Concentration Dynamics and Biodegradability of Dissolved Organic Matter in Wetland Soils Subjected to Experimental Warming

Hang Wang[1], Joseph Holden[2], ZhiJian Zhang[1]*, Meng Li[1], and Xia Li[1]

1. College of Natural Resource and Environmental Sciences, China Academy of West Region Development, ZheJiang University, Yuhangtang Avenue 688, HangZhou, ZheJiang Province, 310058, China.
2. water@leeds, School of Geography, University of Leeds, Leeds, LS2 9JT, UK. Tel: +44 113 343 3317; j.holden@leeds.ac.uk.

*: Corresponding author: ZhiJian Zhang

College of Natural Resource and Environmental Sciences, China Academy of West Region Development, ZheJiang University, Yuhangtang Avenue 688, HangZhou, ZheJiang Province, 310058, China

Tel: +86 571 8697 1854; Fax: +86 571 8697 1719

Email: zhangzhijian@zju.edu.cn

Abstract

Dissolved organic matter (DOM) is the most bioavailable soil organic pool. Understanding how DOM responds to elevated temperature is important for forecasting soil carbon (C) dynamics under climate warming. Here a 4.5-year field microcosm experiment was carried out to examine temporal DOM concentration dynamics in soil pore-water from six different subtropical wetlands. Results are compared between control (ambient temperature) and warmed (+5^{o}C) treatments. UV-visible and fluorescence spectroscopy was performed to reveal DOM structural complexity at the end of the warming incubation. Elevated temperature resulted in initially (1 to 2.5 years) high pore-water DOM concentrations in warmed samples. These effects diminished over longer time periods, which alleviated C loss in dissolved forms under sustained warming. Of the spectral indices, the specific UV absorbance at 280 nm and the humification index were significantly higher, while the signal intensity ratio of the fulvic-like to humic-like fluorescence peak was lower in warmed samples at the end of the study period, compared to the control. Fluorescence regional integration analysis suggested that warming consistently enhanced the contribution of humic-like substances to DOM composition for all tested wetlands. In more mineral-rich wetland soils characterized by low organic contents, the protein-like and soluble microbial byproduct-like substances in DOM were selectively lost in warmed samples. The shrinking of the fulvic-like fluorescence peak under warming compared to the control was mainly observed for organic-enriched soils with a shift in the center position of humic-like peak towards a longer emission wavelength. These spectral fingerprints implied a declined fraction of readily available substrates in DOM allocated to microbial utilization in response to 4.5 years of warming. As a negative feedback, decreased DOM biodegradability may have the potential to counteract initial DOM increases and alleviate C loss in water-saturated wetland soils.

Keywords: dissolved organic matter, wetland, fluorescence, soil carbon, microbial utilization, soil warming

Highlights

1. Stimulatory effect of elevated temperature on DOM release was short-lived.

2. Experimental warming increased DOM humicity and thus decreased its degradability.

3. Different soil types with organic contents had distinct fluorescence fingerprints.

4. Integrated and cumulative fluorescence indices were suitable for determining DOM character.

1. Introduction

Dissolved organic matter (DOM) is a heterogeneous mixture of organic compounds ranging from simple, short-chain molecules to complex fulvic and humic substances leached from soils (Stutter et al., 2007). Though it only represents a small proportion of total soil organic matter in both terrestrial and aquatic ecosystems, DOM links various ecological compartments including soils to water bodies, serves as a crucial indicator of biogeochemical responses to disturbance, and provides carbon (C) and energy for microbial metabolism (Bolan et al., 2011; Kalbitz et al., 2000; Wilson and Xenopoulos, 2009). The average global surface temperature has increased by 0.74^{o}C since 1850 and is likely to increase by another 1.1-6.4^{o}C by the end of this century (Solomon et al., 2007). Wetlands are globally important carbon stores, and many are thought to be highly sensitive to climate change (Erwin, 2009) but we know little about DOM concentration dynamics and the nature of change in DOM properties under warming for a range of wetland types including subtropical wetlands.

Rising temperatures accelerate the microbial decomposition rates of soil organic matter. A key issue is whether the balance of the net soil organic matter store shifts as warmer temperatures may also mean more production of soil organic matter from biotic residues. It is unclear for many wetland systems whether accelerated C loss associated with warming is a transitory phenomenon with almost unchanged soil organic matter contents or whether it is persistent with a net of loss of C from the soil store which is released as CO_2 to the atmosphere (Bengtson and Bengtsson, 2007; Kirschbaum, 2004) and in aquatic forms (Kalbitz et al., 2000; Mulholland, 1997). Data from long-term field warming incubations for mid-latitude hardwood forest soils has demonstrated that the stimulatory effect of rising temperature on increased CO_2

emission rates evident in first few years of warming was reduced so that CO_2 dropped back to similar rates to those before the elevated temperature (Kirschbaum, 2004; Melillo et al., 2002). The reason for long-term reductions in CO_2 emission from wetland soils (after an initial emission increase) was thought to be due to the depletion of available substrate indicating that substrate utilization by microbes is a key mechanism. Better understanding of such processes will aid predictions of soil C cycling dynamics under climate change.

Comparing the soil solid phase and pore-water, DOM in pore-water is probably the most bioavailable pool of soil organic matter (Bolan et al., 2011). The microbial utilization of DOM is controlled by its bioavailability and biodegradability, both of which strongly influence the fate of soil C stocks through influencing microbial feeding and functional physiology (Marschner and Kalbitz, 2003). Increased temperature favors desorption of high-affinity compounds binding to minerals and release of occluded organic matter from soil aggregates (Conant et al., 2011) which enhance pore-water DOM concentrations and thus the substrate bioavailability. Kalbitz et al. (2000) showed, in a review focused on DOM dynamics in soils, that nearly all the results of short-term laboratory studies suggested that a rising temperature may result in increased pore-water DOM concentrations. In field studies, however, multiple factors simultaneously affect DOM concentrations (Stutter et al., 2007), leading to the DOM pool varying with season (Stutter et al., 2007) and soil type (Kalbitz et al., 2000). It is still unclear whether increased pore-water DOM concentrations will persist under more sustained field warming (rather than in short-term studies), given the microbially-mediated decomposition of soil organic matter and the variable levels of stability between different soil organic fractions (von Lutzow and Kogel-Knabner, 2009).

Understanding DOM utilization is limited if we ignore its chemical character related to substrate biodegradability (Kujawinski, 2011). The biodegradability of DOM is strongly affected by its structural complexity (Fellman et al., 2008). The fractions such as low molecular weight monomers with lower aromaticity and less condensed structure can be directly assimilated by microbes, while high molecular weight compounds need to be first broken down, or depolymerized to obtain energy contained within (Marschner and Kalbitz, 2003). DOM with intrinsically low quality usually contains a large proportion of stable aromatic structures, such as lignin compounds, which is more resistant to degradation (Stutter et al., 2007). As a result, decreased DOM biodegradability constrains substrate utilization and further influences C uptake, retention and export (Battin et al., 2008) even without changed DOM concentrations. There has been some DOM characterization work in peatlands subjected to degradation and restoration (Glatzel et al., 2003) which has shown that DOM composition affects CO_2 efflux from peatlands and that DOM composition is also driven by respiration and CO_2 efflux. Work on afforested peatlands showed that peat drying alters the DOM composition with less aromatic and lower molecular weight material (Baker et al., 2008). Seasonal dynamics in DOM investigated by Huang and Chen (2009) for wetlands in the Neponset River Watershed of eastern Massachusets suggested that higher temperature in summer and fall could lead to higher values in fluorescence spectrum intensities of chromophoric DOM compared to those in winter and early spring. So far, information about DOM chemical character in the pore-water of wetland soils under sustained warming is extremely limited, which impairs our understanding of likely wetland soil C cycling in the future.

