However, there is little published work on warming impacts on subtropical wetland biogeochemical cycles. After the reduction of external nutrient loading, P transfer from the sediment into water column becomes a major P source, preventing improvements of water quality for a considerable period. Global warming may change both the direction and strength of P transfer processes at the sediment-water interface, thus further enhancing primary production and accelerating eutrophication in aquatic ecosystems (Elser et al., 2007).

Phosphorus transfer at the sediment-water interface can be enhanced due to changes in environmental conditions such as light, pH, dissolved oxygen, microbial activities and temperature (Sanchez and Boll, 2005; Jiang et al., 2008). For example, light stimulates the growth of autotrophic algae but there is a strong interaction with sediment-derived P fluxes (Cymbola et al., 2008). High pH values result in a competitive exchange of phosphate anions with OH^-, increasing the potential to release P (Christophoridis and Fytianos, 2006). Depletion of dissolved oxygen in

overlying water leads to a decrease in the thickness of the oxidized surface sediment layer, suggesting a redox-sensitive P release (Grunth et al., 2008). Microbial activity may play a vital role in degradation of organic matter, liberating soluble P from soil-microorganism complexes (Pages et al., 2011). Among these various physicochemical factors, elevated temperature is considered one of the main contributors to nutrient fluxes from sediments (Liikanen et al., 2002), through enhancing organic mineralization (Asmus et al., 2000), solute chemical diffusion (Bally et al., 2004), and microbial metabolic processes (Maassen and Balla, 2010). Accordingly, P processes and flux rates within wetland systems will be impacted in response to global warming.

Elevated temperature may account for a large part of the P budget in some water bodies due to P release at the sediment-water interface (Banaszuk and Wysocka-Czubaszek, 2005). Previous climate change studies of P fluxes in wetlands have mainly focused on the impact on the hydrological regime (Aldous et al., 2005; Maassen and Balla, 2010) and seasonal P variations (Davis et al., 2003; Duff et al., 2009). However, few studies have not examined responses to warming when the warming is conducted in comparison to natural variability in temperature over daily cycles. In addition to understanding P biogeochemical characteristics, quantifying P fluxes and their direction of change at the sediment-water interface is a key prerequisite for scientists and policy-markers so they can develop practical P eco-technologies for wetland management under global warming. Previous studies, which have been mainly based on lab-scale temperature manipulation incubation experiments at fixed temperatures (i.e., 16 ℃ and 25 ℃) (Liikanen et al., 2002; Jiang et al., 2008), have demonstrated that elevated temperature has pronounced effects on sediment P dynamics. Studies of microbial shifts in response to

experimental warming (using buried heating cables), such as microbial community composition and soil enzyme activities, have been limited to terrestrial ecosystems (Allison and Treseder, 2008; Frey et al., 2008).

Use of realistic real-time temperature control systems to reflect hourly, daily and seasonal temperature behavior are extremely limited in the literature. Such temperature control would enable experiments that more readily reflect temperature patterns in the natural environment and reduce the chances of experimental artifacts biasing results. In our paper, by means of a novel outdoor microcosm device enabling high resolution temperature control, the objective of our study was to compare P fluxes (benthic P fluxes and potential P fluxes) from six subtropical wetlands located in the delta of the Yangtze River in southeast China, in response to simulated global warming. The corresponding abiotic (P fractions and carbon fractions) and biotic (soil enzyme activities, microbial communities and ratios) factors in sediments were also examined to show their associations with P fluxes. We hypothesize that the global warming scenario of +5℃ will induce greater P fluxes from sediment to water. Such changes in the magnitude and direction of P fluxes are likely to be related to the transformation of sedimentary P forms as well as biological activities, especially for microbes in sediments.

2. Materials and methods

2.1. Microcosm configuration

A custom-built novel microcosm (Fig. S1 & S2) simulating climate warming at a minute-scale for both daily and seasonal temperature variations was developed using independently monitored water-bath jackets for this study. The microcosm consisted of four major components: a storage section, a heating section, water circulation, and

a real-time temperature control section. The storage section was composed of two square stainless steel incubation boxes (100 cm in length and width, and 40 cm in height): one for the ambient temperature treatment (Control), and the other for the +5 ℃ -increased temperature treatment (Warmed). The real-time temperature controlling section was composed of a computer (HP a6315cn), a custom-built controller, and two digital temperature probes (NB 407-25a, China). The temperature signals were transmitted through the temperature probes into the controller which regulated the heater and the pump. With lab-designed software written in C++ language, the temperatures in both incubation boxes were continuously recorded and the temperature differences were compared by signals of temperature probes at two-minute intervals for this study. The temperature difference between the two incubation boxes was set at 5±1 ℃. Except for the computer and the controller, the rest of the microcosm components were set up outdoors in May 2008. Data in this paper were collected from May 2008 to Nov 2009. While daily temperature variability over a 24-hour period is projected to increase under future climate (Fischer and Schar, 2009), our novel microcosm provided a significant advance of the experiments used in previous studies. By having the Warmed treatment track the ambient Control continuously provided a much more natural setting for the experiments, with more realistic simulations (Fig. S2), compared to studies which just set at fixed and constant temperatures (e.g., 15 ℃, 20 ℃, 25 ℃)

2.2. Study sites and sampling regime

The study sites were located in the southern region of the Taihu Lake Basin and the NingShao plain within the delta of the Yangtze River. The climate in this area is subtropical monsoon with an annual average rainfall of 1350 mm and an annual

146 average temperature of 26 ℃ in summer and 4 ℃ in winter (2004-2008 inclusive).
147 Six wetland sites, with a large spatial and temporal variability in sediment-water
148 nutrient exchanges, were chosen for wetland soil sampling (Table S1, Fig. S3). Those
149 tested wetlands belong to typical subtropical wetlands with different uses and nutrient
150 status. YaTang riverine (YT) wetland suffered pollution from duck farms and other
151 rural activities, while XiaZhuhu (XZ) wetland was threatened by aquaculture, typical
152 of contaminated wetlands. The wetlands of JinHu (JH), BaoYang (BY), XiXi (XX),
153 and ShiJiu (SJ) were generally preserved for tourism and used as water reservoirs,
154 typical of recovered wetlands. SJ acts as a waterlogged paddy field with the lowest
155 organic matter and nutrient contents of the six wetlands.

156 Transparent PVC wetland columns (45.0 cm in height and 10.0 cm in internal
157 diameter) were prefabricated before sampling. Sediment cores of 0-15 cm from the
158 surface were collected with a stainless steel column sampler. After most of the larger
159 visible roots, stones, and macrobenthos were removed from the surface, each
160 sediment core (mixed with its zoobenthos community, mainly including Olisochaeta,
161 Crustacea and Mollusca) were divided into a 0-5 cm top-sediment and 5-15 cm
162 sub-sediment manually. Then the 5-15 cm of sub-sediment was first transferred into
163 each PVC wetland column, whereafter the 0-5 cm of top-sediment was carefully
164 refilled to the remaining 0-5 cm column space of the sediment layer. After refilling the
165 20-cm-depth of sediment, each column was filled with 20 cm of the ambient
166 overlying water. The field sampling was conducted in May 2008. All of the wetland
167 columns were shipped back to the laboratory within 3 h where three replicates of each
168 wetland sample were placed inside each of the two incubation boxes. For porewater
169 sampling, an orbicular Teflon tube (TCN-350 Nanjing: 1.0 mm in diameter, 1.0 μm in
170 aperture) (Song et al., 2003) was horizontally imbedded into the sediment in each

column at a depth of 5 cm (Fig. S2). Some floating-leaved (e.g., *Lemna minor L.*, *Trapa spp.*) and submerged (e.g., *Ceratphyllum d*emersum L.) aquatic vegetation was found growing after two months of incubation.

