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Experimental warming increases CO_2 saturation in a shallow prairie pond

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Abstract There is an urgent need to understand the effect of climate warming on the carbon dynamics of lakes and ponds in order to assess contributions to global carbon budgets. Currently, we are unable to predict how the exchange of carbon gases (i.e. CO_2) across the air–water boundary and organic carbon storage in the sediments will be altered with realistic warming scenarios downscaled from climatic models. Given the prevalence of shallow systems and tight atmospheric coupling, we conducted a mesocosm experiment to test the impacts of warming on CO_2 saturation in a shallow prairie pond. We outline and test three possible scenarios for the effect of warming on the CO_2 saturation of ponds, resulting in either an increase, decrease or no net effect for CO_2 saturation. We show that with approximately a two-degree (°C) increase in average water temperature, the pCO_2 of the warmed mesocosms was nine times greater than the ambient temperature mesocosms by the end of the 5-week experiment. Changes in water colour (a measure of dissolved organic carbon) in warmed systems indicate that decomposition of organic matter in the sediments and water column was the main contributor to the increase in pCO_2 in the warmed mesocosms. Our results show that with warming, the release of CO_2 from shallow ponds to the atmosphere will increase and carbon storage in the sediments will decrease, altering the current functioning of shallow prairie ponds and influencing the contribution of ponds to the global carbon cycle.

Keywords Carbon dioxide saturation · Shallow lakes and ponds · Climate change · Sediment organic matter · Mesocosm · Decomposition · Warming

Introduction

Freshwaters have recently gained recognition as important processors of carbon at the global scale (Alin and Johnson 2007; Cole et al. 2007; Dean and Gorham 1998; Einsele et al. 2001). Their role in terms of carbon storage, flux and transport has been elucidated more clearly, indicating that freshwaters currently not only release a globally significant amount of carbon from the terrestrial catchment to the atmosphere, but also store a large amount of allochthonous and autochthonous carbon in sediments (Alin and

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Johnson 2007; Cole et al. 2007; Dean and Gorham 1998; Einsele et al. 2001). The importance of freshwaters for both carbon storage and release makes studies aimed at understanding the effect of warming on the net carbon balance of lakes and ponds crucial for predicting future global carbon cycling (Falkowski et al. 2000).

Currently, the vast majority of lakes worldwide are supersaturated with respect to CO_2, causing CO_2 to be released to the atmosphere (Cole et al. 1994). In terms of carbon cycling within a lake, supersaturation generally indicates net heterotrophy, where organic carbon from external sources (allochthonous) or from pools of stored carbon (i.e. sediment organic matter or DOC accumulated in the water column) is respired and released to the atmosphere at a greater rate than autochthonous carbon uptake and storage (Carpenter et al. 2005; Cole et al. 2000, 2002; del Giorgio and Peters 1993; Duarte and Prairie 2005; Lennon and Pfaff 2005). Undersaturation indicates net autotrophy where more autochthonously generated carbon is stored in the sediments, or water column, than is respired and released to the atmosphere (Flanagan et al. 2006; Hanson et al. 2004; Schindler et al. 1997). If warming disproportionately alters any of these rates, processes or inputs, it will alter the CO_2 saturation of lakes and consequently the net exchange with the atmosphere.

Whether the CO_2 saturation of lakes and ponds will be altered by climate warming and will consequently function differently in the global carbon cycle remains uncertain. Current expectations for how the CO_2 saturation of lakes and ponds will change with warming have revolved primarily around understanding how inputs of organic matter will be altered under a warmed climate due to either direct effects of warming or changes in precipitation patterns (Dillon and Molot 2005; Evans et al. 2005; Moore et al. 1998; Sobek et al. 2005). However, within lakes and ponds, if temperature is currently a limiting process, warming will accelerate multiple biological processes that either absorb or release CO_2 and any non-uniformity either spatially or temporally in the acceleration of CO_2 uptake and release rates will change CO_2 saturation. Being able to understand the conditions that promote acceleration of certain processes over and above, others will help us to predict CO_2 saturation in freshwaters under a changed climate.

In a previous greenhouse mesocosm study, we showed that fully enclosed mesocosms of two depths differed in their response to warming (Flanagan and McCauley 2008). The CO_2 concentration of shallower mesocosms decreased when warmed due to elevated carbon sequestration. The deeper mesocosms, on the other hand, showed no response to warming (Flanagan and McCauley 2008). Here, we examine how the response to warming in shallow systems may differ when the warming manipulation is conducted in situ in a shallow prairie pond, thereby, examining the effect of warming in a more complex system, with more realistic conditions including the presence of an existing sediment pool. Shallow lakes are expected to be particularly influenced by climate change as the water temperature is more closely coupled to the atmospheric temperatures. This strong anticipated effect, in addition to the knowledge that the majority of the world's lakes are shallow (Downing et al. 2006), makes understanding the effect of climate warming on shallow lakes particularly important.