Spectroscopy can be used to describe the quality of DOM since the optical properties of a

chromophoric group are closely related with the chemical and structural character of organic matter (Kothawala et al., 2012). As a highly-sensitive tool, fluorescence spectroscopy allows identification of different compounds belonging to specific regions, and helps evaluate the humicity of water samples (Chen et al., 2003; Fellman et al., 2010). Strong signal intensities of protein-like and soluble microbial byproduct-like fluorescence would suggest that the DOM contains a large hydrophilic fraction, such as carbohydrates, organic acids and proteins of relatively high biodegradability. Enriched aromatic and hydrophobic structures in DOM related to terrestrial-derived humic-like fluorescence indicate an increase in water humicity. These signatures combined with informative spectral indices from integrated UV-visible absorbance and fluorescence measurements provide a basis for estimating DOM biodegradability (Wilson and Xenopoulos, 2009).

In this study, a real-time temperature controlled incubation system (Zhang et al., 2012) was developed outdoors in May 2008 simulating warming scenarios to investigate the dynamics of soil pore-water DOM concentration and its chemical character over 4.5-years of incubation. UV-visible and fluorescence spectroscopy were used to distinguish different classes of DOM character. Six subtropical wetlands covering a broad gradient of soil organic matter contents (14.6 to 114 g kg^{-1} dry soil, **Table 1**) were selected, given the potentially high variability of pore-water DOM concentrations in wetlands. The objectives of this study were to: (i) test whether DOM concentrations were persistently higher in warmed samples compared to the control during 4.5-years of experimental warming; (ii) test whether warming induced changes in DOM chemical character after 4.5-years of incubations; (iii) test whether there were distinct differences in DOM response to warming between wetland soil types and (iv) to investigate

why any changes in DOM occurred.

2. Material and methods

2.1. Microcosm configuration and sample description

A custom-built novel microcosm was used to simulate climate warming (Zhang et al., 2012). The microcosm involved samples being kept at current ambient temperature conditions (control) and simulated warming conditions which were continuously 5^{o}C above ambient temperature (warmed). Specifics regarding the configuration and corresponding operation of this microcosm system have been reported previously (Zhang et al., 2012). The microcosm maintained hydrological characteristics and a humid habitat for microbial growth, offering a high resolution temperature comparison, good repeatability, and the capability to simulate warming conditions with temperature of both the control and warmed treatments 'naturally' varying on a daily and seasonal basis. Transparent PVC wetland columns filled with selected wetland soils (20 cm in depth) and corresponding overlying water (20 cm in depth) were put into the microcosm system in May 2008 and have been in continuous operation since then. The details for preparing the wetland columns (with 6 replicates for each wetland site) were described previously (Zhang et al., 2012).

Samples were taken from study sites located in the southern region of the Taihu Lake Basin within the delta of the Yangtze River, in China. Six wetlands, with shallow water bodies of 0.8-1.5 m in depth, differing in land use and nutrient status were selected (**Table 1**). In brief, YaTang riverine (YT) wetland is a polluted duck farm, while XiaZhuhu (XZ) wetland is threatened by aquaculture and anthropogenic nutrient inputs. The soils in YT and XZ have

significantly higher organic matter, nutrient (i.e., phosphorus and nitrogen) and water contents compared to others (**Table 1**). The wetlands named as BaoYang (BY), XiXi (XX), JinHu (JH), and ShiJiu (SJ) are generally preserved for tourism and used as water reservoirs, typical of recovered wetlands. SJ was formerly a paddy field with the lowest organic matter among the six studied wetlands.

2.2. Non-destructive sampling for water chemical analysis

For soil pore-water sampling, a soil solution sampler (0.5 μm porous polyacrylonitrile hollow fiber, Chinese Academy of Sciences, Nanjing) described by Song (2003) was horizontally embedded into the soil in each column at a fixed depth of 5 cm. About 30 mL of pore-water was sampled from each wetland column on seven occassions (both winter and summer) of between July 2009 and December 2012 inclusive) during 55 months of incubations for DOM concentration analysis. At the end of the incubation (December, 2012), the sampled pore-water was also used for UV-visible and fluorescence spectral analysis. All of the following measurements were conducted after filtration of pore-water through a 0.45 μm filter.

DOM concentration. Dissolved organic C was analyzed using a Shimadzu TOC 5000 analyzer (Shimadzu Scientific Instruments, Columbia, USA) after acidifying (10% HCl) and purging with inert gas to remove any inorganic C. The quantification of organic matter concentration is usually based on the C content. Therefore, we converted the dissolved organic C into DOM throughout the manuscript by multiplying by a factor of 1.72, in order to be consistent with spectroscopic analysis, which includes the whole DOM fraction (Kothawala et al., 2012).

UV-visible spectra. UV-visible absorbance spectra were measured spanning 200 to 400 nm at 0.2 nm intervals using a UV-2550 spectrophotometer (SHIMADZU Corporation, Japan). Samples were put into a 1 cm quartz cuvette and distilled water was used as the blank. Specific UV absorbance (SUVA), including $SUVA_{254}$ and $SUVA_{280}$ were calculated as the absorbance at the wavelength of 254 nm and 280 nm normalized for dissolved organic C concentration, respectively (Weishaar et al., 2003). The slope ($S_{280-400}$) of the absorbance spectrum curve was calculated for the spectrum region between 280 and 400 nm (Stedmon et al., 2000). The A_{253}/A_{203} value is the ratio of absorbance at 253 and 203 nm (He et al., 2013). Of four UV-visible spectral indices ($SUVA_{280}$, $SUVA_{254}$, $S_{280-400}$, and A_{253}/A_{203}), $SUVA_{280}$ and $SUVA_{254}$ are strongly correlated with aromaticity and molecular weight (Chin et al., 1994; Weishaar et al., 2003). For UV-visible spectrum curves, the absorption is generally the highest in the ultraviolet region and decreases to near zero in the red region. Therefore, $S_{280-400}$ is used to evaluate how steep the absorption decreases with increasing wavelength (Stedmon et al., 2000). A high A_{253}/A_{203} ratio indicates the presence of polar functional groups, such as hydroxyl, carbonyl, and carboxyl on the aromatic ring, while a low ratio is related with the substitution with aliphatic and methylene groups on the aromatic ring, and thus an increase in DOM humicity (Minero et al., 2007).

Fluorescence spectra. For fluorescence intensity measurement, three dimensional excitation-emission matrices (3D EEM) were studied using a Hitachi F-4500 fluorescence spectrophotometer (Hitachi High-Technologies Corporation, Japan), and the corresponding contour map was visualized by Sigmaplot 12.0. The excitation wavelengths spanned from 200 to 450 nm, and 300 to 600 nm for emission wavelengths with both at 5 nm increments.

Excitation and emission slit widths were set to 2.5 nm with default values for integration time. Before measurement, manufacturer supplied correction factors were used to correct excitation and emission intensities for instrument-specific biases. The raw data were corrected for inner-filter effects due to the absorption of incident and emitted light by colored organic matter suspended within the sample cuvette using absorbance measurements (Ohno, 2002) after normalizing for dissolved organic C concentration. Raman scatter effects of fluorescence were removed by dividing by the Raman area of a Milli-Q water integrated at an excitation of 350 nm, and over an emission range of 380 to 420 nm (Lawaetz and Stedmon, 2009). The fluorescence index (FI) was calculated as the ratio of emission intensity at 470 and 520 nm at fixed excitation wavelength of 370 nm. The freshness index (β: α) was calculated as the ratio of emission intensity at 380 nm divided by the intensity maximum between 420 and 435 nm at fixed excitation wavelength of 310 nm. The humification index (HIX) is the integrated area under spectra at emission wavelengths from 435 to 480 nm divided by the sum of the area at emission wavelengths from 435 to 480 nm and 300 to 345 nm at a fixed excitation wavelength of 254 nm (Ohno, 2002). Fluorescence peak A, which is associated with fulvic-like components, and peak C, which is attributed to humic-like substances falling within the certain EEM regions (Chen et al., 2003) were acquired by instrument automated "peak-picking" by scrolling to peak locations on the 3D EEM and finding the fluorescence peak intensity. The ratio of fluorescence signal intensity of peak A to peak C (I_A/I_C) was calculated. The above fluorescence spectral indices describe the different aspects of the DOM chemical character as shown by Wilson et al. (2009). Briefly, FI is strongly correlated with degree of structural conjugation, and β: α is an indicator of autochthonous C inputs associated with

microbial-originated sources of DOM. HIX increases with increasing aromaticity, while I_A/I_C is negatively related to the degree of DOM humicity.