After the first six months of incubation, water samples were taken from the wetland columns once every two months for an annual cycle of 2009. About 100 mL of overlying water was sampled from each wetland column 5 cm below the water surface with a syringe. For porewater sampling, the sampler described by Song et al. (2003) was used. A standard 50-mL plastic syringe (-0.05 MPa vacuum pressure) was connected to the suction sampler, then about 30 mL of porewater for each wetland column was collected in the orbicular Teflon tube (Song et al., 2003). In addition, approximately 100 g of top 5 cm fresh sediment were manually sampled for each column after 14-months of incubation. All water and sediment samples were frozen at -15 ℃ prior to further analysis. After each sample was collected, the overlying water remaining inside the wetland column was carefully emptied into a plastic graduated flask by siphon action and the corresponding amount of overlying water was recorded. Then each wetland column was carefully refilled with commercial mineral water (P concentration level less than 0.005 mg L^{-1}; Nongfu Company, China) up to 20 cm in depth for avoiding disturbance on water-sediment interface. Based on the general hydrological regime of the normal water season around the sampling sites, the periodic replacement of overlying water at a 60-day interval was conducted to simulate the general hydraulic cycles, and make sure that phosphorus equilibrium across the interface was fully achieved over this period.

2.3. Water analysis

After thawing, one-half of each overlying water sample was filtered through a 0.45

196 μm filter, and the other half was kept as an unfiltered sample. Unfiltered overlying
197 water and porewater were both digested using a well reported method (Haygarth and
198 Jarvis, 1997) for measurements of total P (TP) and a continuous flow analyzer
199 (Autoanalyzer III, BRAN+LUEBBE, Germany) was used spectrophotometrically at
200 880 nm (Murphy and Riley, 1962). Phosphorus in undigested and filtered samples was
201 measured directly as mentioned above. This form of P is generally defined as the
202 dissolved reactive P (DRP), representing the bioavailable P fraction for algae in
203 surface water (Wu and Huh, 2007). The detection limits for TP and DRP
204 concentrations in porewater and overlying water were in a range from 1 μg L^{-1} to 10
205 mg L^{-1}. The relative deviation of blank concentrations of deionized water as a
206 reference was less than 5%.
207
208 *2.4. Sediment analysis*
209 Measurements of organic matter and TP were taken on the fresh sediment samples
210 that were thawed, air-dried, ground, and sieved according to standard methods (Bao,
211 2000). The P fractions in the sediment samples were determined using a sequential
212 extraction procedure (Rydin, 2000). The residual-P (Res-P) was determined by
213 calculating the remainder of TP minus extracted P fractions. The accuracy of the sum
214 calculation for P fractions was checked by TP analysis to make sure there was an
215 experimental error of less than 10%. According to the susceptibility of organic carbon
216 to $KMnO_4$ oxidation, the contents of three fractions of labile organic carbon in six
217 wetland columns, namely highly labile organic carbon (HLOC), mid-labile organic
218 carbon (MLOC) and labile organic carbon (LOC), were determined using 33, 167 and
219 333 mmol L^{-1} $KMnO_4$, respectively (Logninow et al., 1987).
220 Using a modified method (Sardans et al., 2006), the activities of neutral and

alkaline phosphatases in sediment samples were determined spectrophotometrically based on the release of *p*-nitrophenol from the model substrate *p*-nitrophenyl phosphate (pNPP). The reaction mixture for the sediments contained: 1 g sediment, 2.6 mL 0.05 mol L^{-1} Tris-buffer (pH=7.4 or 8.4), 0.03 mL 0.1 mol L^{-1} $MgCl_2$, and 0.1 mL 10 mmol L^{-1} pNPP. Results of the specific phosphatase activity and the maximum reaction rate (V_{max}, Michaelis-Menten equation) were expressed as μmol *p*-nitrophenyl per gram of sediment per hour (μmol g^{-1} h^{-1}). Based on the reported protocol (He et al., 2007), the phospholipid fatty acids (PLFAs) were extracted from freeze-dried sediment with 1:2:0.8 (v/v/v) chloroform:methanol:citrate buffer (0.15 mol L^{-1}, pH 4.0). Following the elution of neutral lipids and glycolipids, phospholipids were eluted with 8 mL methanol and dried under N_2. PLFAs were subsequently derivatized by mild-alkali methanolysis. The resulting fatty acid methyl esters were then separated and identified by Agilent 6890N gas chromatography (Agilent, Wilmington, DE, USA) fitted with a MIDI Sherlock® microbial identification system (Version 4.5, MIDI, Newark, NJ, USA). The main microbial taxa were indicated by the referred individual PLFA biomarkers. A quality control procedure was applied throughout sampling, preparation and measurement to ensure our laboratory data. The precision for both water and sediments analysis was within 5% by relative standard deviation of duplicates through repeated measurements.

2.5. Phosphorus budgets and statistical analysis

In order to study P fluxes across the sediment-water interface, benthic P fluxes (F_b, μg cm^{-2} d^{-1}) were calculated according to the following equation (Clavero et al., 1999):

$$F_b = (C_{out} - C_0) \times V/(S \times T) \quad (1)$$

where C_{out} is the P concentration in the overlying water (mg L^{-1}), C_0 is the P

concentration of commercial mineral water (mg L^{-1}), *V* is the volume of overlying water (mL), *S* is the area of wetland columns (cm^2) and *T* is the intervals of two sampling dates (d). The first replacement of overlying water with commercial mineral water was on Dec-26-2008. The benthic P fluxes represented the actual P fluxes from six wetland columns across the sediment-water interface during periods of approximately 60 days.

Potential P fluxes (F_P, μg cm^{-2} d^{-1}) were used to evaluate the risk of P transfer potential from porewater to overlying water according to the following equation:

$$F_P = (C_{in} - C_{out}) \times V/(S \times T) \quad (2)$$

where C_{in} is the P concentration in the porewater (mg L^{-1}), and other variables are as mentioned above. Potential P fluxes defined here provide a good indicator to assess P concentration gradients between overlying water and porewater, and high values suggested high risk of P transfer.

Data collected from water and sediment samples were expressed as the mean plus standard errors. A paired Student *t*-test was used to compare the effects of warming on P concentrations in porewater and overlying water, accumulative P fluxes, P and carbon fractions, and microbial communities as well as enzyme activities. A repeated two-way ANOVA was conducted with SPSS software (version 15.0) to examine the effects of experimental warming, sampling dates (seasonality) and their interaction on P fluxes. The statistical tests were considered significant at the $p < 0.05$ level.

3. Results

3.1. Overall P variations pattern in water

The data calculated from the mean value of six tested wetlands for each sampling date showed that TP concentrations in overlying water were generally higher under

271 warming with a range of 0.10 mg L^{-1} (Nov-16) to 1.88 mg L^{-1} (Jul-16), compared to
272 those in the control of 0.07 mg L^{-1} (Nov-16) to 1.62 mg L^{-1} (Jul-16) (Fig. 1). A similar
273 tendency for DRP was also observed (Fig. 1, column chart). For site-specific, mean
274 values of TP concentration during the investigation period reached a range of 0.12 mg
275 L^{-1} (SJ) to 4.10 mg L^{-1} (YT) in the warmed compared to a range of 0.09 mg L^{-1} (SJ) to
276 3.25 mg L^{-1} (YT) in the control.

277 When subjected to warming, P concentrations in porewater (Fig. 1, line chart)
278 calculated from the mean value of different sampling dates for each wetland increased
279 from 22% (YT) to 64% (JH) for TP, and 20% (YT) to 71% (XZ) for DRP in the
280 warmed treatment over the annual cycle of 2009 except for DRP in the SJ wetland.
281 The observed P concentrations in porewater were approximately one order of
282 magnitude higher than those in overlying water, indicating a high concentration
283 gradient existed between overlying water and porewater. Taking BY wetland as an
284 example, P concentrations in porewater were much higher than in overlying water by
285 1.54-fold (Jul-16) and 11.0-fold (Nov-16) (Fig. 1). A significant (p = 0.045)
286 relationship between total P stored in sediments (Table S1) and P concentrations in
287 porewater was found among the tested wetlands, while a marginal (p = 0.075)
288 relationship was found for those in overlying water, suggesting that P was first
289 transferred from sediments into porewater, then upward toward overlying water.
290 These findings were supported by the positive correlations between HPO_4^{2-}
291 concentrations in the porewater and seasonal temperature variability in muddy and
292 sandy sediments (Serpa et al., 2007).