In mesocosms experiencing ambient and experimentally warmed temperature treatments, we measure total phosphorus, dissolved phosphorus, zooplankton biomass, algal concentration, CO_2 saturation and water colour (as an indicator of the amount of coloured dissolved organic carbon present; Pace and Cole 2002) over the duration of the experiment (15 May to 19 June 2007). By using mesocosms, we isolate a column of water and sediments to strictly examine the effect of warming on internal processes rather than external loading of carbon. Changes in net CO_2 saturation resulting from warming can therefore then determined to be due to changes in processes occurring in either the water column or the sediments, or both.

Predicting the net effect of warming on the multiple biological processes important for controlling CO_2 saturation is difficult. Considering this difficulty, we outline three possible scenarios for the change in CO_2 saturation with warming assuming biological rates are positively related to temperature: (1) primary production rates increase by more than total (pelagic and benthic) respiration rates and the average CO_2 saturation of warmed mesocosms is lower than ambient mesocosms, (2) total respiration rates increase by more than primary production rates and warmed mesocosms have higher average CO_2 saturation than ambient mesocosms, (3) primary production and respiration rates increase by the same amount and, consequently, there is no change in CO_2 saturation of warmed mesocosms. There are many

biological mechanisms that could give rise to each of these three scenarios, but both scenarios 1 and 2 will result if either respiration (scenario 1) or primary production rates (scenario 2) are limited by a factor other than temperature (i.e. substrate quality, nutrient levels, light etc), and therefore do not increase as strongly with warming. Without further experimentation, we will not be able to determine the exact mechanism causing the observed scenario, but rather we show the general response of CO_2 saturation to warming in a shallow pond and contrast this to our previous mesocosm experiment excluding sediments (Flanagan and McCauley 2008).

Materials and methods

Description of the pond

The University of Calgary campus pond is a small, shallow pond typical of many naturally occurring prairie ponds. It is $\sim$200 m^2 in surface area with a maximum depth of 3 m. The phytoplankton community is dominated by Chrysophytes, Cryptophytes and Diatoms (Van der Meulen 2003). The zooplankton community is dominated by *Daphnia*, *Ceriodaphnia* and both calanoid and cyclopoid copepods and ostracods. The pond contains no vertebrate predators, and the major invertebrate predators are *Chaoborus* and *Notonecta*. Chlorophyll *a* values typically range from less than 1 to $\sim$20 μg l^{-1} depending on the time of year. Mean water total phosphorus is typically $\sim$40 μg l^{-1}.

Experimental set-up

The experiment was conducted in the early open water period from 15 May to 19 June 2007. Cylindrical, open-bottomed high density polyethylene mesocosms (84 cm tall with a diameter of 89 cm containing 521 l) were inserted $\sim$0.2 m into the sediments of the shallow pond. The mesocosm bottoms were removed to allow inclusion of the pond sediments and exchange of materials between sediments and surface waters (Fig. 1). A total of eight mesocosms were installed, with four replicates of an 'ambient' (unheated) treatment and four replicates of a 'warm' treatment. Mesocosms were placed at the same depth following the perimeter of the pond. The warming treatment was applied in an alternating pattern of 'warm' and 'ambient' to ensure that any differences in substrate quality along the shore were controlled. The experiment used existing pond algal, bacterial and zooplankton communities and densities as the initial conditions. Sampling of the mesocosms began 3 days after mesocosm installation in the pond. Installation of the mesocosms did cause some temporary sediment disturbance, but the sediments were settled by the time first sampling occurred.The experiment was conducted for a total of 5 weeks, with heating beginning between the first and second week of the experiment.

Warmed mesocosms were heated with stainless steel coils placed just above the sediment surface. Warm water was continually pumped through the coils from a reserve heating bin, containing an independent supply of water. The heat was maintained in the reserve bins by submersible water heaters that were programmed to maintain the heating bin water at 5°C warmer than the ambient water temperature.

Sampling procedure

Mesocosms were sampled twice weekly for algal biomass (measured as chlorophyll *a*), zooplankton biomass, colour and temperature, and once weekly for pCO_2, total and dissolved phosphorus. Mesocosm walls were scrubbed once weekly to minimize periphytic growth and carbon/nutrient sequestration (as per Flanagan and McCauley 2008).

Temperature profiles were measured with a temperature probe (YSI 58, Yellow Springs, OH) to quantify the temperature manipulation. Measurements were taken just below the water surface, in the middle of the mesocosms and just above the sediments; only the values from the middle of the mesocosms are reported as these measures best represent the average heating manipulation. Continuous recording temperature loggers (ibutton, Maxim Innovation, CA) were also installed in the middle of two warmed mesocosms and two ambient mesocosms. These loggers recorded the temperature every 30 min and captured the diurnal temperature fluctuations and differences between warmed and ambient mesocosms on a finer time scale.