The fluorescence regional integration (FRI) technique was adopted to further analyze the EEM spectra. According to the approach described by Chen et al. (2003), each EEM spectrum was divided into five regions (Region I-V). The integrated volume beneath each region was quantitatively calculated in a unit of AU-nm^2-[mg/L C]$^{-1}$ after being normalized to dissolved organic C concentration using MATLAB R2010b. We divided the calculated volume by the relative region area (nm^2) in order to reduce the effects of secondary or tertiary excitation-emissions responses on the extension of fluorescence peak shoulders at longer wavelengths. The percent fluorescence response ($P_{i,n}$ which refers to the proportion of above area-normalized values in Region i to the entire Region in EEM) was represented in this study. The operationally defined five regions are as follows (Chen et al., 2003): the regions under excitation (Ex) and emission (Em) coordinates (~250/~380, Ex/Em) at both shorter excitation and emission wavelengths represent aromatic proteins (i.e., tyrosine and tryptophan, Region I and II respectively); the regions under coordinates (~250/380~, Ex/Em) at shorter excitation wavelengths and longer emission wavelengths are associated to fulvic acid-like materials (Region III); the regions at the intermediate excitation wavelengths and shorter emission wavelengths (250-280/~380 nm, Ex/Em) are related to soluble microbial byproducts (Region IV); the regions at both longer excitation and emission wavelengths (280~/380~, Ex/Em) correspond to humic-like substances (Region V).

2.3. Statistical analyses

A preliminary three-way ANOVA was used to examine the total differences in DOM concentrations varied with site (wetlands), treatment (control vs. warmed), and sampling time as three factors. In a specific sampling time, differences in DOM concentrations, spectral indices and integrated volume beneath specific regions were examined by a two-way ANOVA with site and treatment as two factors. If the ANOVA result was significant ($p < 0.05$), Student-Newman-Keuls (S-N-K) was further used for multiple comparisons between groups. Student's *t*-tests were used to test for differences in DOM concentrations and chemical character for each specific wetland between treatments.

3. Results

3.1. DOM concentration dynamics

DOM concentrations during 2009 to 2012 varied significantly with site ($p < 0.001$), treatment ($p < 0.001$), and sampling time ($p < 0.002$). Multiple comparisons showed that the mean DOM concentrations were the highest in YT, and the lowest in SJ ($p < 0.001$) among the six wetlands. Warming impacts on DOM concentrations depended on sampling time as revealed by significant ($p < 0.001$) interaction effects between treatment and sampling time. On average the treatment samples had DOM which was 14.5% greater than the controls in July 2009 ($p = 0.001$, **Fig. 1A**). The maximum differences between treatments as a whole were observed in samples taken in November 2010 (26.8% higher in warmed relative to the control, **Fig. 1H**). The two winter sampling occassions after November 2010 (February 2011, December 2012) both indicate declining winter DOM concentration differences between treatments (**Fig, 1H**) with the 7.92% difference in December 2012 between controls and treatments being

insiginificant (p = 0.579, **Fig. 1G**) when the DOM dataset is considered as a whole. Similarly, after August 2010 the differences in summer samples between treatments were successively smaller in 2011 and 2012 (July 2012 only 4.4% and not significant (p = 0.611)). Warming effects on DOM concentrations were site-specific (p = 0.067). For all sampling occassions from July 2011 onwards differences in DOM concentrations between treatments for each wetland were insignificant except for BY (**Fig. 1E-G**). YT and XZ, characterized as organic-enriched wetlands, showed the strongest responses of DOM release to warming in the initial years of the experiment (2009-2010, **Fig. 1A-E**), and then the warming effects on DOM concentration gradually became smaller in the proceeding years (2011-2012, **Fig. 1F, G**). For SJ and JH, characterized as organic-poor wetlands, there were no significant differences in DOM concentration between treatments throughout all observed years (**Fig. 1A-G**).

3.2. UV-visible absorbance and spectral indices

UV-visible absorbance of DOM at 254 nm (A_{254}) was consistently higher (p < 0.001) in warmed samples relative to the control at the end of the experiment, especially for XZ and YT (**Table 2**). The patterns of differences were similar for $SUVA_{280}$ (p = 0.010) and $SUVA_{254}$ (p = 0.008). The ratio of A_{253}/A_{203} was lower (p = 0.026) by 52.3% under warming when comparing the mean values from six wetlands between treatments (**Table 2**). Decreased (p < 0.05) $S_{280\text{-}400}$ values between treatments were only observed in XZ .

3.3. Fluorescence specific components and spectral indices

Of the fluorescence spectral indices (FI, β: α, HIX, and I_A/I_C), FI and β: α remained unchanged between treatments for all samples (**Table 3**). HIX in warmed samples was higher (by 22.6%,

$p < 0.001$) than those in the control by comparing the mean values from six wetlands between treatments, while I_A/I_C was lower ($p < 0.001$) under warming, especially for XZ and YT (**Table 3**). Compared to UV-visible spectral indices in **Table 2** as well as FI and β: α in **Table 3**, HIX had a relatively low coefficient of variation both within-samples (0.38% to 8.86%, replicates for each wetland) and between-samples (4.68%, among six tested wetlands). In contrast, I_A/I_C varied greatly among these wetlands ($p < 0.001$, S-N-K test) from 1.76 (JH) to 0.67 (YT) (**Table 3**).

For FRI analysis, the $P_{V,n}$ values in region V were consistently higher ($p < 0.05$ or 0.01) in all warmed samples, ranging from 6.81 (JH) to 14.2 (YT), compared to the control, which varied from 5.42 (SJ) to 11.1 (YT) (**Table 4**). For other regions, $P_{I,n}$ and $P_{II,n}$ in region I and II were marginally ($p = 0.098$ and 0.072, respectively) decreased under warming in all tested wetlands as a total, and were especially lower for warmed SJ and JH samples relative to the control (**Table 4**). Meanwhile, $P_{III,n}$ in region III were exclusively lower ($p < 0.05$) in warmed XZ and YT samples, and $P_{IV,n}$ in region IV were lower ($p < 0.05$) only in warmed SJ samples when compared to the control. Similar to HIX, $P_{i,n}$ values had a relatively low coefficient of variation within-samples (0.25% to 30.4%). For between-samples, $P_{V,n}$ in YT and XZ were much higher than those in SJ and JH, while in contrast $P_{I,n}$ were the lowest in YT among tested wetlands (**Table 4**). To confirm these differences, we compared the EEM of SJ samples (as an example) with YT samples (**Fig. 2**). For SJ samples, the soluble microbial byproduct-like fluorescent components (with center peak located in 275/305, E_X/E_M, in Region IV), and the protein-like fluorescent components (with center peak located in 230/335, E_X/E_M, in Region II) in the control (**Fig. 2A**) were not present or overlapped in the warmed samples (**Fig. 2B**). For

YT samples, the center position of the humic-like peak (i.e., peak C) shifted from E_X/E_M, 325/410 (control, **Fig. 2C**) to E_X/E_M, 330/425 (warmed, **Fig. 2D**), a shift of the emission spectra toward longer wavelengths, which is also defined as a red shift in fluorescence spectrum. Such shifts in the position of humic-like peak were also observed in XZ (**Table 3**). Meanwhile, the fulvic-like peak (i.e., peak A) area in Region III was more contracted in the warmed (**Fig. 2D**), compared to the control (**Fig. 2C**).