293 For seasonal P variations, samples taken in the summer tended to have higher P
294 concentrations in the overlying water for each wetland column than samples taken in
295 the winter and spring (Fig. 1, YT as an example), which is similar to findings reported

by Duff et al. (2009). A *t*-test of six tested wetlands for each sampling date showed that significant differences in P concentrations of overlying water between warmed and control treatments were mainly found on Jul-16 (p = 0.049 for TP and p = 0.042 for DRP) and Nov-16 (p = 0.033 for TP and p = 0.023 for DRP), suggesting a greater influence of experimental warming on P concentrations of overlying water in wetland columns occurred under the extreme temperature conditions. Seasonal P variations in the porewater were correlated with seasonality to a lesser extent, compared to the seasonal P variations in the overlying water (Fig. 1).

3.2. Phosphorus fluxes in water

3.2.1. Benthic phosphorus fluxes (F_b)

The benthic fluxes of TP and DRP were generally greater in warmed treatments compared to those in the control for each wetland and sampling date (e.g., JH wetland, Nov-16) over the annual cycle of 2009 (Table 1). Data calculated from the mean value of different sampling dates for each wetland showed TP fluxes in warmed treatments ranged from 0.010 μg cm^{-2} d^{-1} (SJ) to 1.732 μg cm^{-2} d^{-1} (YT) compared to a range of 0.008 μg cm^{-2} d^{-1} (SJ) to 1.528 μg cm^{-2} d^{-1} (YT) in the control. The repeated two-way ANOVA (Table S2) revealed significant differences in TP fluxes between warmed and control treatments in XZ ($F_{1,5}$ = 9.07, p = 0.006), XX ($F_{1,5}$ = 5.34, p = 0.029), and SJ ($F_{1,5}$ = 6.88, p = 0.015), the means of which were greater by 60%, 33% and 38%, respectively. The statistical analysis further showed that seasonality (sampling dates) significantly affected the benthic P fluxes within six wetland columns ($F_{5,5}$ < 2.62, p < 0.001, Table S2). When calculating the mean value of the six tested wetlands for warmed treatments at each sampling date, the highest benthic TP fluxes (0.361 μg cm^{-2} d^{-1}) were found in the summer (Jul-16), and the lowest benthic TP fluxes (0.019

μg cm^{-2} d^{-1}) were in the winter (Nov-16), which agrees well with the P concentrations in the overlying water (Fig. 1). However, there were no significant interaction effects between treatments (Warmed and Control) and sampling dates (seasonality) on benthic P fluxes for each wetland column (Table S2). The dependence of warming impacts on the seasons for benthic P fluxes in the long-term needs further study.

The accumulative benthic P output (ABO) for TP over the annual cycle of 2009 for six wetland columns under warming ranged from 7.76 μg cm^{-2} y^{-1} (SJ) to 283 μg cm^{-2} y^{-1} (YT), while it ranged from 5.84 μg cm^{-2} y^{-1} (SJ) to 224 μg cm^{-2} y^{-1} (YT) for the control (Table 1). For DRP, the range was 3.43 μg cm^{-2} y^{-1} (SJ) to 207 μg cm^{-2} y^{-1} (YT) under warming, and 3.05 μg cm^{-2} y^{-1} (SJ) to 167 μg cm^{-2} y^{-1} (YT) under the control. The statistical analysis for each wetland column showed that significant increases in ABO under warmed treatments were found in the XZ (p = 0.015) and XX (p = 0.031) wetland columns for TP, and the JH (p = 0.014), XZ (p = 0.037) and BY (p = 0.048) wetland columns for DRP (Table 1). Seasonally, the wetland columns in the summer contributed for a large proportion of ABO. For warmed treatment of JH wetland column, 42.3% of ABO for TP and 47.9% for DRP were released in the early summer (May-16); whereas only 6.70% of ABO for TP and 7.47% for DRP were released in the winter (Nov-16). A similar tendency was also observed in other wetland columns.

3.2.2. Potential phosphorus fluxes (F_p)

The potential TP fluxes (Table 2) derived from the mean value of different sampling dates for each wetland ranged from 0.069 μg cm^{-2} d^{-1} (SJ) to 3.68 μg cm^{-2} d^{-1} (YT) under warmed treatments, and 0.053 μg cm^{-2} d^{-1} (SJ) to 3.10 μg cm^{-2} d^{-1} (YT) under control treatments. The repeated two-way ANOVA (Table S3) showed that experimental warming significantly increased the potential P fluxes, except for YT

($F_{1,5}$ = 3.40, p = 0.077, DRP) and SJ ($F_{1,5}$ = 2.34, p = 0.139, DRP). Additionally, seasonality (sampling dates) significantly ($F_{5,5}$ < 2.62, p < 0.001) affected the potential fluxes of TP and DRP for each wetland column (Table S3). Strong interactions between experimental warming and sampling dates were also found in the wetland columns, except for those from YT ($F_{5,5}$ = 2.42, p = 0.065, DRP), and XX ($F_{5,5}$ = 2.25, p = 0.082, DRP), suggesting that effects of experimental warming on potential P fluxes were enhanced by seasonality. For the SJ wetland column, the P concentrations in porewater were less than in the overlying water, so that negative potential fluxes were obtained (Table 2). Though solute diffusion is a rate-limiting step in P transfer from porewater into overlying water (Reddy et al., 1996), negative potential fluxes suggested that in the SJ wetland, P was quickly transported from porewater to overlying water, while P release from sediments into porewater is relatively slow and delayed. This phenomenon may be related to the lowest TP and organic carbon contents in SJ wetland sediment (Table S1).

The accumulative potential P output (APO) of TP for each wetland column under warmed treatments were found in a range of 15.8 (SJ) to 1097 μg cm^{-2} y^{-1} (YT), while the range was from 12.3 (SJ) to 907 μg cm^{-2} y^{-1} (YT) for control treatments (Table 2). For DRP, the range was 4.06 μg cm^{-2} y^{-1} (SJ) to 808 μg cm^{-2} y^{-1} (YT) under warmed treatments, and 4.18 μg cm^{-2} y^{-1} (SJ) to 677 μg cm^{-2} y^{-1} (YT) under control treatments, respectively. The statistical analysis (Table 2) showed that significant differences in APO between the two treatments occurred for the JH (p = 0.031), XZ (p = 0.002), YT (p = 0.017), XX (p = 0.002), and BY (p = 0.042) wetland columns for TP with values 77%, 53%, 21%, 65%, and 66% greater under warmed treatments, respectively. The statistical results for DRP are also shown in Table 2. The amount of potential TP output was in a decreasing order of YT>XX>XZ>BY>JH>SJ, which corresponded

371 with TP contents in six sediment samples as shown in Table S1, expect for at XX. The
372 relatively high amount of potential TP output in the XX wetland indicated the P
373 concentration gradient between porewater and overlying water was large, despite
374 relatively low TP contents in the XX sediment.
375
376 *3.3. TP analysis and P fractions in the sediments*
377 The concentrations of TP in six wetland sediments decreased in both the control
378 and warmed treatments following P transfer after an annual cycle (Table S1 & Table
379 3). However, the decreased amount of TP for warmed treatments was higher than for
380 control treatments. Taking JH as an example, 51.2 mg kg^{-1} (9.8%) of sediment TP was
381 lost under warming, compared to 37.2 mg kg^{-1} (6.3%) of P in the control.
382 For P fractions in sediments, the ratio of NH_4Cl-P to TP was very low
383 (0.12%-2.20%), while the contents of NaOH-P (Al-P) and BD-P (Fe-P) accounted for
384 a large proportion of TP for both warmed and control treatments. When subjected to
385 warming, shifts in absolute P fractions were found. The mean value of NH_4Cl-P in six
386 wetland columns increased from 6.50 mg kg^{-1} in control treatments to 10.0 mg kg^{-1} in
387 warmed treatments. The BD-P decreased under warming, ranging from 33.8 (SJ) to
388 731 mg kg^{-1} (YT); while it ranged from 37.2 (SJ) to 799 mg kg^{-1} (YT) in the control
389 (Table 3). The Student *t*-test showed that significant differences in BD-P contents (mg
390 kg^{-1}) between warmed and the control were found in JH (p = 0.008), XZ (p = 0.048)
391 and XX (p = 0.023) wetland columns. NaOH-P contents in six wetland sediments
392 generally increased under warming, especially for XZ (p = 0.014), XX (p = 0.049)
393 and SJ (p = 0.042). Comparing all P fractions, it seemed that the decrease in BD-P
394 was accompanied by an increase in NaOH-P. The ratio of NaOH-P to TP (%) is
395 consistently higher for the wetlands under warming, ranging from 53% (XX) to 22%

(YT), compared to 48% (XX) to 21% (YT) in the control.

