Metabolic and physiochemical responses to a whole-lake experimental increase in dissolved organic carbon in a north-temperate lake

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Abstract

Over the last several decades, many lakes globally have increased in dissolved organic carbon (DOC), calling into question how lake functions may respond to increasing DOC. Unfortunately, our basis for making predictions is limited to spatial surveys, modeling, and laboratory experiments, which may not accurately capture important whole-ecosystem processes. In this article, we present data on metabolic and physiochemical responses of a multiyear experimental whole-lake increase in DOC concentration. Unexpectedly, we observed an increase in pelagic gross primary production, likely due to a small increase in phosphorus as well as a surprising lack of change in epilimnetic light climate. We also speculate on the importance of lake size modifying the relationship between light climate and elevated DOC. A larger increase in ecosystem respiration resulted in an increased heterotrophy for the treatment basin. The magnitude of the increase in heterotrophy was extremely close to the excess DOC load to the treatment basin, indicating that changes in heterotrophy may be predictable if allochthonous carbon loads are well-constrained. Elevated DOC concentration also reduced thermocline and mixed layer depth and reduced whole-lake temperature. Results from this experiment were quantitatively different, and sometimes even in the opposite direction, from expectations based on cross-system surveys and bottle experiments, emphasizing the importance of whole-ecosystem experiments in understanding ecosystem response to environmental change.

Many northern hemisphere lakes have experienced a gradual increase in dissolved organic carbon (DOC) concentration over the past several decades, a phenomenon termed "global browning" (Evans et al. 2006; Roulet and Moore 2006; Monteith et al. 2007). The increase in DOC concentration has been attributed to a recovery from acidification (Evans et al. 2006; Monteith et al. 2007), increased catchment terrestrial primary production (Freeman et al. 2004), high nitrogen loads affecting soil decomposition (Findlay 2005), ecosystem effects of climate change (Urban et al. 2011), and changes in catchment hydrology (Evans et al. 2005). Although the mechanism for global browning is important to understand and currently still debated, the ecological consequences of increased DOC concentration on lake processes are poorly understood.

DOC has both abiotic and biotic effects on lake ecosystems, and comparative studies suggest DOC as a master variable in structuring aquatic ecosystems (Solomon et al. 2015). Abiotic effects of DOC on lake ecosystems are expressed through its light attenuating properties, as the absorption of solar radiation affects the vertical distribution of light and heat, and in turn, affects a host of other lake ecosystem functions. For example, highly colored north-temperate lakes had reduced epilimnetic depth, temperature, and irradiance compared with clearer lakes (Houser 2006). Additionally, modeling of a north-temperate bog lake showed that a 50% reduction in DOC concentration caused a deepening of the mixed layer depth by 44% and a warmer whole-lake water temperature (Read and Rose 2013). These changes in temperature and light regimes have strong implications for lake food webs as DOC concentration has been shown to limit primary productivity via light attenuation (del Giorgio and Peters 1994; Ask et al. 2009; Godwin et al. 2014), consequently reducing invertebrate and fish productivity (Karlsson et al. 2009; Finstad et al. 2013; Kelly et al. 2014). Additionally,

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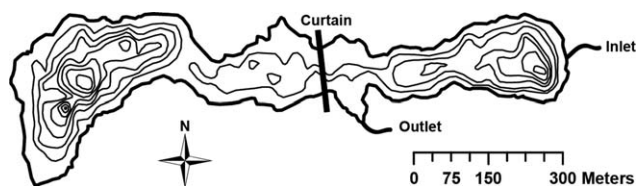Additional Supporting Information may be found in the online version of this article.

Fig. 1. Bathymetric map of Long Lake. The curtain divides Long Lake into two basins of nearly equal size. The East basin (treatment basin) receives a majority of the terrestrial dissolved organic carbon load delivered from the inlet located on the East end of the treatment basin. Contour lines represent 2 m depth intervals and the map is projected using Albers Equal Area Conic.

altered temperature regimes caused by elevated DOC concentration have direct control on biological process rates such as bacterial respiration (Hanson et al. 2011).