Dissolved CO_2 concentrations were determined by calculation from pH and alkalinity, measured by titration with 0.1 N HCl to an end point pH of 3.5

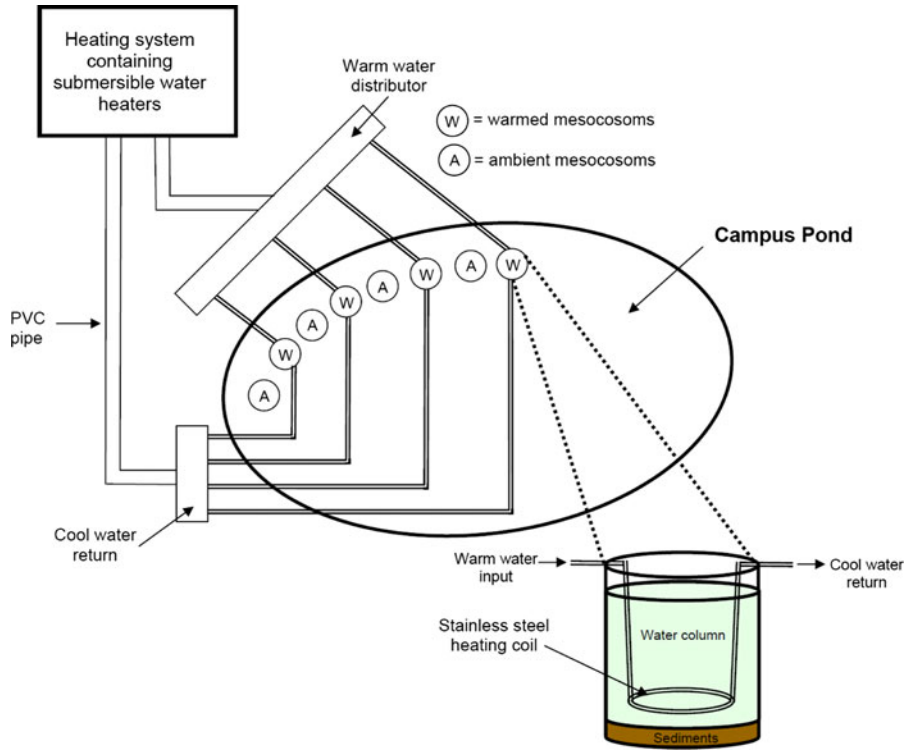

Fig. 1 Bird's eye view of the University of Calgary campus pond experimental set-up and heating system, with a profile of a warm mesocosm to show the location of heating coils

(Thomas and Lynch 1960; Clesceri et al. 1998). Mesocosms were sampled between 07:30 and 08:30 h each week to ensure that there was no confounding effect of sampling time on CO_2 concentrations. Water samples were taken from the middle of the mesocosms, with the 250-ml Nalgene® sampling containers inverted very slowly so as to minimize the amount of gases introduced into the water column due to sampling. Samples were placed on ice and in the dark for transport. All samples were warmed to room temperature prior to analysis to ensure that differences measured between treatments were not a result of the temperature effect on CO_2 concentrations. Measures of CO_2 concentration from the alkalinity calculation (in mg $CO_2\ l^{-1}$) were converted to partial pressures of CO_2 (pCO_2 in µatm) according to Henry's Law (Stumm and Morgan 1996) for ease of comparison with atmospheric CO_2 levels in terms of undersaturation or oversaturation of CO_2 in the water column.

Water colour was measured on the dissolved fraction of the pond water (filtered through 1.2-µm GF filter) sampled from the middle of the mesocosm using a 250-ml Nalgene® sampling bottle. Colour was measured as absorbance on a spectrophotometer (Shimadzu UV 1700, Suzhou, Jiangau, China) at a wavelength of 440 nm in a 10-cm cuvette, according to Cuthbert and Delgiorgio (1992). This measure determines the brown colour of dissolved substances and estimates the humic and fulvic compounds in the pond water (Pace and Cole 2002). Values reported for colour are expressed as a wavelength-specific absorption coefficient (m^{-1}):

$$a_{440} = 2.303X\,(\text{absorbance at 440 nm} \div 0.1\,\text{m})$$

Total and dissolved phosphorus of the water column were measured according to the standard molybdate reaction method (Strickland and Parsons 1968) from samples taken in the middle of the mesocosms using a 1-l Nalgene® sampling bottle.

Mesocosms were mixed by hand for about 1 min prior to sampling for zooplankton and algal biomass; however, hand mixing was done carefully so as to not disturb the sediments on the bottom. The mesocosms were sampled from the centre using a 1-l Nalgene® bottle. Zooplankton from a 1 l sample were counted live and returned to the mesocosms. Taxa were identified to the level of family. Biomass estimates for the zooplankton community were calculated according to McCauley (1984). Differences in

community composition between warm and ambient mesocosms were examined by conducting Repeated measures ANOVAs (RM-ANOVA) on the four most abundant families through time.

To measure algal biomass, a 1 l sample was taken from the middle of the mesocosms using a dark brown Nalgene® sampling bottle, and a subsample was analysed for the chlorophyll *a* content using 90% acetone extraction and fluorescence on a fluorometer (Sequoia-Turner Model 450, Mountain View, CA). Total chlorophyll *a* and edible chlorophyll *a* were both measured. The edible fraction of the total chlorophyll *a* was determined based on size fractionation through a 35-μm Nitex® mesh (McCauley and Murdoch 1990).