3.4. Phosphatase activities in sediments

Generally, the experimental warming promoted the excretion of neutral phosphatase in sediments collected from those tested wetland columns (Fig. 2). The statistical analysis showed that significant differences in neutral phosphatases were found in the JH (p = 0.004), XX (p = 0.025), BY (p = 0.039) and SJ (p = 0.003) wetland columns, and the values for these sediments were 35%, 52%, 17% and 100% greater for the warmed compared to the control treatments, respectively. As for alkaline phosphatases, there was no significant difference between control and warmed treatments for the top layer of sediment from the six wetland columns.

In the SJ wetland column, neutral and alkaline phosphatases in the warmed treatment were much higher (2.0 times and 2.9 times for neutral and alkaline phosphatases, respectively) compared to those in control treatment. However, no significant differences in neutral or alkaline phosphatases were found in the YT wetland column. The SJ wetland column had the lowest TP content (346 mg kg^{-1}) and organic matter (14.6 mg kg^{-1}), while the YT had the highest TP content (2530 mg kg^{-1}) and organic matter (114 mg kg^{-1}), as shown in Table S1. Therefore, warming can drive the higher secretion of phosphatase when P contents are low in sediments.

3.5. Microbial community changes in sediments

The total amount of PLFAs and microbial community compositions in six wetland sediments are shown in Table 4. Subjected to experimental warming, the total PLFAs increased significantly (p < 0.01) in the JH, XZ, XX, and SJ wetland columns. Among these wetland sediments, total PLFAs increased from 30.0 nmol g^{-1} (SJ) to 135 nmol

421 g^{-1} (XX) in warmed treatments compared to those in control treatments. The typical
422 microbial groups, such as bacteria and fungi in JH, XZ, XX, and SJ wetland
423 sediments were much higher in warmed treatments than in control treatments ($p <$
424 0.01), except for the YT ($p = 0.795$) and BY ($p = 0.273$) columns.
425 The average ratio of bacteria to total biomass indicated by characteristic fatty acids
426 (i15:0+a15:0)/16:0 was 18% lower in warmed treatments than in control treatments (p
427 < 0.001). The relative amount of fungi indicated by the ratio of fungi to bacteria was
428 significantly ($p = 0.004$) greater in warmed treatments, varying from 0.13 (BY) to
429 0.38 nmol g^{-1} (SJ), compared to 0.14 (JH) to 0.17 nmol g^{-1} (XZ) in control treatments.
430 However, no significant difference ($p = 0.816$) was found in the ratio of aerobic to
431 anaerobic organisms indicated by the ratio of monounsaturated PLFAs to branched
432 PLFAs (Table 4). The experimental warming led to an increase in the microbial
433 biomass and a shift in the microbial community with a relative dominance of fungi
434 and a relative decline in the bacterial abundance.
435
436 *3.6. Dynamics of three fractions of organic carbon*
437 The contents of three fractions of organic carbon (HLOC, MLOC and LOC) in
438 sediments from six wetland columns are shown in Fig. 3. Under warmed treatments,
439 HLOC was generally greater except for in the XX sediment; however, there were no
440 significant differences between the two treatments for the six wetlands according to
441 Student *t*-tests. For MLOC, significantly lower values were found in BY ($p = 0.008$)
442 and JH ($p = 0.035$) sediments under warmed treatments compared to the control
443 treatments. The LOC in sediments from the six wetlands ranged from 13.4 mg g^{-1} (JH)
444 to 33.0 mg g^{-1} (YT) under warming; and from 6.13 mg g^{-1} (SJ) to 29.2 mg g^{-1} (YT) for
445 the control treatments. The statistical analysis showed that LOC under warmed

446 treatments was significantly increased by 110% (p = 0.005), 60% (p = 0.001), 85% (p
447 = 0.007), 73% (p = 0.002) and 133% (p = 0.042) for the JH, XZ, XX, BY and SJ
448 wetland columns, respectively, but was not different between treatments for YT (p =
449 0.252). The low labile organic carbon (LLOC) calculated as LOC minus HLOC and
450 MLOC (not shown in Fig. 3) significantly increased at the same rate as the changes in
451 LOC between two treatments.
452

453 # 4. Discussion

454 ## *4.1. Phosphorus flux response to experimental warming and the implications for*
455 *wetland function*

456 After periodically replacing overlying water, the equilibrium of P between sediment
457 and overlying water at the sediment-water interface was disturbed; which drove P
458 diffusing from the porewater to the overlying water, followed by the P transfer from
459 sediment into porewater. In six wetland columns, the average P concentrations in the
460 porewater were approximately one order of magnitude higher than those in the
461 overlying water (Fig. 1). Moreover, an average 31.1% greater concentration of TP in
462 the overlying water compared to a 42.7% greater concentration for porewater in six
463 wetland columns was observed under warming (Fig. 1), indicating an increased P
464 concentration gradient under warmed treatments. Solute diffusion, as a result of high
465 concentration gradients between porewater and overlying water (Bally et al., 2004;
466 Serpa et al., 2007), regulated the P transport across the sediment-water interface
467 (Serpa et al., 2007; Pages et al., 2011). For this reason, P which had accumulated in
468 the porewater could be transferred into overlying water under warming, thereby
469 creating the risk of P release under climate change in aquatic ecosystems. This is
470 indicated by the significant differences in potential P fluxes (p < 0.05) as shown in

Table 2.

On an annual time scale, the highest benthic P fluxes of the six wetlands were generally found in the summer (Jul-16; Table 1). However, the highest potential P fluxes were found in the spring (Mar-16; Table 2), suggesting that a large amount of P was not transported into overlying water, but accumulated in the porewater in this season. It seemed that the response of P in the overlying water to experimental warming was relatively slow and delayed compared to P in the porewater since solute diffusion is generally a rate-limiting step and the indirect effect of experimental warming on P in the overlying water compared to in the porewater.

For seasonal P variations, significant differences in benthic and potential P fluxes between sampling dates were found, especially for samples collected in the summer and winter. In previous studies, the highly positive benthic fluxes of soluble reactive P in the lowland Wood River Wetland, Oregon, for example, averaged 46 mg m^{-2} d^{-1} in June and August when water temperature was the highest (Duff et al., 2009). A lowland river case study indicated that P fluxes at 7.6 mg m^{-2} d^{-1} in the summer was generally higher than fluxes measured in early autumn (about 3.1 mg m^{-2} d^{-1}) (Banaszuk and Wysocka-Czubaszek, 2005). Additionally, the high temperature may facilitate the P transfer from sediments into both porewater (Table 2) and water columns (Table 1), but to a different degree. The average ABO of the six wetlands was greater by 12.9 μg cm^{-2} y^{-1} (28%) and 8.26 μg cm^{-2} y^{-1} (25%) for TP and DRP under warmed conditions, respectively. For APO, the value was greater by 54.6 μg cm^{-2} y^{-1} (29%) for TP and 33.3 μg cm^{-2} y^{-1} (24%) for DRP. Further analysis indicated that significant differences for all wetland columns between control and warmed treatments were found in APO (p = 0.034 for TP and p = 0.049 for DRP) than ABO (p = 0.051 for TP). Therefore, we can infer that the current trend of global warming

would make it more difficult to maintain the stability of wetland ecosystems. Data comparison also shows that the factors of increase on APO by experimental warming in YT (the highest value of sediment TP in Table S1) were found to be 21% for TP and 19% for DRP, while the related ranges of 29% (SJ) to 77% (JH), and 28% (XZ) to 85% (BY) were found for the rest of the tested sediments, respectively, except for DRP in SJ (Table 2). Potential P fluxes in nutrient-enriched wetlands may therefore be relatively less impacted by warming.