Along with abiotic impacts on lake ecosystems, DOC can also act as a resource for aquatic consumers including microbes and zooplankton. For example, DOC concentration is often positively correlated with whole-lake respiration (Hanson et al. 2003; however, see Solomon et al. 2013) and can support a vast majority of bacterial production (Hessen 1992; Cole et al. 2006; Berggren et al. 2010). Excess microbial respiration increases the amount of greenhouse gas emission to the atmosphere (Tranvik 1992; Cole et al. 1994). Upper trophic levels are impacted by the excess microbial energy as high energy mobilization from bacteria can support a large portion of zooplankton biomass (Berggren et al. 2010), however, reliance on terrestrial carbon may result in reduced zooplankton growth due to low food quality (Brett et al. 2009; Kelly et al. 2014).

The diverse effects of DOC on lakes makes predicting how lake ecosystem function will respond to changing DOC concentration difficult, especially as a majority of our knowledge is based on cross-system comparative studies (e.g., Hanson et al. 2003; Kelly et al. 2014). As lake ecosystems vary in many characteristics (e.g., size, shape, food web structure, and nutrient loads), cross-system observational surveys of the effects of elevated DOC concentration may not adequately represent a single lake's response through time, leaving us uncertain about how lakes will respond to changing DOC concentration (Solomon et al. 2015). Here, we present results from a whole-lake experiment designed to test the effects of elevated DOC concentration on lake physiochemical variables and ecosystem metabolism. Based on cross-system observational and modeling studies, we predicted that elevated DOC concentration would reduce light and heat penetration, thus making the thermocline shallower, the epilimnion darker, and the whole-lake temperature colder. We further hypothesized that the resulting darker epilimnion would reduce pelagic primary production via light limitation, and the excess allochthonous DOC would subsidize heterotrophic bacterial activity, consequently increasing lake respiration and heterotrophy.

Methods

Site and experimental description

The experimental lake, Long Lake, is located at the University of Notre Dame Environmental Research Center (UNDERC, 46°13′N, 89°32′W) in Gogebic County, Michigan, U.S.A. Long Lake is an 8.1 ha seepage lake with a mean depth of 3.8 m and a maximum depth of 14 m occurring at both ends of the hourglass-shaped lake (Fig. 1). The lake's 16.4 ha watershed is characterized as a second-growth mixed hardwood forest. We divided Long Lake into two basins of nearly equal size with a $1/16^{th}$ inch nylon mesh curtain in the spring of 2011 (Memphis Net & Twine). The West basin (reference basin) is 4.9 ha while the East basin (treatment basin) is 3.2 ha. The reference basin's watershed is dominated by coniferous forest (63.2%; dominate species: balsam fir, *Abies balsamea*) while the treatment basin's watershed is dominated by deciduous forest (74.1%; dominate species: red and sugar maple, *Acer rubrum* and *Acer saccharum*) (Christensen et al. 1996). The mesh curtain allowed exchange of water between the two basins while keeping fish in their respective basins for two sampling seasons of pre-manipulation data.

In the autumn of 2012, we replaced the permeable curtain with an impermeable curtain, which we expected to naturally increase DOC concentration in the treatment basin based on results from Christensen et al. (1996). The impermeable curtain was a continuous piece of high-density polyester weave (Curry Industries) weighted with sand bags on the lake sediments and extending approximately 30 cm above the lake surface by floats sewn into the curtain. The curtain extended at least 15 m onshore to ensure lake division with fluctuating water levels. The natural increase in DOC in the treatment basin was driven by an intermittent inlet located at the East end of the treatment basin (Fig. 1). This intermittent inlet drains a 0.7 ha mixed hardwood wetland and contributes a majority of the terrestrial DOC load to Long Lake while contributing a small fraction of the hydrologic load. This inlet had highly colored water (mean water color at 440 nm: 58.35 m^{-1}) with DOC and nutrient concentrations that were much higher (mean DOC: 75.0 mg C L^{-1}; mean total phosphorus (TP): 54.6 μg P L^{-1}; mean total nitrogen: 2194.4 μg N L^{-1}) than pre-manipulation lake water (mean DOC: 8.0 mg C L^{-1}; mean TP: 16.7 μg P L^{-1}; mean total nitrogen: 446.6 μg N L^{-1}). We collected two sampling seasons (2013 and 2014) of post-manipulation data in both basins.