Statistical analysis

All analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, NC, USA). Mixed model RM-ANOVAs were performed on temperature, pCO_2, colour, total and dissolved phosphorus, chlorophyll *a* and zooplankton biomass to examine average treatment effects on each of these variables while controlling for within subject time-dependent correlations (Littell et al. 1998). The variance–covariance structure was modelled as one of four types: antedependence (ANTE(1)), unstructured (UN), heterogeneous autoregressive (ARH(1)) or spatial power (SP(POW)), according to the biological expectations for time dependence and limitations of the dataset. Selection of the most appropriate variance–covariance structure was determined based on the lowest value of the Akaike Information Criteria for small samples (AICC) (Wang and Goonewardenen 2004). The degrees of freedom were adjusted for non-independence using the Kenward Rogers method.

Results

The heating system successfully established a temperature difference between the ambient and warm treatments (Fig. 2); the mean (±SE) temperature in the warm mesocosms was 19.3 (±0.21)°C, which was 1.9 (±0.29)°C higher than in the ambient mesocosms (17.4 (±0.21)°C) when examined over the duration of the experiment (RM-ANOVA, $F = 42.71$, $df = 1,8.38$, $P < 0.0001$).The temperature profiles (taken just below the surface, in the middle of the mesocosm and just above the sediment surface) indicated a slightly larger increase in temperature just above the sediments with the warming manipulation; however, due to convective mixing of the water column, the heating treatment was relatively uniform throughout the mesocosm.

Warming significantly influenced the mean partial pressure of CO_2 in the experimental mesocosms (Fig. 3). The mean (±SE) pCO_2 in the ambient and warm mesocosms was initially 18,293 (±2,434) μatm and 18,245 (±2,434) μatm, respectively; however, by the end of the experiment, the mean (±SE) pCO_2 in ambient mesocosms was 1,438 (±2,434) μatm and the warm mesocosms had a pCO_2 of 12,983 (±2,434) μatm. Warming caused the overall average pCO_2 to nearly double when examined over the duration of the experiment (RM-ANOVA, $F = 8.55$, $df = 1$, 5.55, $P = 0.029$). Furthermore, when we examined the trend in CO_2 with warming as two diverging trajectories over time (interaction between time and treatment in RM-ANOVA), we see that pCO_2 values were significantly higher in the warm mesocosms from the third week of the experiment onwards (Table 1). By the end of the fifth week of the experiment, the warm mesocosms had an average of nine times the pCO_2 of the ambient mesocosms (orthogonal contrast of warmed and ambient mesocosms on 13 June, $t = 3.35$, $df = 14.3$, $P = 0.005$). Alkalinity measures used to determine the CO_2 concentrations ranged from ~90 to 200 mg $CaCO_3$ l^{-1} and pH measured ranged from ~7 to 8.5.

The water colour also differed with warming (Fig. 4). The warm mesocosms had significantly higher mean (±SE) colour (1.931 (±0.039) m^{-1}) than the ambient mesocosms (1.700 (±0.039) m^{-1}) when examined over the entire experiment (RM-ANOVA, $F = 17.64$, $df = 1$, 36.1, $P = 0.0002$). Although the interaction between the treatment effect and time was non-significant (RM-ANOVA, $F = 1.89$, $df = 9,17.2$, $P = 0.1223$), pairwise comparisons of the ambient and warm mesocosms showed a significant difference emerging between ambient and warm mesocosms for the last two sampling dates (orthogonal contrast on 11 June, $t = 2.51$, $df = 6$, $P = 0.0460$, orthogonal contrast on 13 June, $t = 2.77$, $df = 6$, $P = 0.0323$).

Total phosphorus in the mesocosms ranged from 30 to 60 μg l^{-1}. Dissolved phosphorus ranged from

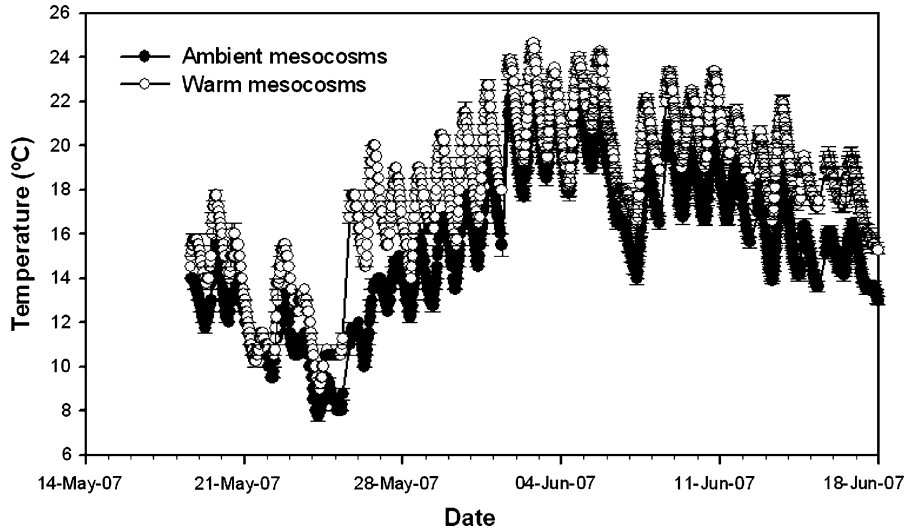

Fig. 2 Average temperature (°C, two replicates per treatment, with SE bars shown) for ambient (*closed circles*) and warm (*open circles*) mesocosms for the duration of the experimentally warmed treatment period