4.2. Mechanisms of phosphorus fluxes under warmer conditions

P fluxes between sediment and the water column in wetland environments (Fig. S4) are controlled by a combination of abiotic and biological processes (Aldous et al., 2005; Lindstrom and White, 2011), leading to sediment-water P exchanges (Grunth et al., 2008). After 14-months of incubation, TP contents in sediment decreased for both control and warmed treatments (Table 3). The warming promoted more P release, resulting in a greater decrease in TP concentrations in warmed sediments compared to the ambient control (Table S1 & Table 3). However, the detection of small changes in TP is insufficient to predict the P behaviors (Kaiserli et al., 2002) because of the high background concentrations of TP in sediments compared to those in the overlying water and porewater. As a potential source of dissolved P in the porewater, NH_4Cl-P increased in warmed treatments. The decline of BD-P in sediments may be due to changes in the redox potential of sediment. With the increase in temperature, microbial activities are enhanced which could further result in O_2 depletion and declined oxygen solubility (Grunth et al., 2008; Qian et al., 2010). The P accumulated in the sorbed form on iron oxides in the sediments may be transferred into porewater, or even transported into overlying water when temperature increases (Maassen and

Balla, 2010; Pages et al., 2011). Since BD-P (accounted for 29.4% of TP in six wetland columns under warmed treatments) was one of the main P fractions (Table 3), the experimental warming led to the P transfer at the sediment-water interface which was highly related to BD-P. NaOH-P contains not only the P bound to metal oxides Al, but the P in microorganisms, including poly-P (Banaszuk and Wysocka-Czubaszek, 2005) which is part of microbial biomass. NaOH-P was increased partly due to the adsorption of BD-P by Al hydroxides (Hansen et al., 2003) and partly because of the enhanced microbial biomass (Table 4). The experimental warming mainly led to the transformation and redistribution of P fractions within the potentially mobile P, while changes in HCl-P and Res-P were to a lesser extent.

Several studies have shown enhanced enzyme activities under experimental warming (Bell et al., 2010; Maassen and Balla, 2010; Coolen et al., 2011). In our study, experimental warming significantly increased the neutral phosphatase in the JH, XX, BY and SJ wetland columns, especially for SJ. Similar findings were reported by Sardans et al. (2006) who observed an increase in alkaline phosphatase activities (22%) for a shrubland soil in spring within a one-year period of warming. Biological phosphatase secretion is due to low P availability in soil (Zhang et al., 2007). This might explain the increase of phosphatase in the SJ wetland columns under the warmed treatment. Since microbes play a critical role in P uptake and release (Sondergaard et al., 2003; Jiang et al., 2008), slight changes in microbial biomass or community structure may affect the cycling of P in sediments. In our study, the microbial biomass (total PLFAs) increased (Table 4) under the experimental warming, which is consistent with previous investigations (Jiang et al., 2008). An increase in microbial biomass and a significant change in phosphatase were also observed in a warming and nitrogen addition experiment in an old field (Bell et al., 2010). In

addition, a significant increase (p = 0.004) in the ratio of fungi to bacteria under warmed treatments was observed in our study. The relatively higher abundances of fungi (Table 4) could increase the efficiency of P-hydrolyzing enzymes in sediment (Zhang et al., 2005; Allison and Treseder, 2008), which may help explain the relatively higher P concentrations in the porewater under a warmer condition.

Climate warming is shown to change the P transfer directions and strengths in the tested wetland sediments, but the extent of which depends on nutrient state. PLFAs and enzyme activity analysis showed no clear response to experimental warming in the nutrient-enriched treatments, such as the YT wetland column. In our study, the highest P fluxes (μg cm^{-2} d^{-1}) were always found in YT compared to other wetland columns. However, the experimental warming significantly increased the neutral and alkaline phosphatases, and microbial biomass in wetlands characterized by low-P and median-P, such as in the JH, XX and SJ wetlands. This indicates that microbial activities in the low-P and median-P sediments are more susceptible to global warming. The previous field survey (Wang et al., 2010) showed that 52% of sediments sampled from the tested wetland ecosystems were found as sediment TP contents less than or equal to that of JH (Table S1), which were commonly utilized as drinking water reservoirs and/or nature reserves. The implication is that ecological management within these regions might face more severe challenges with global warming due to microorganism-driven P biogeochemical changes.

Labile organic carbon is believed to be one of the most active fractions of soil organic carbon (Tirol-Padre and Ladha, 2004) and is sensitive to changes in environmental factors, such as soil moisture and temperature (Plante et al., 2010). Previous studies showed that the microbial activity was limited by the supply of biologically available substrates and the microbial biomass may be concomitant with

571 labile carbon contents (Allison and Treseder, 2008; Conant et al., 2011; Pages et al.,
572 2011). In our study, the LOC of sediments from five out of six wetlands increased
573 significantly between two treatments. The higher concentrations of microbial biomass
574 are most likely to be due to an increase in availability of labile organic carbon; such a
575 concussion is supported by PLFAs analysis. Similarly, the increased microbial activity
576 under warming may also accelerate the decomposition of organic matter stored in
577 sediments, thus influencing labile organic carbon in an interactive way. The average
578 total amounts of PLFAs for the wetland columns studied increased by 37% (Table 4),
579 accompanied by a 72% increase in labile organic carbon (Fig. 3C) under warmed
580 treatments. In addition, the correlation coefficient between LOC and LLOC was 0.973
581 for control treatments and 0.802 for warmed treatments, indicating that experimental
582 warming increased the contents of LOC mainly due to increases of LLOC; this might
583 be explained by decomposition of sediments since no significant difference was found
584 in the contents of HLOC.

585 Under climate change, increased CO_2 concentrations, the dynamics of carbon
586 pools in wetland sediments (Hirota et al., 2007; Allison and Treseder, 2008; Coolen
587 et al., 2011) as well as net primary production (Wania et al., 2009) may change the
588 traits of wetland processes related to plant-sediment-microorganism-water
589 interactions. Our short-term (14 month) warming experiment demonstrated that more
590 P was transferred from sediment into waterbodies, which may induce faster growth of
591 phytoplankton and hydrophytes in wetland ecosystems. Phosphorus pools among the
592 different phases at the interface of sediment and water in these wetland ecosystems
593 may be re-allocated given a higher accumulation of organic substrate. In addition,
594 biomass and C assimilation of aquatic plants (such as *A. filiculoides*) in wetlands have
595 been shown to significantly increase with elevated CO_2 and temperature (Cheng et al.,

2010). Phosphorus dynamics among soil microorganisms are generally regulated by biogenetic element balances (particularly for C-N-P), according to ecological stoichiometry (Sinsabaugh et al., 2009). A proportion of P in the aquatic phase might be 'returned' into sediment by ecological stoichiometry forces, which could offset some impacts of warming. Warming may redistribute global and regional water resources (Sen, 2009). As such, the sediment redox potential (Niedermeier and Robinson, 2007) would be changed in response to intensified fluctuations of hydrological regimes, leading to a disturbance of the P equilibrium between sediment and water (Trojanowska and Jezierski, 2011) for many tropical wetland ecosystems. Therefore, more research at a mesocosm-scale is needed to further investigate P biogeochemical behavior and corresponding P fluxes in wetlands under warming, particularly when the system is also influenced by changes in hydrology and atmospheric CO_2 concentrations in the long term.

5. Conclusion

The amount of P release from sediment to porewater, followed by the upward transfer into overlying water was generally enhanced in the six subtropical wetland columns under a warmer climate. Seasonality had a great impact on P fluxes ($p < 0.01$) with a peak in summer and trough in winter or spring. However, in response to experimental warming, the potential P fluxes (F_p) revealed a stronger and quicker build-up of P in the porewater compared to P in the overlying water, potentially leading to a high risk of subsequent P transfer from porewater into overlying water. Potential P fluxes in nutrient-enriched wetlands were relatively less impacted by experimental warming, implying that ecological management for 52% of the sampling region might be faced with more severe challenges associated with global warming.