Lake physiochemical sampling

Integrated water samples from the upper mixed layer were collected weekly from May to August from the deepest part of the basins (14 m in both basins) for water chemistry analysis. We measured DOC on the filtrate of lake water passing through pre-combusted (450°C for 4 h) GF/F filters using a total organic carbon analyzer (TOC-V; Shimadzu Scientific Instruments), and TP was measured by colorimetric assay after persulfate digestion of unfiltered water (Menzel and Corwin 1965).

We estimated thermocline depth from high-frequency (10 min) temperature profiles using a fixed temperature chain (Onset HOBO Pendants; Onset Computer Corporation). We only used data from the thermistor chain when there was a complete profile of the lake water column. Due to thermistor malfunctions in 2011 and 2012, we removed several days of temperature profile data from the analysis as missing thermistor data resulted in unrealistic estimates of the thermocline and mixed layer and prohibited comparison between basins. We estimated the thermocline using the function *ts.thermo.depth* from the R package rLakeAnalyzer (Winslow et al. 2014), where we set the minimum density gradient to 0.1 kg m^{-3} m^{-1}, and both the "seasonal" and "na.rm" options to "FALSE." Volume-weighted whole-lake temperature was calculated using the *layer.temperature* function in rLakeAnalyzer (Winslow et al. 2014) and data from weekly temperature profiles measured with a YSI temperature profiler (YSI Professional Plus; YSI Incorporated) at 0.5 m depth intervals from 0 m to 8 m and 1 m depth intervals from 8 m to 14 m. We use weekly temperature profiles as opposed to the high-frequency profiles to calculate whole-lake temperature as the fixed high-frequency temperature chain only measured temperature to a maximum depth of 4 m.

Light climate was calculated as the average light reaching a particle in the surface mixed layer of the lake (assuming a well-mixed layer). The light intensity (I_z) at a given depth (z) at time t was estimated with Eq. 1, where I_0 was the incident photosynthetically active radiation (PAR) measured from a floating platform on the reference basin (Onset HOBO met station, Onset Computer Corporation), and the light extinction coefficient (k_D) was linearly interpolated between weekly estimates to 10-min intervals. We estimated weekly k_D values using water column PAR measured with an underwater quantum PAR sensor (LI-192SA; LI-COR Biosciences) and light meter (LI-250A) every 0.25 m from the surface to 1 m depth and 0.5 m depth intervals from 1 m to a depth which receives less than 1% of incoming light.

$$I_{z,t} = I_{0,t} \times e^{-K_{d,t} \times z} \qquad (1)$$

I_z was integrated over the surface mixed layer depth (z_{mix}) and then divided by z_{mix} to obtain an average light climate reaching a particle in units of mmol PAR m^{-2} s^{-1}. z_{mix} was calculated using the high-frequency thermistor data and the *ts.meta.depths* function from rLakeAnalyzer (Winslow et al. 2014). We aggregated light climate estimates to daily values by taking the mean of all estimates for each day. These estimates of light climate do not take into account reflection at the water surface, however, there was no significant difference in reflection between the basins estimated from difference between incoming PAR and PAR measured just below the water surface (*t*-test *p* value = 0.15; mean percent reflection in treatment basin = 34.5%, mean percent reflection in reference basin = 37.9%). Although the true light climate

may be closer to 62–65% of our reported values, this should not affect any interpretation of light climate differences or lack thereof between the two basins.