4. Discussion

4.1. Effects of experimental warming on DOM concentration dynamics

YT and XZ samples had relatively high organic contents and it is likely that their substrates were capable of more leaching at elevated temperature from the soil solid-phase to pore-water, and had stronger susceptibility to experimental warming compared to other wetland soils over the first 2.5-years of the experiment. In contrast, no significant differences in DOM concentrations between treatments throughout the incubation occurred for organic-poor wetland soils (SJ and JH). When organic matter in soils is relatively low, physical disconnection and spatial inaccessibility is enhanced between soil microbes and substrates (Schmidt et al., 2011). Elevated temperature may, therefore, not be able to promote desorption of soluble compounds bound to minerals and release occluded organic matter from soil aggregates in these soils. Moreover, the more unevenly distributed organics in soil particles and water-saturated pore spaces (Schmidt et al., 2011) may have also enhanced the variability within replicate samples of SJ and JH, leading to statistical insignificance. As a result, organic-poor wetland soils were less responsive to warming in the short-term (1-2.5 years).

However, under sustained warming (4.5-years), there were no significant differences in DOM concentrations between treatments for all but one (BY) of the wetlands.

The impacts of a given elevated temperature will also depend on the ambient temperature, therefore, our +5^{o}C soil warming on the winter samples (such as samples taken in November 2010, February 2011, and December 2012) when ambient water temperatures are around 10^{o}C did not have the same impacts as the warming on the summer samples (taken in July or August of 2009-2012) when ambient water temperatures are around 26^{o}C. Warming effects on DOM release may be greater (when comparing the difference between ambient and warmed treatments) during winter months as indicated by our November 2010 results, suggesting treatment conditions (+5^{o}C warming) could sometimes be a larger factor when ambient temperatures are initially lower and such seasonal factors need to be incorporated into long-term warming experiments on subtropical wetlands. Despite this, our summer patterns of DOM production in different years showed that differences in DOM concentrations between treatments diminished over time after an initial phase of increase in the summers one year and two years after the start of the incubation. For winter DOM concentration differences we only have the November 2010 sampling date as our starting point so we cannot tell if there had been a gradual rise in differences between treatments for successive winters after the start of the incubation in 2008. However, the February 2011 winter sample had a smaller difference between treatments and contols (all site samples combined) than that three months earlier and the December 2012 sample had no significant differences between treatments and contols for DOM concentration.

When DOM was released, the accumulated DOM in pore-water may be transported from

soils into water bodies through vertical diffusion processes under the steep concentration gradient along a soil profile, leading to large aquatic C loss from wetlands downstream to aquatic ecosystems (Mulholland, 1997). The acclimation of DOM concentrations under sustained experimental warming in this study suggests that C loss in dissolved forms could be tempered over time in subtropical wetlands, which may have implications for our predictions of C cycling and C loss under climate change scenarios. Of course, in reality a soil will not be subject to an immediate and sustained 5^{o}C warming under climate change and so more gradual warming effects on some wetland soils over time may result in no observed differences in soil DOM concentration and release compared to baseline conditions due to the feedback effects we have identified.

4.2. Effects of experimental warming on DOM biodegradability

Many wetlands have longer hydrological and biogeochemical residence times than downstream rivers or lakes (Bullock and Acreman, 2003). Before transport into overlying water, DOM in pore-water would go through a high degree of microbial consumption and transformation with a progressed level of biological processing, including microbial uptake of available substrate and the release of microbial metabolites (Fellman et al., 2008). As a result, DOM composition and chemistry may be highly variable, depending on the autochthonous C production, which has a microbial origin, and allochthonous organic matter leaching from solid-phase soil organic matter pools. The allochthonous DOM fraction, largely of terrestrial origin, is resistant to biodegradation due to its high content of complex aromatic structures, including chitin and lignin compounds (Fellman et al., 2010). Not all soluble substrates can be

easily assimilated and metabolized by microbial cells. A large proportion of humic-like substances in DOM increase the difficulty of microbial feeding. Meanwhile, the protein-like fluorescence, which is most closely related to DOM biodegradability (Fellman et al., 2008; Kothawala et al., 2012), was selectively lost in some warmed samples. The red shift reflects higher molecular-weight fractions with an increased degree of water humicity. The water absorption of visible-UV light is due to the ubiquitous aromatic chromophores (primarily humics) in DOM. Weishaar et al. (2003) have shown that percent aromaticity determined by ^{13}C NMR was strongly correlated with SUVA values indicating that SUVA values can be used to reflect the amount of aromatic compounds. These fluorescence fingerprints combined with decreased A_{253}/A_{203} and I_A/I_C ratio provides evidence for a relative shortage of readily available substrates for DOM composition under warming.

Terrestrially-derived soluble humic-like substances can be traced back to the dynamics of solid-phase soil organic C pools. According to different soil intrinsic turnover times (Davidson et al., 2000), soil organic C pools can be divided into labile and recalcitrant C fractions. Recently, studies have suggested that recalcitrant soil organic C pools with complex molecular attributes, characterized by low decomposition rates and requiring high activation energies to react, are intrinsically more sensitive to temperature than the labile pool (von Lutzow and Kogel-Knabner, 2009). It is well known that the newly incorporated fresh litter can be gradually utilized and converted into more stable forms through soil-forming processes. However, these processes may be impaired by global warming with more stable soil organic matter converted into active soil organic matter in dissolved forms through soil organic matter destabilization, such as depolymerization, dissolution and desorption processes (Sollins et al.,

1996). The relatively higher humicity of DOM in the wetland soils we studied suggested the recalcitrant soil fractions may be preferentially leached in response to warming, compared to the labile pools. Consistent with this, laboratory incubation of boreal forest soils has shown that warming led to the leaching of humified soil organic matter incorporated into DOM (Li et al., 2012), which increased the contribution of aromatic contents in the composition of DOM. Moreover, some soluble proteins and simple fatty acids could be rapidly consumed when microbial metabolic rates increase significantly with rising temperature (Gudasz et al., 2010). The shortage of readily available substrate may be a negative feedback which counteracts the early effects of warming on DOM and CO_2 effluxes through diminished microbial activities (Frey et al., 2008; Melillo et al., 2002). In this process, thermal acclimation of microbial physiology may also be one of the key mechanisms leading to gradually diminished C loss under experimental warming (Bradford et al., 2008).

Alternatively to the above, however, there are some papers where substantial C loss has been reported under sustained soil warming (e.g. from boreal permafrost (Schuur et al., 2009), as well as some fertilized soils (Mack et al., 2004)). Most of these studies showed that C transferred into atmosphere came from recalcitrant, old C fractions. Allison et al. (2010) theoretically suggested that waming may lead to omnipotent microbes changing their strategies to utilize more recalcitrant C pools through a changed microbial community or adjustment of C use efficiency when readily available substrate is limited, leading to greater C loss. These findings increase uncertainty in our ability to predict C cycle changes under future climate. Therefore, further research about the dynamics of different soil organic C fractions and associated enzymatic activities as well as microbial community shifts are needed in our

tested wetlands under sustained warming.

4.3. Effect of soil types on DOM concentration and structural complexity

There were only small differences in pore-water DOM concentrations between the six studied wetlands yet the soil organic matter stored in YT was 6.83 times higher than those in SJ. Although most of DOM originates from leaching of solid-phase soil organic pools, the relationship between total organic matter stored in the soils and DOM concentrations in pore-water for the six wetlands was not significant ($p > 0.05$). In contrast, a previous study (Wang et al., 2012) reported that phosphorus concentrations in the pore-water were closely ($p = 0.045$) related with soil total phosphorus contents from these microcosm samples. Our results imply that besides allochthonous precursor organic matter in bulk soils responsible for DOM concentration dynamics, microbial consumption and production of microbial-originated DOM may also greatly influence DOM concentrations, increasing its variability and uncertainty in field study. Rapid turnover of DOM and the resultant large fluctuations in DOM concentrations by microbial activities have been well documented in temperate forest soils (Bengtson and Bengtsson, 2007). SJ had the lowest soil organic contents (14.6 g kg^{-1}) among the six wetlands, while the DOM concentrations in SJ were comparable to those in other wetlands. These findings suggest that even some organic-poor soils may retain relatively high soluble C as available substrates through microbial metabolism. Soluble phosphorus contents in the pore-water of SJ were very low and almost approached detection limits (Wang et al., 2012).