A redistribution of potential mobile P (NH_4Cl-P, BD-P, and NaOH-P) was observed under warmed treatments. The experimental warming enhanced the microbial biomass (increased by 37%) due to an increase (by 72%) in availability of labile organic carbon. Meanwhile the microbial community tended towards a relative dominance of fungi (increased by 4.7%) and a relative decline in bacterial abundance (by 18%) with warming. Measurements of phosphatase excretion, microbial biomass and microbial community shift also showed that wetlands with low or medium P contents are more susceptible to experimental warming compared to P-rich wetlands, indicating that biogeochemical cycling of P at the sediment-water interface highly depends on the original nutrient state of a wetland.

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Figures

Fig. 1 Total phosphorus (TP) and dissolved reactive phosphorus (DRP) concentrations in the overlying water (bar chart) and porewater (line chart) in the microcosm experiment (Control: ambient temperature; Warmed: ambient temperature + 5 ℃) over the annual cycle of 2009.

Fig. 2 A) The activities of neutral (pH 7.4) and B) alkaline phosphatases (pH 8.4) in sediments collected from wetland columns in the microcosm experiment system after 14-months of incubation (Jul/16/2009). Control and Warmed represent the treatments of ambient temperature and ambient temperature +5 ℃, respectively. The abbreviations on the x-axis are the wetland sampling sites located in JinHu (JH), XiaZhuhu (XZ), YaTang (YT), XiXi (XX), BaoYang (BY), and ShiJiu (SJ), respectively.

Fig. 3 The fractionation of organic carbon (mg g^{-1} dry weight) i.e., A) highly labile organic carbon B) mid-labile organic carbon and C) labile organic carbon in sediments collected from wetland columns in the microcosm experiment system after 14-months of incubation (Jul/16/2009). Control and Warmed represent the treatments of ambient temperature and ambient temperature +5 ℃, respectively. The abbreviations on the x-axis are the wetland sampling sites located in JinHu (JH), XiaZhuhu (XZ), YaTang (YT), XiXi (XX), BaoYang (BY), and ShiJiu (SJ).

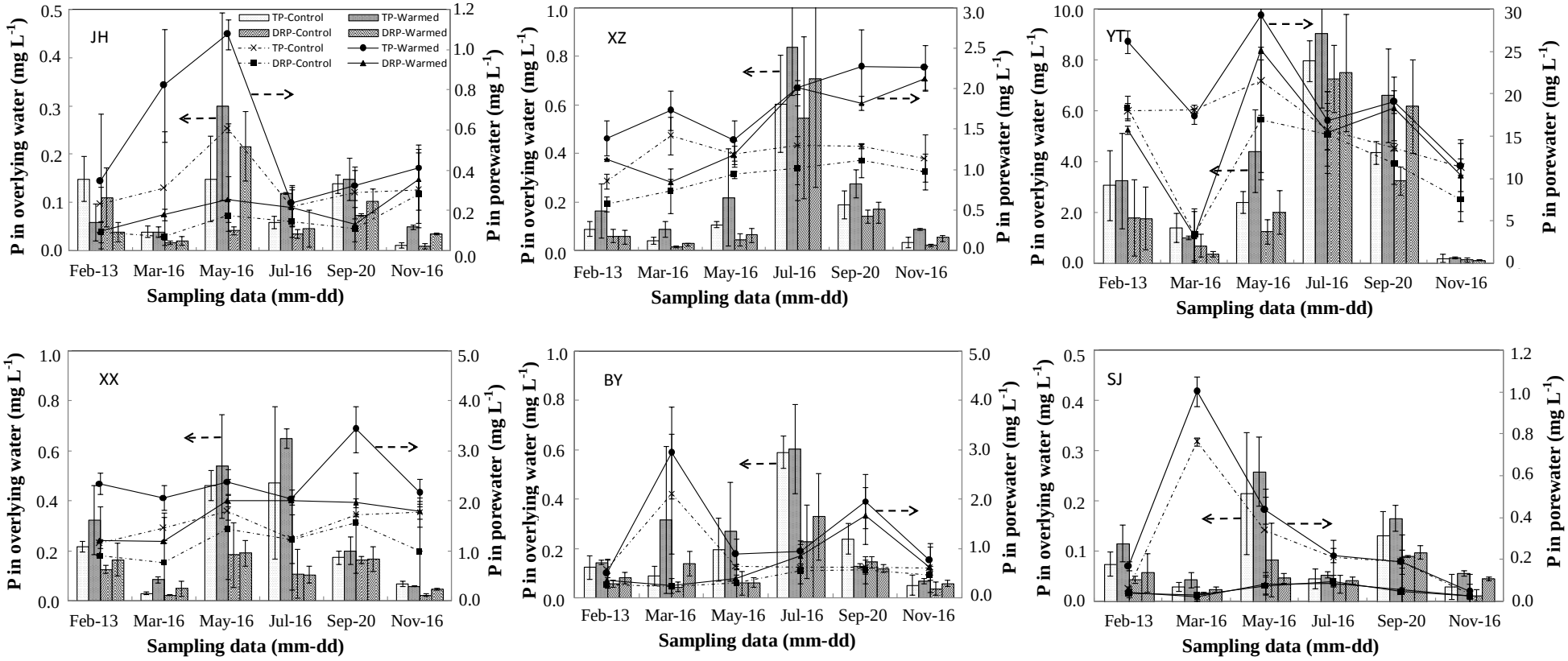
JH
XZ
YT
XX
BY
SJ
TP-Control
DRP-Control
TP-Control
DRP-Control
TP-Warmed
DRP-Warmed
TP-Warmed
DRP-Warmed
P in overlying water (mg L-1)
P in porewater (mg L-1)
Feb-13
Mar-16
May-16
Jul-16
Sep-20
Nov-16
Sampling data (mm-dd)

Figure 1

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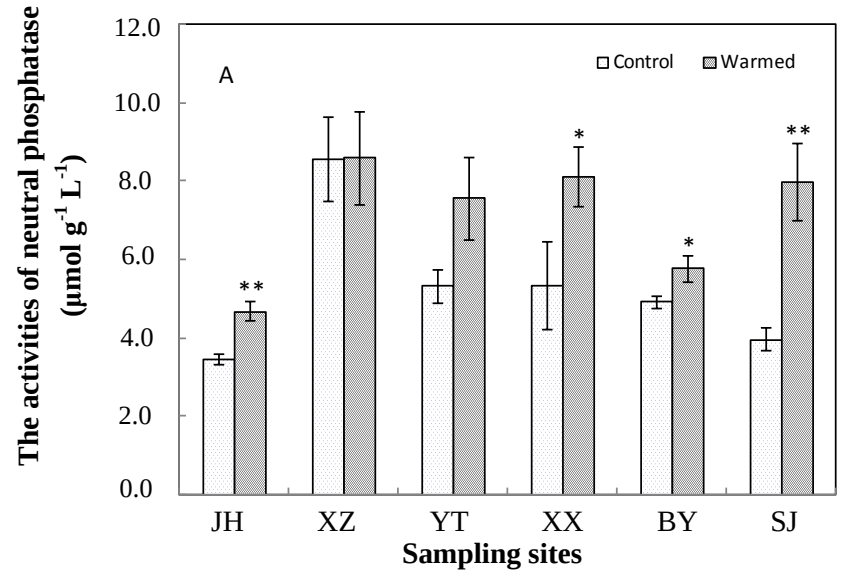

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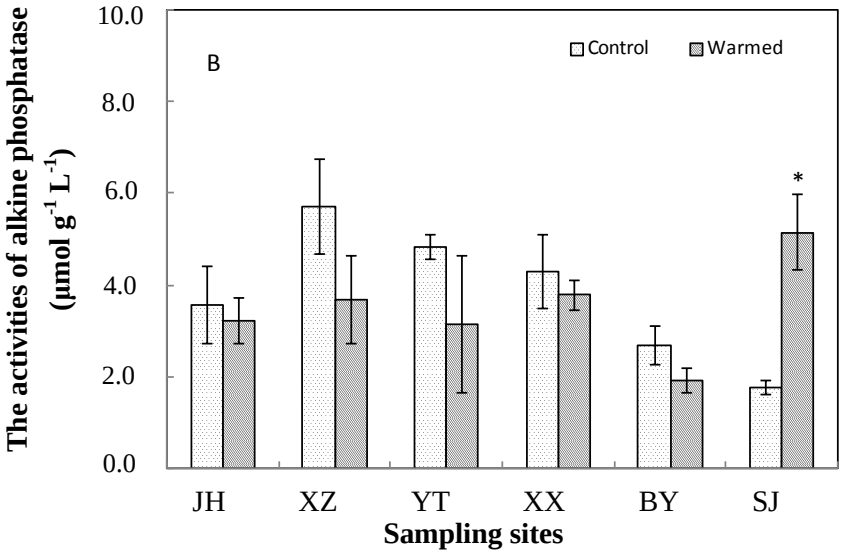