Terrestrial DOC flux estimates

We estimated daily terrestrial DOC flux to Long Lake by autonomously measuring stream discharge and precipitation, and manually sampling groundwater discharge. Water height in the inlet stream was measured every 10 min with a pressure sensor (Onset HOBO U20-001-04 Water Level Data Logger; Onset Computer Corporation) behind a V-notch weir (Daugherty and Ingersoll 1954). Stream discharge (Q) was estimated using Eq. 2:

$$Q = \frac{8}{15} \times \sqrt{2g} \times \tan\left(\frac{\theta}{2}\right) \times H^{5/2} \qquad (2)$$

where g is the acceleration due to gravity (9.81 m^2 s^{-1}), θ is the angle of the V-notch (90° for our weir), and H is water height above the bottom of the V-notch. Q was calibrated using stage-discharge relationship where discharge was measured using a salt slug injection procedure (Moore 2005). Stream DOC concentration was measured weekly to biweekly and linearly interpolated to discharge measurement frequency to estimate inlet stream DOC flux to the lake. The outlet stream has a diffuse flow, which precluded use of a V-notch weir to estimate discharge; instead we estimated outlet discharge using lake a stage-discharge relationship where discharge was measured using a salt slug injection procedure (Moore 2005). Outlet stream DOC flux was estimated as the product of outlet stream discharge and lake DOC concentration.

Groundwater discharge was estimated using seven in-lake piezometers installed in each basin. Hydraulic head (h; vertical distance between depth to water in each piezometer and lake water level) was measured at least monthly and values were averaged across the sampling period. Hydraulic conductivity (k) was estimated for each piezometer using slug tests (Bouwer 1989) and groundwater discharge (q) was estimated using Darcy's law:

$$q = k \times \left(\frac{h}{l}\right) \times a \qquad (3)$$

where l is the piezometer insertion depth (vertical distance between lake sediments and top of piezometer screen opening) and a is the piezometer cross-sectional area. All measurements of hydraulic head in both basins were negative, meaning that groundwater was recharging. Thus, outflow of DOC via groundwater was estimated as the product of lake DOC concentration and q.

Hourly rainfall was measured using a tipping bucket rain gauge (TE525MM-L; Campbell Scientific). Precipitation DOC was measured twice, and we apply the mean of these measurements across the entire time series (mean DOC = 3.2 mg L^{-1}) as an estimate of DOC flux to the lake via precipitation.

Daily terrestrial DOC flux to each basin was estimated as the sum of stream DOC flux and precipitation DOC flux. The mass balance of DOC in each basin was estimated using Eq. 4:

$$GW_{in} + S_{in} + P = GW_{out} + S_{out} + F + R + \Delta S \quad (4)$$

where *GW* is groundwater flux in or out of the lake; *S* is surface flow flux in or out of the lake; *P* is precipitation flux; *F* is DOC flocculation estimated as the product of DOC concentration and a rate constant (0.005 day^{-1}; von Wachenfeldt and Tranvik 2008; Jones et al. 2012); *R* is DOC oxidation including both biological respiration and photo-oxidation estimated from net ecosystem production (NEP); and ΔS is the change in storage of DOC within the lake.