The six wetlands contained DOM with distinct fluorescent fingerprints, including the

identified fluorescent components, and the associated intensities and positioning of specific fluorescence peaks. The DOM in YT had the highest humic-like fluorescence intensities among the six wetlands studied as indicated by the lowest I_A/I_C and the highest $P_{V,n}$ values in Region V. Most humic acids resistant to biodegradation have been found to accumulate in upper organic horizons, and decrease in a soil profile with increasing mineral contents in lower soil horizons, where fulvic acids gradually become the dominant fraction (Ussiri and Johnson, 2003). This partially explains why organic-enriched wetlands (i.e., YT and XZ) have relatively higher humic-like contents than others. We observed the soluble microbial byproduct-like and protein-like fluorophore in SJ and JH wetlands under ambient temperature conditions, while for other wetlands the protein-like fluorescence peaks were almost overlapped by the fulvic-like fluorescence. Consistent with this, $P_{I,n}$ values in Region I associated with protein-like substances were higher for SJ and JH compared to YT. This suggests that the paddy soils of organic-poor wetlands (such as SJ) contain DOM with relatively large fractions of easily-degradable substances among the studied soil types. Minerotrophic wetland soils such as fens and paddy soils, have been shown to possess high primary production and fast turnover rates of nutrients (Aerts et al., 1999). Consistent with this, we also observed that the highly productive emergent macrophytes (including *Acorus calamus* and *Typha angustifolia*) grew in the SJ and JH wetland sites, while most floating-leaf aquatic plants, like *Trapa incisa, Lemna minor,* and *Azolla imbricata* dominated in YT, XZ and XX wetland sites. Some easily-degradable carbohydrates and amino acids through root exudation and litter decay (Eviner and Chapin, 1997) may explain the detected protein-like fluorescent components in DOM from SJ and JH. For YT and XZ, though possessing enriched soil organic matter

contents, the large soil water contents (more than 60%) seems to inhibit the growth of rooted-plants, leading to unexpectedly low protein-like compounds in DOM. A large proportion of easily-degradable DOM is generally "young" and recently fixed (Yano et al., 2000), which implies that the substrates in SJ and JH could be efficiently utilized by plants or microbes, leading to fast DOM turnover rates. As a result, microbial demand for substrates in organic-poor wetlands seems to be more severe than that in organic-enriched wetlands (i.e., YT and XZ).

4.4. Evaluation of different spectrum values

We found that the routinely used spectral indices, including $S_{280\text{-}400}$, FI and β: α failed to capture observed changes in DOM character in response to warming. Microbial utilization and photo-degradation of DOM may both cause $S_{280\text{-}400}$ to decrease through a flattening effect on the slope of spectrum curves, while the removal of some complex compounds is responsible for the increase in $S_{280\text{-}400}$ (Stedmon et al., 2000). Warming did not significantly change the $S_{280\text{-}400}$, except for the XZ wetland soil. FI are derived from three single data points with fluorescence intensities, including two emission wavelengths (470 and 520 nm) and one excitation wavelength (379 nm) falling into the humic-like acid regions (Region V in EEM) (Chen et al., 2003). We observed an increased percentage of humic-like fluorescence intensities and shifted positioning of the humic-like peak cenrter towards longer emission wavelengths (red shift) for warmed samples compared to the control. FI seemed to be insufficient to describe the shape or positioning of these fluorescence peaks and the resultant differences in FI between treatments were negligible. The freshness index (β: α) is calculated

based on the fluorescence peak C and peak M, in which peak C falls into the humic-like acid region (Region V), while the peak M is blue-shifted towards protein-like regions along the emission axis relative to peak C and is largely located at the transitional zone between soluble microbial byproduct-like (Region IV) and humic-like acid regions (Chen et al., 2003; Wilson and Xenopoulos, 2009). Therefore, peak M is fresher than peak C. One of the fluorescence intensity variables used in the freshness index was at the excitation wavelength of 310 nm. However, in this study, some of fluorescence peaks were found at excitation wavelengths less than 300 nm. Therefore, the freshness index may fail to describe warming-induced changes in DOM character. Overall, the common feature in most of the above spectral indices was that only one to three data points from an EEM spectrum were used to quantify fluorescence spectra, which led to large variability in values within-samples and between samples when, effectively, only a small part of the dataset was used. Therefore, the interpretation of these indices to understand the extent of decompostion and production of different DOM structures should be undertaken with caution. We further used FRI technology combined with the HIX value to quantify fluorescence EEM spectra. Both HIX and integrated percent fluorescence distribution had a relatively low coefficient of variation within-samples. We suggest that HIX, calculated from the integrated area of specific region, is more robust for detecting inconspicuous changes in the chemical character of DOM, compared to FI or β: α, calculated from one to three data points. Across a wide range of DOM concentrations from tested wetlands (SJ-YT), percent fluorescence distributions were well verified by the changing patterned viewed within EEM spectra (**Fig. 2**), indicating the method of quantifying cumulative fluorescence intensities under certain fluorescence peaks can be applied to various

water samples (Chen et al., 2003). Through inner filter correction, the influence of high DOM concentrations on HIX values and $P_{i,n}$ values could be more effectively corrected to produce reliable results to describe DOM character shifts under warming.

Conclusions

To our best knowledge, this study is the first to explore the impacts of experimental warming on DOM character for soil pore-water in wetlands. Elevated temperature was associated with an increase in the release of DOM from the solid-phase into pore-water with increasing differences up to 2.5 years into the experiment. After this point the greater DOM in the warmed treatments declined and there was no overall significant difference by the end of the experiment between treatments except for one of the six wetland soils. However, at the end of the experimental period, 55 months after the commencement of a 5^{o}C incubation above ambient conditions, spectral information for spectral indices (i.e., $SUVA_{280}$, $SUVA_{254}$, A_{253}/A_{203}, HIX and I_A/I_C) and regional EEM spectra analysis indicated that the experimental warming increased the DOM humicity with enriched humic-like substances found in warmed samples, where relatively easy degradable substrates, such as protein-like, microbial byproduct-like, and/or fulvic-like compounds were selectively lost or reduced, leading to decreased DOM biodegradability in response to experimental warming. Thus a negative feedback effect has been identified, the magnitiude of which depended to some extent on the nature of the wetland substrate. A preferential loss of easily degradable substances in DOM composition with concomitant decreases in soil DOM degradability under warming could be very important as a process which ought to be included when predicting how wetland C

cycling will operate under future climate change.

ACKNOWLDGEMENTS

The authors wish to thank the National Natural Science Foundation of China (40701162), Public Projects of National Ministry of Environmental Protection (2010467014) and National Ministry of Water Resources (201301092), and the University of Leeds Worldwide University Network Fund for International Collaboration for providing the financial support for this project.

References

Aerts R, Verhoeven JTA, Whigham DF. Plant-mediated controls on nutrient cycling in temperate fens and bogs. Ecology 1999; 80: 2170-2181.

Allison SD, Wallenstein MD, Bradford MA. Soil-carbon response to warming dependent on microbial physiology. Nature Geoscience 2010; 3: 336-340.

Baker A, Bolton L, Newson M, Spencer RGM. Spectrophotometric properties of surface water dissolved organic matter in an afforested upland peat catchment. Hydrological Processes 2008; 22: 2325-2336.