840 Figure 2

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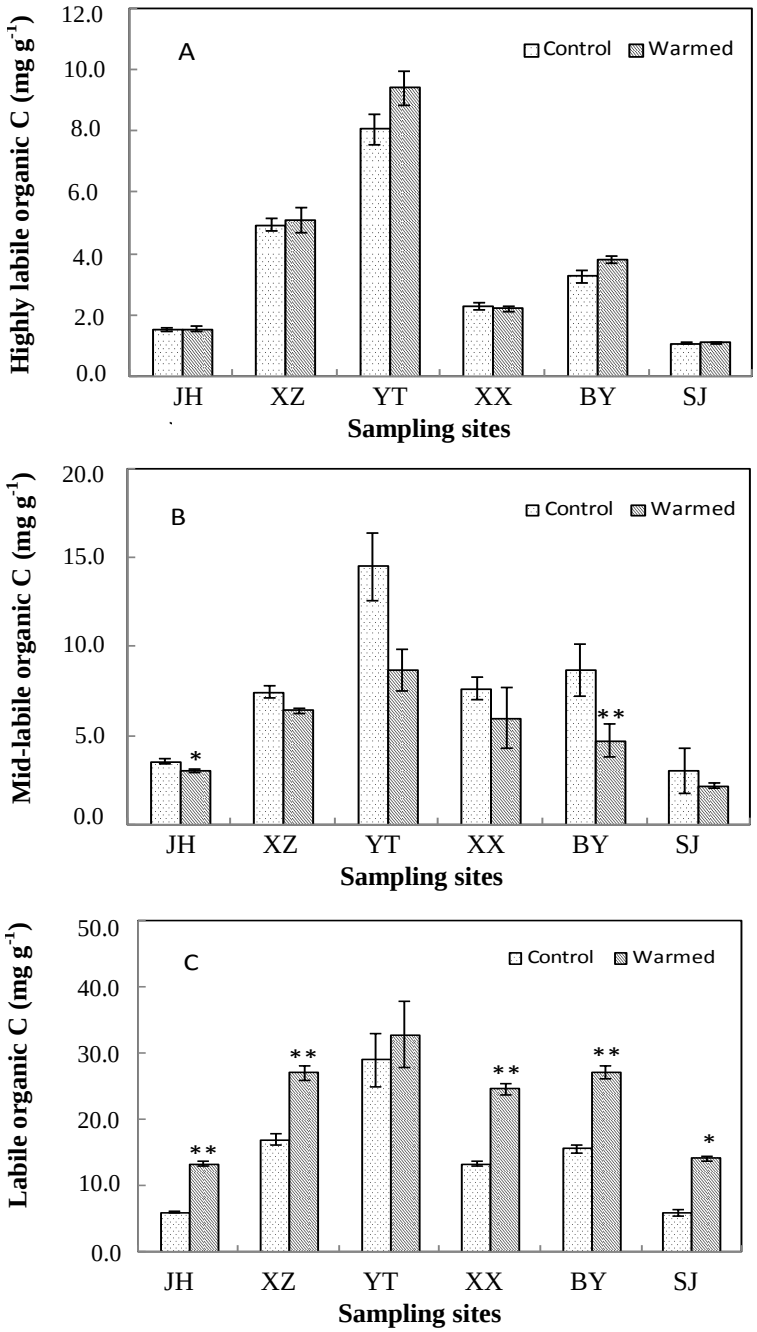

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847 Figure 3

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Table 1 Benthic fluxes of TP and DRP (μg cm^{-2} d^{-1}) at the intervals of the different sampling dates and accumulative benthic P output (ABO) (μg cm^{-2} y^{-1}) after an annual cycle of 2009 from wetland columns in the microcosm experiment (Control: ambient temperature; Warmed: ambient temperature + 5℃) expressed as mean + standard errors. *p* values evaluated significant difference in ABO between two treatments.

Sampling dates	JH wetland column		XZ wetland column		YT wetland column		XX wetland column		BY wetland column		SJ wetland column	
	Control	Warmed	Control	Warmed	Control	Warmed	Control	Warmed	Control	Warmed	Control	Warmed
	TP											
Jan-13	0.036±0.012	0.014±0.009	0.021±0.008	0.039±0.027	0.753±0.335	0.798±0.456	0.053±0.005	0.079±0.034	0.030±0.012	0.034±0.002	0.018±0.006	0.028±0.009
Mar-16	0.013±0.004	0.013±0.004	0.013±0.005	0.030±0.001	0.495±0.199	0.359±0.026	0.010±0.002	0.029±0.005	0.031±0.014	0.110±0.105	0.009±0.003	0.014±0.005
May-16	0.028±0.017	0.057±0.037	0.020±0.002	0.041±0.038	0.464±0.084	0.849±0.311	0.088±0.011	0.103±0.014	0.037±0.025	0.051±0.038	0.041±0.009	0.049±0.013
Jul-16	0.011±0.002	0.022±0.000	0.115±0.038	0.160±0.038	1.528±0.153	1.732±0.555	0.090±0.059	0.124±0.007	0.113±0.017	0.116±0.035	0.008±0.004	0.010±0.001
Sep-20	0.025±0.003	0.026±0.008	0.034±0.010	0.049±0.011	0.784±0.082	1.188±0.330	0.031±0.005	0.036±0.010	0.043±0.011	0.023±0.002	0.023±0.009	0.029±0.005
Nov-16	0.002±0.001	0.010±0.000	0.006±0.004	0.018±0.000	0.045±0.030	0.050±0.008	0.014±0.002	0.011±0.000	0.010±0.009	0.013±0.002	0.005±0.005	0.011±0.000
ABO*	6.14±1.06	8.08±1.69	12.0±1.95	19.0±3.50	223±21.7	283±47.9	16.1±2.48	21.1±2.62	14.7±2.22	17.4±4.09	5.84±1.42	7.76±0.91
p	0.077		0.015		0.051		0.031		0.179		0.053	
	DRP											
Jan-13	0.027±0.015	0.009±0.005	0.014±0.007	0.013±0.008	0.439±0.367	0.435±0.303	0.030±0.004	0.040±0.016	0.014±0.003	0.019±0.006	0.010±0.002	0.013±0.009
Mar-16	0.005±0.001	0.006±0.003	0.004±0.001	0.009±0.003	0.249±0.151	0.134±0.036	0.007±0.001	0.016±0.010	0.015±0.007	0.047±0.017	0.005±0.001	0.008±0.002
May-16	0.008±0.002	0.041±0.012	0.008±0.005	0.012±0.007	0.244±0.092	0.387±0.165	0.035±0.025	0.036±0.010	0.011±0.009	0.011±0.004	0.015±0.014	0.008±0.002
Jul-16	0.006±0.002	0.008±0.007	0.104±0.064	0.135±0.086	1.394±0.252	1.437±0.443	0.020±0.019	0.020±0.006	0.043±0.029	0.063±0.034	0.006±0.003	0.008±0.001
Sep-20	0.013±0.001	0.018±0.004	0.025±0.005	0.030±0.011	0.587±0.100	1.111±0.330	0.029±0.002	0.030±0.009	0.026±0.004	0.021±0.003	0.016±0.000	0.017±0.002
Nov-16	0.001±0.001	0.007±0.000	0.004±0.001	0.011±0.004	0.033±0.018	0.030±0.003	0.004±0.001	0.009±0.001	0.007±0.006	0.012±0.003	0.002±0.002	0.009±0.001
ABO	3.18±0.51	5.16±0.96	9.33±2.43	12.3±3.46	167±25.2	207±36.6	7.00±1.53	8.05±1.34	6.38±1.63	8.87±1.67	3.05±0.65	3.43±0.44
p	0.014		0.037		0.087		0.208		0.048		0.204	