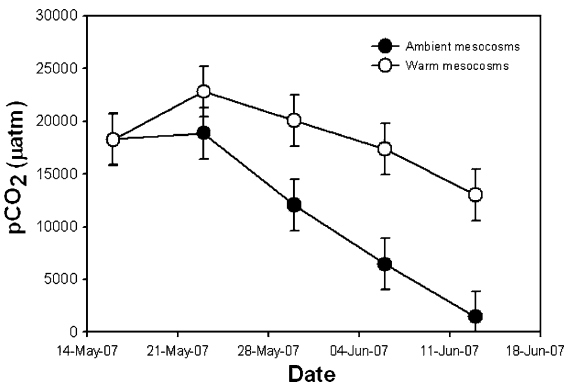

Fig. 3 The average pCO_2 (µatm) of ambient (*closed circles*) and warm (*open circles*) mesocosms including the SE of the four replicates for the 5 weeks of the experiment

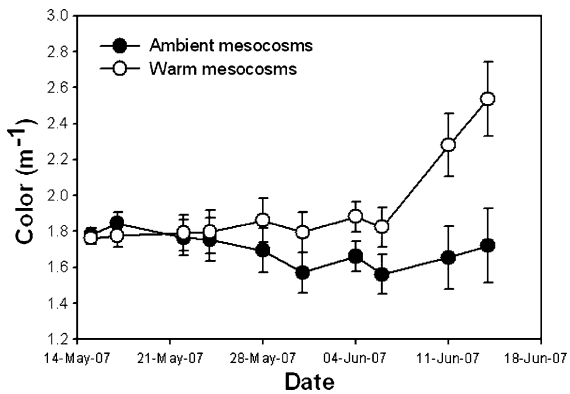

Fig. 4 The average colour (m^{-1}) for the ambient (*closed circles*) and warm (*open circles*) mesocosms, SE bars of four replicates shown for the 5 weeks of the experiment

Table 1 Pairwise contrasts between mean ambient and mean warm pCO_2 for the 5 weeks of the experiment

Week	Difference (warm–ambient)	SE	*df*	*t*	*P*
1	−47	3,442	14.3	−0.01	0.9892
2	29,757	3,442	14.3	1.14	0.2734
3	60,795	3,442	14.3	2.33	0.0351
4	82,812	3,442	14.3	3.17	0.0067
5	87,615	3,442	14.3	3.35	0.0046

15 to 30 µg l^{-1}. Total phosphorus and dissolved phosphorus were log transformed to meet the assumption of normality in the RM-ANOVA analysis. There was no significant effect of warming on either the log(dissolved phosphorus) (RM-ANOVA, $F = 3.49$. $df = 1,6.41$, $P = 0.1079$) or log(total phosphorus) (RM-ANOVA, $F = 1.73$, $df = 1$, 6.92, $P = 0.2308$) (Fig. 5). The log(dissolved phosphorus) also showed no significant trend with time (RM-ANOVA, $F = 1.98$, $df = 3,18.1$, $P = 0.1523$) and no significant interaction between treatment effects and time (RM-ANOVA, $F = 0.52$, $df = 3$, 18.1, $P = 0.6713$). Log (total phosphorus) did show an effect of time (RM-ANOVA, $F = 14.81$, $df = 3,17.9$, $P < 0.0001$), with log(total phosphorus) decreasing as the experiment progressed. There was no interaction between treatment effects and time for log(total phosphorus) (RM-ANOVA, $F = 1.70$, $df = 3,17.9$, $P = 0.2038$).

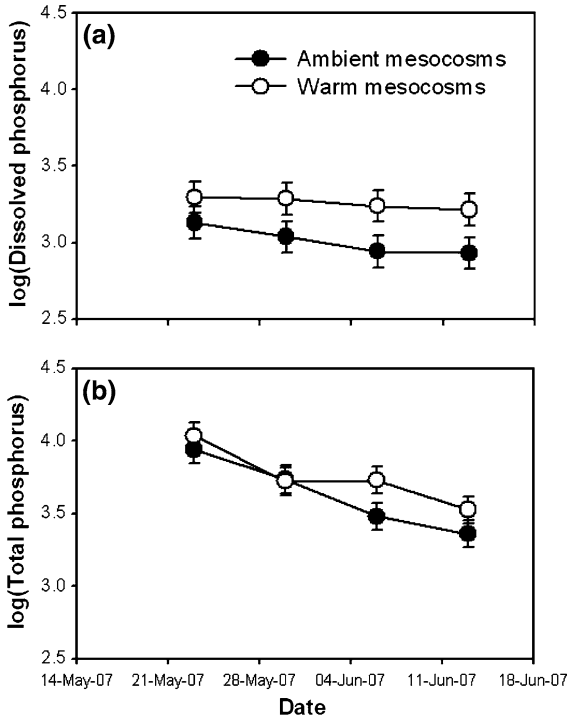

Fig. 5 The average **a** log dissolved phosphorus (μg l^{-1}) and **b** log total phosphorus (μg l^{-1}) for ambient (*closed circles*) and warm (*open circles*) mesocosms. SE bars of four replicates shown