Battin TJ, Kaplan LA, Findlay S, Hopkinson CS, Marti E, Packman AI, et al. Biophysical controls on organic carbon fluxes in fluvial networks. Nature Geoscience 2008; 1: 95-100.

Bengtson P, Bengtsson G. Rapid turnover of DOC in temperate forests accounts for increased CO_2 production at elevated temperatures. Ecology Letters 2007; 10: 783-790.

Bolan NS, Adriano DC, Kunhikrishnan A, James T, McDowell R, Senesi N. Dissolved organic matter: biogeochemistry, dynamics, and environmental significance in soils. Advances in Agronomy 2011; 110: 1-75.

Bradford MA, Davies CA, Frey SD, Maddox TR, Melillo JM, Mohan JE, et al. Thermal adaptation of soil microbial respiration to elevated temperature. Ecology Letters 2008; 11: 1316-1327.

Bullock A, Acreman M. The role of wetlands in the hydrological cycle. Hydrology and Earth System Sciences 2003; 7: 358-389.

Chen W, Westerhoff P, Leenheer JA, Booksh K. Fluorescence excitation - Emission matrix regional integration to quantify spectra for dissolved organic matter. Environmental Science & Technology 2003; 37: 5701-5710.

Chin YP, Aiken G, Oloughlin E. Molecular-weight, polydispersity, and spectroscopic properties of

aquatic humic substances. Environmental Science & Technology 1994; 28: 1853-1858.
Conant RT, Ryan MG, Agren GI, Birge HE, Davidson EA, Eliasson PE, et al. Temperature and soil organic matter decomposition rates - synthesis of current knowledge and a way forward. Global Change Biology 2011; 17: 3392-3404.
Davidson EA, Trumbore SE, Amundson R. Soil warming and organic carbon content. Nature 2000; 408: 789-90.
Erwin KL. Wetlands and global climate change: the role of wetland restoration in a changing world. Wetlands Ecology and Management 2009; 17: 71-84.
Eviner VT, Chapin FS. Nitrogen cycle - Plant-microbial interactions. Nature 1997; 385: 26-27.
Fellman JB, D'Amore DV, Hood E, Boone RD. Fluorescence characteristics and biodegradability of dissolved organic matter in forest and wetland soils from coastal temperate watersheds in southeast Alaska. Biogeochemistry 2008; 88: 169-184.
Fellman JB, Hood E, Spencer RGM. Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review. Limnology and Oceanography 2010; 55: 2452-2462.
Frey SD, Drijber R, Smith H, Melillo J. Microbial biomass, functional capacity, and community structure after 12 years of soil warming. Soil Biology & Biochemistry 2008; 40: 2904-2907.
Glatzel S, Kalbitz K, Dalva M, Moore T. Dissolved organic matter properties and their relationship to carbon dioxide efflux from restored peat bogs. Geoderma 2003; 113: 397-411.
Gudasz C, Bastviken D, Steger K, Premke K, Sobek S, Tranvik LJ. Temperature-controlled organic carbon mineralization in lake sediments. Nature 2010; 466: 478-U3.
He XS, Xi BD, Jiang YH, He LS, Li D, Pan HW, et al. Structural transformation study of water-extractable organic matter during the industrial composting of cattle manure. Microchemical Journal 2013; 106: 160-166.
Huang W, Chen RF. Sources and transformations of chromophoric dissolved organic matter in the Neponset River Watershed. Journal of Geophysical Research-Biogeosciences 2009; 114.
Kalbitz K, Solinger S, Park JH, Michalzik B, Matzner E. Controls on the dynamics of dissolved organic matter in soils: A review. Soil Science 2000; 165: 277-304.
Kirschbaum MUF. Soil respiration under prolonged soil warming: are rate reductions caused by acclimation or substrate loss? Global Change Biology 2004; 10: 1870-1877.
Kothawala DN, von Wachenfeldt E, Koehler B, Tranvik LJ. Selective loss and preservation of lake water dissolved organic matter fluorescence during long-term dark incubations. Science of the Total Environment 2012; 433: 238-246.
Kujawinski EB. The impact of microbial metabolism on marine dissolved organic matter. Annual Review of Marine Science, Vol 3 2011; 3: 567-599.
Lawaetz AJ, Stedmon CA. Fluorescence intensity calibration using the raman scatter peak of water. Applied Spectroscopy 2009; 63: 936-940.
Li JW, Ziegler S, Lane CS, Billings SA. Warming-enhanced preferential microbial mineralization of

humified boreal forest soil organic matter: Interpretation of soil profiles along a climate transect using laboratory incubations. Journal of Geophysical Research-Biogeosciences 2012; 117.
Mack MC, Schuur EAG, Bret-Harte MS, Shaver GR, Chapin FS. Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. Nature 2004; 431: 440-443.
Marschner B, Kalbitz K. Controls of bioavailability and biodegradability of dissolved organic matter in soils. Geoderma 2003; 113: 211-235.
Melillo JM, Steudler PA, Aber JD, Newkirk K, Lux H, Bowles FP, et al. Soil warming and carbon-cycle feedbacks to the climate system. Science 2002; 298: 2173-2176.
Minero C, Lauri V, Falletti G, Maurino V, Pelizzetti E, Vione D. Spectrophotometric characterisation of surface lakewater samples: Implications for the quantification of nitrate and the properties of dissolved organic matter. Annali Di Chimica 2007; 97: 1107-1116.
Mulholland PJ. Dissolved organic matter concentration and flux in streams. Journal of the North American Benthological Society 1997; 16: 131-141.
Ohno T. Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter. Environmental Science & Technology 2002; 36: 742-746.
Schmidt MWI, Torn MS, Abiven S, Dittmar T, Guggenberger G, Janssens IA, et al. Persistence of soil organic matter as an ecosystem property. Nature 2011; 478: 49-56.
Schuur EAG, Vogel JG, Crummer KG, Lee H, Sickman JO, Osterkamp TE. The effect of permafrost thaw on old carbon release and net carbon exchange from tundra. Nature 2009; 459: 556-559.
Sollins P, Homann P, Caldwell BA. Stabilization and destabilization of soil organic matter: Mechanisms and controls. Geoderma 1996; 74: 65-105.
Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor,M., et al. Climate change 2007: the physical science basis. Contribution of Working Group to the Fourth Assessment Report of the IPCC. Cambridge University Press, Cambridge, United Kingdom 2007.
Song J, Luo YM, Zhao QG, Christie P. Novel use of soil moisture samplers for studies on anaerobic ammonium fluxes across lake sediment-water interfaces. Chemosphere 2003; 50: 711-715.
Stedmon CA, Markager S, Kaas H. Optical properties and signatures of chromophoric dissolved organic matter (CDOM) in Danish coastal waters. Estuarine Coastal and Shelf Science 2000; 51: 267-278.
Stutter MI, Lumsdon DG, Cooper RJ. Temperature and soil moisture effects on dissolved organic matter release from a moorland Podzol O horizon under field and controlled laboratory conditions. European Journal of Soil Science 2007; 58: 1007-1016.
Ussiri DAN, Johnson CE. Characterization of organic matter in a northern hardwood forest soil by C-13 NMR spectroscopy and chemical methods. Geoderma 2003; 111: 123-149.
von Lutzow M, Kogel-Knabner I. Temperature sensitivity of soil organic matter decomposition-what do we know? Biology and Fertility of Soils 2009; 46: 1-15.
Wang H, Holden J, Spera K, Xu X, Wang Z, Luan J, et al. Phosphorus fluxes at the sediment–water interface in subtropical wetlands subjected to experimental warming: A microcosm study.