*ABO refers to the sum of six batches of data on benthic fluxes of TP/DRP

Table 2 Potential fluxes of TP and DRP (μg cm^{-2} d^{-1}) at the intervals of the different sampling dates and accumulative potential P output (APO) (μg cm^{-2} y^{-1}) after an annual cycle of 2009 from wetland columns in the microcosm experiment (Control: ambient temperature; Warmed: ambient temperature + 5℃) expressed as mean + standard errors. *p* values evaluated significant difference in APO between two treatments

Sampling dates	JH wetland column		XZ wetland column		YT wetland column		XX wetland column		BY wetland column		SJ wetland column	
	Control	Warmed	Control	Warmed	Control	Warmed	Control	Warmed	Control	Warmed	Control	Warmed
	TP											
Jan-13	0.020±0.011	0.070±0.041	0.186±0.011	0.299±0.026	3.654±0.111	5.600±0.159	0.227±0.018	0.495±0.046	0.113±0.021	0.086±0.036	-0.004±0.002	0.013±0.003
Mar-16	0.094±0.049	0.273±0.049	0.479±0.040	0.571±0.043	5.820±0.094	5.685±0.154	0.495±0.046	0.689±0.051	0.706±0.213	0.916±0.162	0.255±0.003	0.334±0.012
May-16	0.089±0.002	0.149±0.007	0.208±0.016	0.219±0.024	3.668±0.382	4.750±0.423	0.253±0.026	0.353±0.044	0.085±0.005	0.118±0.029	0.024±0.019	0.034±0.006
Jul-16	0.031±0.009	0.022±0.008	0.133±0.047	0.226±0.003	1.493±0.217	1.495±0.328	0.147±0.018	0.267±0.002	0.005±0.031	0.063±0.022	0.031±0.008	0.032±0.003
Sep-20	0.027±0.012	0.032±0.007	0.197±0.002	0.359±0.041	1.654±0.015	2.237±0.118	0.278±0.011	0.582±0.064	0.069±0.015	0.329±0.057	0.010±0.002	0.004±0.005
Nov-16	0.060±0.019	0.075±0.012	0.227±0.030	0.445±0.028	2.287±0.290	2.296±0.095	0.351±0.073	0.431±0.026	0.111±0.005	0.140±0.026	0.001±0.002	-0.002±0.004
APO*	16.3±4.63	28.8±5.62	70.4±7.46	107±9.02	907±62.0	1097±71.5	85.3±9.80	141±12.0	44.7±11.3	74.0±15.0	12.3±2.1	15.8±1.6
p	0.031		0.002		0.017		0.002		0.042		0.067	
	DRP											
Jan-13	-0.005±0.006	0.014±0.000	0.126±0.018	0.262±0.012	4.046±0.179	3.420±0.055	0.188±0.004	0.252±0.041	0.050±0.002	0.065±0.019	-0.001±0.001	-0.002±0.002
Mar-16	0.018±0.003	0.056±0.005	0.251±0.018	0.285±0.048	0.953±0.523	0.976±0.522	0.259±0.022	0.396±0.031	0.062±0.007	0.039±0.027	0.005±0.004	-0.001±0.003
May-16	0.026±0.008	0.008±0.011	0.171±0.003	0.212±0.004	3.012±0.673	4.434±1.377	0.239±0.043	0.347±0.074	0.046±0.004	0.062±0.007	-0.002±0.003	0.007±0.004
Jul-16	0.021±0.007	0.032±0.009	0.090±0.003	0.250±0.038	1.530±0.356	1.526±0.461	0.214±0.017	0.365±0.010	0.061±0.002	0.098±0.024	0.011±0.002	0.008±0.001
Sep-20	0.006±0.000	0.005±0.008	0.174±0.016	0.296±0.019	1.533±0.213	2.176±0.334	0.251±0.013	0.323±0.013	0.076±0.028	0.278±0.059	-0.008±0.004	-0.008±0.001
Nov-16	0.056±0.015	0.066±0.016	0.196±0.023	0.424±0.023	1.519±0.267	2.106±0.433	0.198±0.026	0.360±0.041	0.087±0.010	0.114±0.045	0.003±0.002	-0.004±0.001
APO	6.74±2.13	8.92±2.72	53.5±4.20	68.3±7.15	677±116	808±176	69.9±6.83	105±11.2	20.6±2.95	38.1±9.80	4.18±0.78	4.06±0.70
p	0.326		0.027		0.333		0.005		0.031		0.477	

*APO refers to the sum of six batches of data on potential fluxes of TP/DRP

Table 3 TP analysis and P fractions (mg kg^{-1} dry weight) in sediments from six wetland columns (JH, XZ, YT, XX, BT, SJ) in the microcosm experiment (Control: ambient temperature; Warmed: ambient temperature + 5 ℃) after 14-months of incubation (Jul/16/2009) expressed as mean + standard errors.

Samples		TP	Potentially mobile phosphorus			HCl-P	Res-P (calculated)
			NH_4Cl-P*	BD-P	NaOH-P		
JH	Control	542±39.0	1.19±0.03	95.4±6.77	198±16.5	146±29.0	101±7.27
	Warmed	528±44.5	1.23±0.04	68.3±8.82	218±18.9	136±18.2	104±12.0
XZ	Control	861±66.2	1.30±0.28	422±31.1	251±23.1	81±10.0	105±8.1
	Warmed	817±41.3	1.85±0.37	354±34.7	310±15.3	60±4.12	91±5.75
YT	Control	2418±108	32.9±5.88	799±63.7	498±28.3	978±27.8	110±21.1
	Warmed	2398±91.5	53.1±9.93	731±67.7	528±11.7	949±33.5	137±19.2
XX	Control	491±42.27	1.44±0.23	144±18.7	235±9.33	54±4.48	57±9.17
	Warmed	488±24.2	1.96±0.56	103±12.2	256±11.0	51.6±2.14	66±12.9
BY	Control	769±38.3	1.69±0.17	281±23.6	329±16.7	37.6±2.50	120±15.3
	Warmed	721±31.8	2.10±0.51	258±29.5	325±10.0	50.4±9.85	86±13.4
SJ	Control	339±14.4	0.52±0.10	37.2±4.83	117±6.04	39.0±4.17	145±13.1
	Warmed	315±5.27	0.38±0.08	33.8±3.62	133±8.78	33.3±1.94	114±6.11

*: NH_4Cl: loosely sorbed-P; BD-P: P forms sensitive to low redox potential such as P bound to Fe(III) hydroxides; NaOH-P: containing organic P (such as phosphomonoester, poly-P) and Al oxides bound P (i.e., Al-P); and HCl-P and Res-P: Ca bound P (i.e., Ca-P) and residual inert P.

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865 **Table 4** The effect of temperature regime on the total amount of PLFAs (nmol g^{-1}) and typical microbial groups (nmol g^{-1}) and the ratios of

866 characteristic fatty acids (i15:0+a15:0)/16:0, monounsaturated PLFAs to branched PLFAs, and fungal to bacterial fatty acids in the wetland

867 sediments in the microcosm experiment system after 14-months of incubation (Jul/16/2009). Control and Warmed represent the treatments

868 of ambient temperature and ambient temperature +5 ℃, respectively.

Microbial groups and ratios	JH		XZ		YT		XX		BY		SJ	
	Control	Warmed	Control	Warmed	Control	Warmed	Control	Warmed	Control	Warmed	Control	Warmed
Total PLFAs	37.6	51.2	60.3	95.0	272	260	64.8	135	208	205	11.1	30.0
Bacteria	22.5	24.0	38.6	48.1	157	112	42.5	60.0	141	115	4.83	12.0
Fungi	3.20	5.00	6.66	11.0	25.2	36.0	6.10	21.0	19.6	15.0	0.72	4.60
(i15:0+a15:0)/16:0	0.54	0.42	0.49	0.45	0.67	0.46	0.67	0.47	0.53	0.67	0.50	0.29
Fungi/bacteria	0.14	0.21	0.17	0.23	0.16	0.32	0.14	0.35	0.14	0.13	0.15	0.38
Monounsaturated/branched	1.28	2.04	2.82	2.08	1.66	2.23	1.68	2.40	2.89	1.75	1.91	1.68

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