Total chlorophyll *a* in the mesocosms ranged from 1 to 40 μg l^{-1} and edible chlorophyll *a* ranged from 1 to 7 μg l^{-1}. Total and edible chlorophyll *a* was log transformed to meet the assumption of normality for the RM-ANOVA analysis. There was no significant effect of warming on log(total chlorophyll *a*) (RM-ANOVA, $F = 2.13$, $df = 1,30.4$, $P = 0.1546$) (Fig. 6a). There was also no interaction between sampling date and warming for log(total chlorophyll *a*) (RM-ANOVA, $F = 0.60$, $df = 9,16.9$, $P = 0.7766$). However, there was a significant trend with time for log(total chlorophyll *a*)(RM-ANOVA, $F = 5.26$, $df = 9,16.9$, $P = 0.0017$). Log(edible chlorophyll *a*) also showed no effect of warming (RM-ANOVA, $F = 2.21$, $df = 1,20$, $P = 0.1525$) (Fig. 6b) or interaction between warming and sampling date (RM-ANOVA, $F = 1.76$, $df = 9,46.8$, $P = 0.1010$). There was an effect of time, however, on the log(edible chlorophyll *a*) (RM-ANOVA, $F = 4.80$, $df = 9,46.8$, $P = 0.0001$).

Zooplankton biomass ranged from 0.05 to 2.5 mg l^{-1}. Zooplankton biomass was log transformed

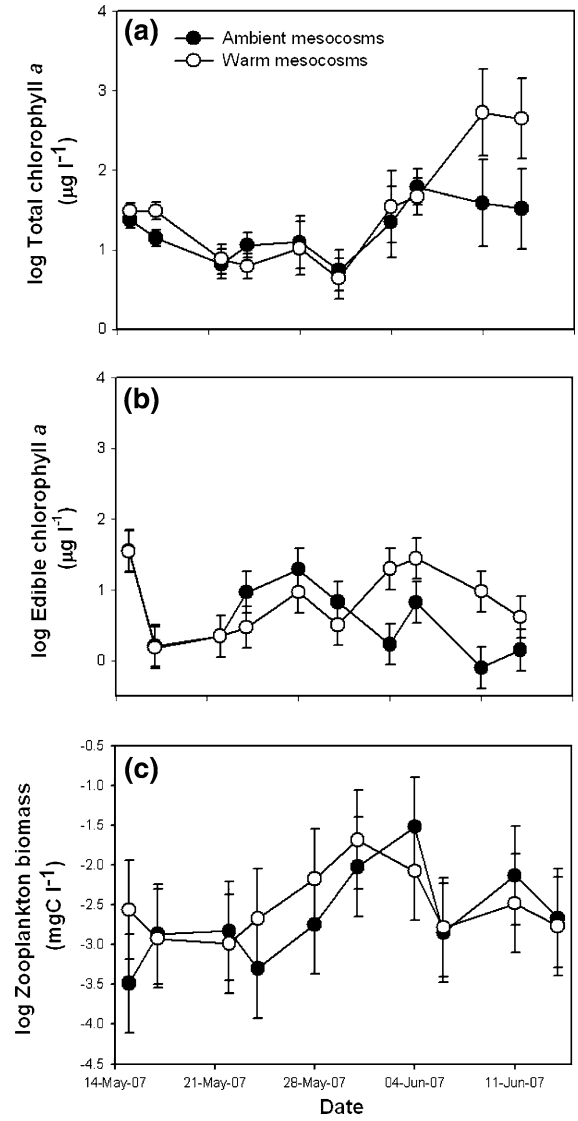

Fig. 6 The average concentration of **a** log total chlorophyll *a* (μg l^{-1}), **b** log edible chlorophyll *a* (μg l^{-1}) and **c** log zooplankton biomass (mg C l^{-1}) for the ambient and warmed treatments SE bars of four replicates shown

to meet the assumptions of RM-ANOVA. Log(zooplankton biomass) did not show a significant effect of warming (RM-ANOVA, $F = 0.06$, $df = 1,9.76$, $P = 0.8130$) (Fig. 6c). There was also no trend in log(zooplankton biomass) with time (RM-ANOVA, $F = 1.79$, $df = 9,49.6$, $P = 0.0944$) or any interaction between warming and time (RM-ANOVA, $F = 0.71$, $df = 9,49.6$, $P = 0.6949$).