Chemosphere 2012.
Weishaar JL, Aiken GR, Bergamaschi BA, Fram MS, Fujii R, Mopper K. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environmental Science & Technology 2003; 37: 4702-4708.
Wilson HF, Xenopoulos MA. Effects of agricultural land use on the composition of fluvial dissolved organic matter. Nature Geoscience 2009; 2: 37-41.
Yano Y, McDowell WH, Aber JD. Biodegradable dissolved organic carbon in forest soil solution and effects of chronic nitrogen deposition. Soil Biology & Biochemistry 2000; 32: 1743-1751.
Zhang ZJ, Wang ZD, Holden J, Xu XH, Wang H, Ruan JH, et al. The release of phosphorus from sediment into water in subtropical wetlands: a warming microcosm experiment. Hydrological Processes 2012; 26: 15-26.

Table 1. Descriptions of the study sites for sampling in May 2008 and the basic soil chemical properties[a].

Wetland ID	County	Latitude and longitude	Main wetland use	Annual mean water depth (m)	Annual mean flow velocity, (m min^{-1})	pH	Organic matter (g kg^{-1})	Total nitrogen (g kg^{-1})	Total phosphorus (mg kg^{-1})	Water contents (%)
ShiJiu multipond wetland (SJ)	JiaXing	120°41'31"E, 30°53'55"N	Water reservoir	1.20	1.68	7.3**a**	14.6**a**	1.45**a**	346**a**	32.6**a**
JinHu (JH)	ShaoXing	120°33'32"E, 30°01'58"N	Water reservoir	2.50	0.05	7.2**a**	25.5**a**	1.89**a**	579**b**	35.1**a**
XiXi national wetland park (XX)	HangZhou	120°03'59"E, 30°16'23"N	Tourism	0.85	0.10	7.4**a**	32.6**b**	3.87**c**	521**b**	55.0**b**
BaoYang riverine wetland (BY)	ChangXing	119°54'24"E, 31°04'31"N	Water reservoir	0.68	1.32	7.1**a**	39.3**b**	2.40**b**	833**c**	54.5**b**
XiaZhuhu (XZ)	DeQing	120°02'54"E, 30°31'28"N	Tourism and aquaculture	1.50	0.12	7.3**a**	64.7**c**	4.32**c**	906**c**	64.5**c**
YaTang riverine wetland (YT)	TongXiang	120°29'13"E, 30°43'15"N	Mixed use	0.80	1.02	7.4**a**	114**d**	6.81**d**	2530**d**	68.7**c**

[a]Different letters labeled in the column of soil chemical properties, i.e., "pH", "organic matter", "total nitrogen", "total phosphorus" and "water contents" indicate significant differences in the means between study sites by one-way analysis of variance (ANOVA) with study site as a factor and Student-Newman-Keuls (SNK) method was conducted for multiple comparisons. The organic matter, total nitrogen, total phosphorus in wetland soils were calculated based on dry soils, while water contents were calculated based on fresh soils.

Table 2. Mean ± standard error of UV-visible absorbance at 254 nm wavelength in a 1cm quartz cuvette ($A_{254,}$ cm^{-1}), as well as dissolved organic carbon concentration-normalized UV-visible spectral indices, including the specific UV-visible absorbance at 280 nm ($SUVA_{280}$, L mg^{-1} m^{-1}) and at 254 nm wavelength ($SUVA_{254}$, L mg^{-1} m^{-1}), UV-visible spectral slope between 280 and 400 nm wavelengths ($S_{280\text{-}400}$) and the ratio of absorbance at 253 and 203 nm wavelength (A_{253}/A_{203}) measured in soil pore-water of the six studied wetlands (i.e., SJ, JH, XX, BY, XZ, and YT) between treatments (control *vs.* warmed). Water samples were collected on Dec-08-2012, 4.5-years from the onset of experimental warming incubation. For each wetland, bold indicates a significant difference in the warming treatment compared to the control with asterisks indicating * $p < 0.05$, or ** $p < 0.01$ by Student's *t*-test. The bottom row indicates *p*-values from ANOVA conducted to test for differences between treatments across all wetlands as a whole.

Wetlands	A_{254}		$SUVA_{280}$		$SUVA_{254}$		$S_{280\text{-}400}$		A_{253}/A_{203}	
	Control	Warmed	Control	Warmed	Control	Warmed	Control	Warmed	Control	Warmed
SJ	0.17 ± 0.05	0.23 ± 0.06	0.32 ± 0.06	0.57 ± 0.14	0.37 ± 0.08	**0.70* ± 0.17**	9.70 ± 3.80	6.70 ± 1.81	0.95 ± 0.12	**0.50* ± 0.12**
JH	0.13 ± 0.01	0.17 ± 0.04	0.30 ± 0.04	0.30 ± 0.07	0.35 ± 0.03	0.40 ± 0.10	20.1 ± 2.2	16.3 ± 2.6	0.93 ± 0.04	**0.68* ± 0.09**
XX	0.14 ± 0.01	**0.28** ± 0.03**	0.33 ± 0.04	**0.53* ± 0.05**	0.44 ± 0.05	**0.69* ± 0.04**	11.8 ± 1.5	9.83 ± 3.35	2.93 ± 1.13	**0.63* ± 0.14**
BY	0.12 ± 0.01	**0.21** ± 0.01**	0.27 ± 0.02	**0.35* ± 0.02**	0.38 ± 0.03	**0.47* ± 0.03**	10.3 ± 1.5	13.5 ± 3.8	0.87 ± 0.31	0.49 ± 0.08
XZ	0.36 ± 0.06	**0.54* ± 0.00**	0.84 ± 0.28	1.01 ± 0.14	0.96 ± 0.33	1.21 ± 0.19	12.6 ± 2.6	**7.40* ± 1.41**	0.89 ± 0.09	**0.63* ± 0.03**
YT	0.50 ± 0.06	**0.81* ± 0.03**	0.74 ± 0.11	**1.24* ± 0.21**	0.93 ± 0.14	**1.56* ± 0.27**	16.4 ± 2.7	15.8 ± 0.7	0.44 ± 0.02	0.41 ± 0.01
p-value	**< 0.001** (Increase)		**0.010** (Increase)		**0.008** (Increase)		0.839 (No change)		**0.026** (Decrease)	

Table 3. Mean ± standard error of dissolved organic carbon concentration-normalized fluorescence spectral indices, including fluorescence index (FI), freshness index (β: α), and humification index (HIX), as well as the fluorescence intensity ratio of peak A to peak C (I_A/I_C). The position of peak A and peak C falling within the specific 3D EEM regions are expressed as excitation and emission coordinates (Ex/Em) represented in the columns on the right. Water samples were collected on Dec-08-2012. For each wetland, bold indicates a significant difference in the warming treatment compared to the control with asterisks indicating * $p < 0.05$, or ** $p < 0.01$. The bottom row indicates *p*-values from ANOVA conducted to test for differences between treatments across all wetlands as a whole.

Wetlands	FI		β: α		HIX		I_A/I_C		Peak A		Peak C	
	Control	Warmed	Control	Warmed	Control	Warmed	Control	Warmed	Control	Warmed	Control	Warmed
SJ	2.12 ± 0.06	2.11 ± 0.09	0.64 ± 0.04	0.52 ± 0.10	0.91 ± 0.01	0.90 ± 0.01	n.d.	1.42 ± 0.10	240/415	240/415	n.d.	315/400
JH	2.24 ± 0.01	2.09 ± 0.10	0.98 ± 0.23	1.48 ± 1.36	0.82 ± 0.03	**0.89* ± 0.02**	n.d.	1.76 ± 0.12	235/400	230/410	n.d.	295/395
XX	1.97 ± 0.37	2.16 ± 0.07	0.67 ± 0.14	0.51 ± 0.08	0.78 ± 0.04	**0.87* ± 0.03**	1.74 ± 0.06	n.d.	240/420	235/400	315/400	n.d.
BY	2.22 ± 0.04	2.14 ± 0.02	0.47 ± 0.04	0.71 ± 0.46	0.84 ± 0.02	0.85 ± 0.06	1.68 ± 0.22	1.42 ± 0.06	245/435	240/400	300/400	300/410
XZ	2.27 ± 0.03	2.25 ± 0.02	0.72 ± 0.01	0.70 ± 0.02	0.88 ± 0.01	**0.89* ± 0.00**	1.43 ± 0.08	**1.12** ± 0.06**	230/400	250/430	305/415	330/440
YT	2.08 ± 0.16	2.15 ± 0.07	0.65 ± 0.06	0.62 ± 0.01	0.89 ± 0.02	**0.92* ± 0.00**	0.87 ± 0.08	**0.67* ± 0.03**	245/410	250/415	325/410	330/425
p-value	0.979 (No change)		0.645 (No change)		**< 0.001** (Increase)		**< 0.001** (Decrease)		Not Available			

Table 4. Mean ± standard error of percent fluorescence distribution ($P_{i,n}$, %) in the specific 3D EEM regions (i.e., Region I-V) of the entire EEM spectra. Water samples were collected on Dec-08-2012. For each wetland, bold indicates a significant difference in the warming treatment compared to the control with asterisks indicating * $p < 0.05$, or ** $p < 0.01$. The bottom row indicates *p*-values from ANOVA conducted to test for differences between treatments across all wetlands as a whole.