Lake metabolism estimates

In each basin, we measured high-frequency (10-min interval) dissolved oxygen (DO) at a fixed depth (0.7 m) in the epilimnion (YSI 6600 V2 Sonde, YSI Incorporated), temperature profiles using a fixed temperature chain (Onset HOBO Pendants, Onset Computer Corporation), and meteorological parameters including wind speed, wind direction, air temperature, PAR, relative humidity, and barometric pressure from a floating platform on the reference basin (Onset HOBO met station, Onset Computer Corporation). All high frequency data was adjusted for sensor drift, if there was any, at weekly intervals. We estimated rates of gross primary production (GPP), respiration (R), and the difference between the two, termed net ecosystem production (NEP = GPP − R), by fitting a maximum likelihood metabolism model to the high-frequency DO cycles as described by Solomon et al. (2013). We estimated uncertainties in daily metabolism using a bootstrapping routine, where we create 1000 bootstrapped time series of DO data to which we fit the metabolism model, producing a distribution of 1000 GPP, R, and NEP estimates for each metabolism day. We report metabolism estimates in volumetric units (mg O$_2$ L^{-1} day^{-1}) and areal units (mg O$_2$ m^{-2} day^{-1}) as the product of volumetric rates and epilimnetic depth. We use linearly interpolated weekly temperature profiles as opposed to the high-frequency profiles in our epilimnetic depth calculation because the high-frequency profiles often detect microstratification that is shallower than the DO sensor placement (0.7 m).

Background respiration

In addition to total respiration, we calculated the background respiration for each lake-year based on methods from Solomon et al. (2013). Background respiration (β_0 in Eq. 5) is lake ecosystem respiration that is theoretically supported by recalcitrant allochthonous and autochthonous organic matter, and can be generally described as the *y*-intercept of the linear regression fit between R and GPP (standardized to 20°C, R_{20} and GPP_{20}; Holtgrieve et al. 2010), or in other words the hypothetical rate of R when GPP is zero (Eq. 5).

$$R_{20,t} = \alpha \times R_{20,t-1} + \beta_0 + \beta_1 \times GPP_{20,t} + \varepsilon; \quad \varepsilon \sim N(0, \sigma^2) \quad (5)$$

For each lake-year and for pre- and post-manipulation periods, we fit linear regression models with Gaussian error distribution to each of the 1000 bootstrapped sets of R_{20} and GPP_{20} to incorporate the uncertainty in the estimates of R and GPP into the uncertainty in the estimated background respiration. Additional parameters in Eq. 5 include the auto-correlation coefficient (α) and the rate of R_{20} per GPP_{20} (β_1). We then tested for significant differences in background respiration between the treatment and reference basins by checking for overlap in 95% confidence intervals calculated from these bootstrapped distributions of background respiration estimates. We used the R statistical package for all model fitting and subsequent analyses (R Core Team 2014). All the R code and data used in analyses are available on GitHub at https://github.com/jzwart/LongLakeMetab.

Steady state assumption

To compare our results to previously established lake processes, we occasionally compared our observed mean changes in metabolic rates to mean differences in chemical concentrations (DOC and TP) multiplied by literature-derived process rates. For comparison to NEP, we used the basin difference change in the DOC pool multiplied by the turnover rate of DOC typically reported for north-temperate lakes (0.005 day^{-1}; Hanson et al. 2011). For comparison to GPP, we used the change in inter-basin difference of TP converted to carbon fixation (or GPP) using the Redfield ratio (C : P, 106 : 1; Redfield 1934). In these comparisons, we assumed a steady state, "black box" ecosystem in which the difference in constituent concentrations between the treatment and reference basins was maintained by consistent fluxes throughout the pre- and post-manipulation periods. For example, on average DOC increased in the treatment basin by 4.2 mg L^{-1} from the pre- to post-manipulation periods and we assumed that this increase was sustained by consistent external DOC inflow to the lake supporting consistent internal fluxes of DOC oxidation. This assumption is a simplification; in reality there was variation in the constituent concentration differences caused by variation in constituent influxes and internal processes, in both pre- and post-manipulation periods. However, our use of the mean of many observed differences pre- and post-manipulation likely meets our steady state assumption and allows comparison of our observed mean metabolic differences to literature-derived, stead-state expectations.