The four most abundant families of zooplankton (*Daphnia*, *Ceriodaphnia*, copepods and *Chydoridae*)

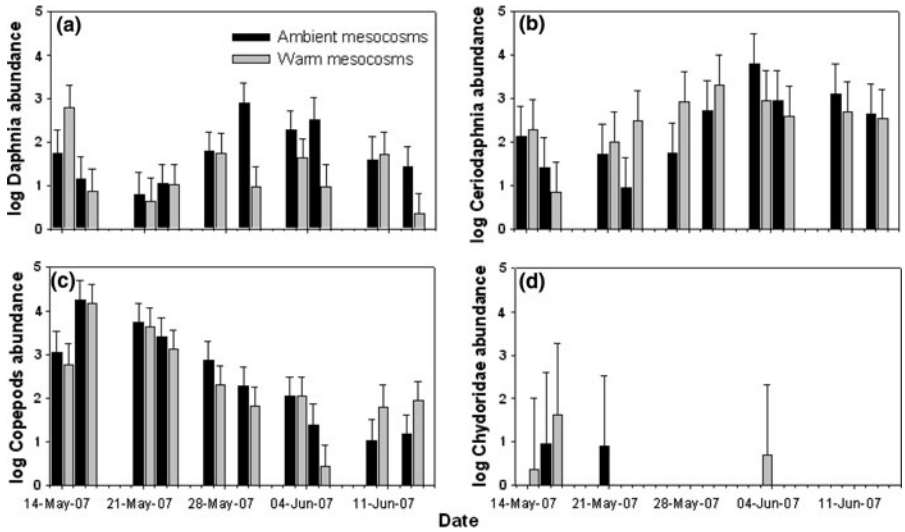

Fig. 7 The average log abundances of **a** *Daphnia*, **b** *Ceriodaphnia*, **c** copepods and **d** *Chydoridae* for the ambient (*black bars*) and warm (*grey bars*) mesocosms. SE bars of four replicates shown

were analysed for changes in community composition with warming (Fig. 7). These four families consistently comprised over 80% of the total abundance of all taxa present. All analyses are done on the log(abundances) for each species. Log(*Daphnia* abundance) showed no effect of warming (RM-ANOVA, $F = 3.02$, $df = 1,\ 14.8$, $P = 0.1028$). However, there was an effect of sampling date (RM-ANOVA, $F = 2.32$, $df = 9,37.3$, $P = 0.0347$). There was no interaction between warming and sampling date (RM-ANOVA, $F = 1.68$, $df = 9,37.3$, $P = 0.1285$). Pairwise contrasts between ambient and warm mesocosms showed a significant difference between log(*Daphnia* abundance) on a single sample date (30 May 2007) (orthogonal contrast, $t = -3.01$, $df = 42.3$, $P = 0.004$). No other contrasts produced significant differences between ambient and warm mesocosms. Log(*Ceriodaphnia* abundance) showed no significant effect of warming (RM-ANOVA, $F = 0.07$, $df = 1,10.4$, $P = 0.8002$), no effect of sampling date (RM-ANOVA, $F = 1.92$, $df = 9,45.2$, $P = 0.0725$) and no interaction between warming and time (RM-ANOVA, $F = 0.84$, $df = 9,45.2$, $P = 0.5850$). No pairwise contrasts between ambient and warmed mesocosms were significantly different. Log(copepod abundance) also had no effect of warming (RM-ANOVA, $F = 0.19$, $df = 1,12.8$, $P = 0.6671$). There was, however, a significant effect of sampling date (RM-ANOVA, $F = 7.79$, $df = 9,37.5$, $P < 0.0001$), but no interaction between warming and sampling date (RM-ANOVA, $F = 0.51$, $df = 9,37.5$, $P = 0.8554$). No pairwise contrasts between ambient and warm mesocosms were significant. Log(*Chydoridae* abundance) also was not significantly different between ambient and warm mesocosms (RM-ANOVA, $F = 1.36$, $df = 1,13.5$, $P = 0.2637$). There was an effect of sampling date (RM-ANOVA, $F = 9.90$, $df = 9,31.9$, $P < 0.0001$) but no interaction between treatment and sampling date (RM-ANOVA, $F = 1.60$, $df = 9,31.9$, $P = 0.1556$).

Discussion

The pCO_2 in our shallow prairie pond experiment responded dramatically to warming. With approximately a 2°C increase in average temperature, warmed mesocosms maintained average pCO_2 levels above ambient mesocosm pCO_2. This result suggests climate warming may directly influence exchange of CO_2 with the atmosphere via changes in internal processing of organic carbon in shallow ponds, causing ponds to release more CO_2 to the atmosphere than has previously been observed.