Wetlands	Region I		Region II		Region III		Region IV		Region V	
	Control	Warmed	Control	Warmed	Control	Warmed	Control	Warmed	Control	Warmed
SJ	24.9 ± 0.7	**23.0* ± 0.4**	26.8 ± 0.4	**25.7* ± 0.3**	25.8 ± 0.2	26.5 ± 0.1	17.0 ± 0.3	**16.5* ± 0.1**	5.42 ± 0.97	**8.27** ± 1.04**
JH	23.9 ± 1.2	23.7 ± 0.2	26.5 ± 0.3	**25.9* ± 0.3**	27.7 ± 0.1	27.5 ± 0.0	15.6 ± 0.6	16.0 ± 0.5	6.37 ± 0.89	6.81 ± 0.03
XX	25.2 ± 0.5	24.1 ± 0.1	26.9 ± 0.3	26.7 ± 0.2	24.4 ± 1.0	26.4 ± 0.0	17.9 ± 1.6	15.5 ± 0.1	5.56 ± 0.28	**7.36** ± 0.25**
BY	22.5 ± 1.8	22.5 ± 1.0	28.6 ± 0.6	27.5 ± 0.1	24.3 ± 1.2	24.9 ± 0.6	17.9 ± 1.5	17.5 ± 0.7	6.66 ± 0.32	**7.62* ± 0.79**
XZ	24.3 ± 0.7	**22.3* ± 0.5**	26.4 ± 0.4	25.9 ± 0.7	26.5 ± 0.2	**25.8* ± 0.0**	15.4 ± 0.3	15.9 ± 0.2	7.43 ± 1.02	**10.1** ± 1.1**
YT	20.9 ± 1.3	19.7 ± 1.1	26.6 ± 0.5	25.4 ± 1.0	24.0 ± 0.8	**22.4* ± 1.4**	17.3 ± 0.8	18.4 ± 1.0	11.1 ± 1.8	**14.2** ± 2.5**
p-value	0.098 (Marginal decrease)		0.072 (Marginal decrease)		0.722 (No change)		0.696 (No change)		**0.014** (Increase)	

Fig. 1 DOM concentration dynamics during 4.5-years of warming incubations, including July 2009 (A), August 2010 (B), November 2010 (C), February 2011 (D), July 2011 (E), July 2012 (F) and December 2012 (G). For each panel of A-G, two-way ANOVA was conducted with F and *p* values shown of differences in mean values of DOM concentrations from six wetlands between control and warmed treatments. Asterisks represent significant (*$p < 0.05$, **$p < 0.01$) Student's *t*-test differences in means for each wetland between treatments. The percent changes in mean values of DOM concentrations from six wetlands between treatments at each sampling month through time are shown in panel H. Vertical bars in each panel show the 95% confidence interval.

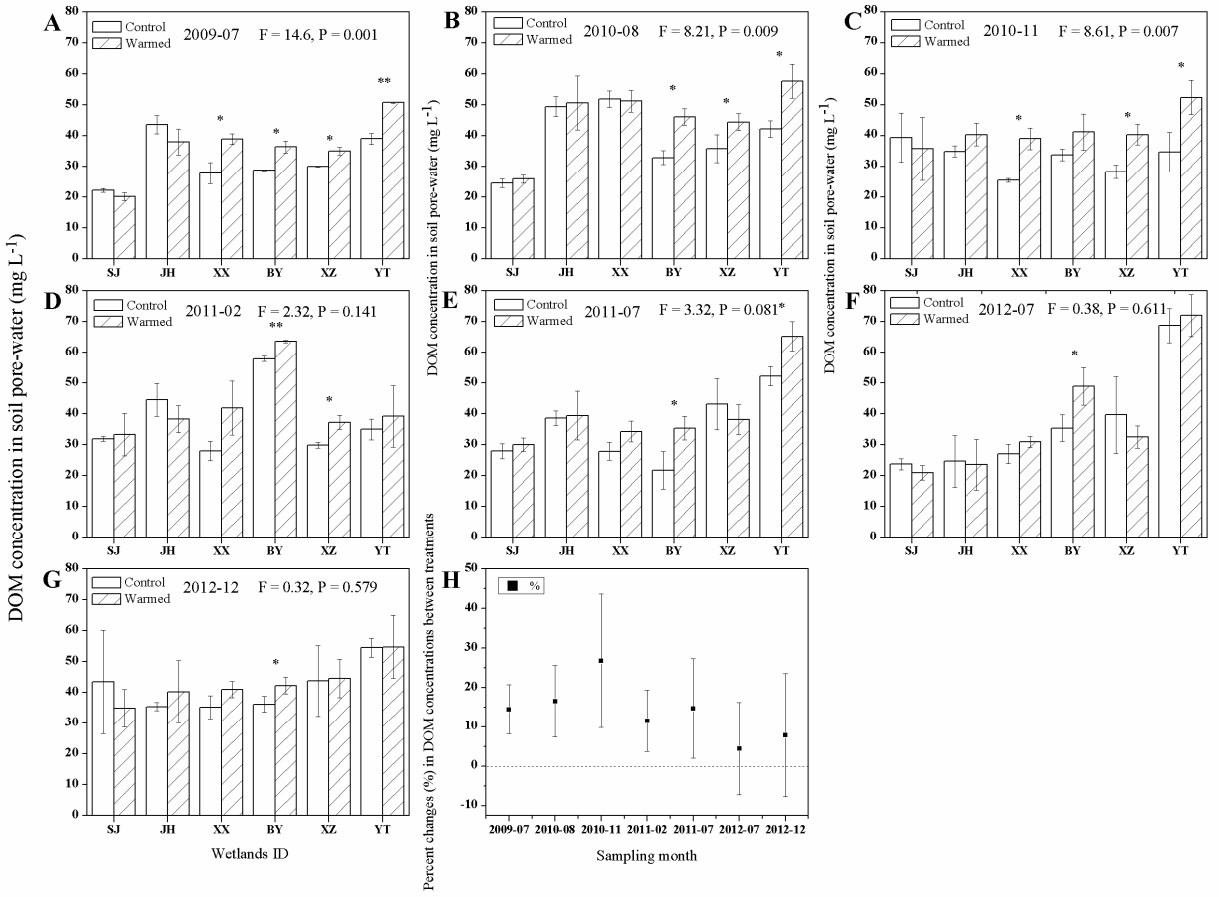

Fig. 2 Excitation-emission matrix fluorescence spectra for soil pore-water in SJ (control: A and warmed: B) and YT (control: C and warmed: D), two typical wetlands in this study. Spectra are examples from each of the replicates. The fluorescence intensities of each EEM panel are corrected for inner-filter effects using absorbance measurements, and the raw data are transformed between 0 and 1, and thus intensities here are unitless.