Both ambient and warmed mesocosms were initially supersaturated with respect to atmospheric pCO_2 ($\sim$390 μatm). The pCO_2 at the start of the

experiment was approximately 30 times atmospheric pCO_2 ($\sim$18,250 μatm). While this is high, it is well within the range of pCO_2 values for lakes worldwide (17–65,250 μatm) (Sobek et al. 2005). Over the duration of the experiment, warm mesocosms maintained significantly higher pCO_2 than ambient mesocosms; by the end of the fifth week of the experiment, pCO_2 in warm mesocosms was approximately nine times the pCO_2 of ambient mesocosms. We expected both primary production and respiration rates to increase with warming, however, we deduce based on the direction of the change in pCO_2 with warming (an increase in saturation), that respiration rates increased more than primary production causing pCO_2 to be elevated in warmed mesocosms. Consequently, in this experiment, the change in pCO_2 indicates that respiration may have been limited by temperature to a greater extent than primary productivity (which may have been limited by nutrient concentrations and/or light penetration, for example) causing respiration to increase over-and-above increases in primary productivity with warming.

The colour of the mesocosms was also significantly influenced by warming, with warmed mesocosms having higher average colour. Water colour, as measured, is indicative of the amount of coloured dissolved organic carbon (CDOC) present in the water column, typically in the form of humic and fulvic acids (Cuthbert and Delgiorgio 1992; Pace and Cole 2002). CDOC forms as a by-product of degradation/decomposition of organic matter (Jansson et al. 2000; Koelmans and Prevo 2003), and in this experiment, because mesocosms were closed to all external organic carbon sources (i.e. runoff of organic material from the surrounding terrestrial landscape), the organic carbon substrate being degraded and producing CDOC was carbon in mesocosm sediments and water column (Cuthbert and Delgiorgio 1992; Eiler et al. 2003; Houser et al. 2003; Pace and Cole 2002). The measured increase in colour with warming provides evidence that increased decomposition of organic matter within the mesocosms fuelled elevated CO_2 production in warmed mesocosm causing pCO_2 to remain higher relative to ambient mesocosms. In terms of carbon cycling in lakes and ponds, this would shift the role of lakes towards increased CO_2 emission to the atmosphere and reduced storage in sediments, which if the response occurred for lakes and ponds on a wide scale may have important implications for the role lakes and ponds play in the global carbon cycle (Cole et al. 2007).

The nutrient levels for both dissolved and total phosphorus showed no effect of warming. In addition, the lack of any significant differences between zooplankton biomass, zooplankton community structure and chlorophyll *a* indicates that the food web played a relatively minor role in the response of the mesocosms to warming. There was a dynamical response in the community composition over time, with *Ceriodaphnia* abundance increasing and copepod abundance decreasing; however, there was no shift in community composition with warming. It appears that the primary response to warming was that of microbial activity on organic substrates in the sediments and water column altering mesocosm colour and pCO_2. The lack of response in the zooplankton community to warming may have been due to direct competitive interactions between algae and bacteria (Carpenter et al. 1998; Cotner and Biddanda 2002; Grover 2000; Klug 2005), photoinhibition of algae due to increased colour (Houser et al. 2003) or nutrient substrate limitation rather than temperature limitation on the growth rates.

The observed dramatic increase in average pCO_2 with warming is likely a 'transient response' that could be very important in calculating annual changes in pond carbon budgets. Elevated respiration due to decomposition of the sediments and water column organic carbon can only be sustained as long as there is suitable organic matter available for decomposition. Based on this experiment, it appears that some proportion of the large amount of organic carbon currently stored in the sediments and water column of shallow lakes and ponds will likely decompose under a warmer climate increasing the CO_2 flux to the atmosphere and decreasing carbon storage. However, seasonal effects on these release rates, as well as, the duration (extent) of this efflux remain unknown.

In light of our two experiments, understanding conditions that promote the response of increased CO_2 sequestration as was observed in our previous experiment (Flanagan and McCauley 2008) versus enhanced CO_2 release observed in this experiment is important. It appears based on the difference in response to pCO_2 to warming between the two experiments that the availability of sediments for respiration is critical in determining the direction of the response of pCO_2 to

warming. Whether organic carbon in the sediments is readily available when ponds are warmed will likely depend on the physical proximity of sediments to surface waters (i.e. depth, proportion of lake that is littoral zone), light penetration, extent of warming of sediments and changes in thermal stratification. However, to generalize these results to the global carbon cycle, we need to better understand how the physical conditions of lakes and ponds would promote or hinder decomposition of the sediments under warmed conditions and how the measured effect of warming is altered when considered in the context of lakes and ponds embedded in a landscape. In addition, changes in organic carbon input to lakes with climate change will also influence the net carbon exchange from lakes and ponds. Integrating the effects of both changes in internal processes due to direct effects of warming as well as changes in loading to lakes will be necessary for understanding how the pCO_2 of lakes will change. Further experimentation is required to determine how these factors interact.

Shallow lakes are expected to be particularly influenced by climate warming (Baulch et al. 2005; Carvalho and Kirika 2003; Gyllstrom et al. 2005; Moss et al. 2003). The fact that the global distribution of lakes is dominated by millions of small water bodies (Downing et al. 2006) suggests that the response observed in this experiment to warming may be important to carbon cycling on a global scale.

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