Whole-ecosystem experiment interpretation

Experimentation at the ecosystem scale is challenging because replication oftentimes is not possible and other factors are beyond control (e.g., climate). The tradeoff between realism and degree of experimental control in experiments
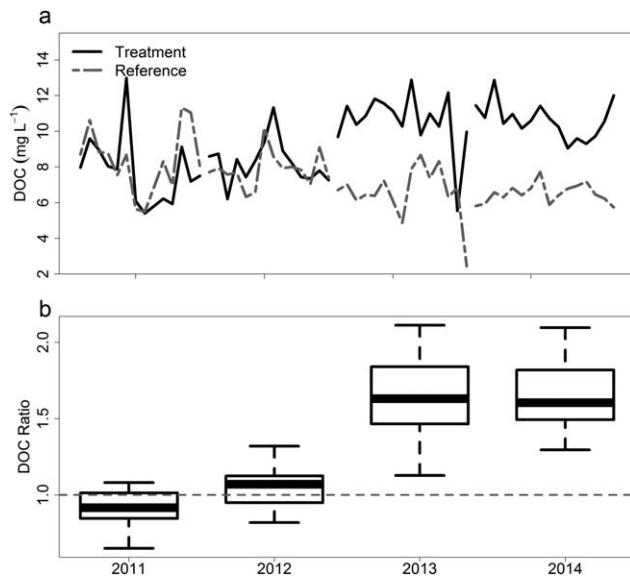
Fig. 2. (A) Time series of dissolved organic carbon (DOC) of the treatment and reference basins over the 4-year whole-ecosystem experiment. Lake basins were hydrologically isolated during the fall of 2012. Note that measurements were made between May 1 and September 30 each year and winter periods lacking observations are not shown along the x-axis. (B) The distribution of weekly, summertime DOC ratios (treatment: reference). Wide bars represent the median, with boxes showing the interquartile range, and the whiskers encompass the 95% interval for each year.

of differing scale has long been recognized (Carpenter 1998; Schindler 1998). To overcome the challenges of whole-ecosystem experiments, a reference, rather than control ecosystem, is used, and the ecosystem responses to the experimental treatment are evaluated as the difference between the treatment and reference ecosystems (Carpenter et al. 1989). This "controls" for any variability due to sources other than our experimental manipulation. For ease of language, we refer to the between-basin difference in a response variable as either the "increase" or "decrease" of the treatment basin in relation to the reference basin, despite the post-manipulation difference between basins resulting from simultaneous change in both basins. For example, our whole-lake manipulation increased DOC in the treatment basin from 8.0 mg L^{-1} to 10.6 mg L^{-1} while reducing DOC in the reference basin from 8.1 mg L^{-1} to 6.5 mg L^{-1}, and we refer to this divergence in DOC as an increase by 4.2 mg L^{-1}.

As there was a decrease in DOC concentration in our reference lake, we also discuss our metabolism results in light of an alternative reference lake, Paul Lake. We produced metabolism estimates using methods described above for Paul Lake, which has been used as a reference lake in many whole-lake experiments at UNDERC over 30+ years (e.g., Carpenter et al. 1987). We evaluated the treatment effect on GPP, R, and NEP by comparing differences in paired sam-

pling events pre- and post-manipulation in the treatment basin and Paul Lake using the same statistical analysis described below.

Statistical analysis

We evaluated the treatment effect on all physiochemical variables by comparing differences in paired sampling events pre- and post-manipulation in the treatment and reference basins using the Welch *t*-test (Stewart-Oaten et al. 1992). The Welch *t*-test is robust to large differences in the mean and variances in the pre- and post-manipulation periods. In the results, we report sample size of the pre- (*n* pre) and post-manipulation (*n* post) periods from the paired sampling time points.

We evaluated the treatment effect on metabolism variables using two approaches. First, we use the Welch *t*-test in the same fashion as the physiochemical variables. This test is not strictly valid as all metabolism estimates and high-frequency physiochemical variables were significantly temporally autocorrelated and failed to uphold the Welch *t*-test assumption of independence (Stewart-Oaten et al. 1992). Thus, we also implemented a test that explicitly accounts for autocorrelation by comparing an AR(1) model to an AR(1) plus treatment model using likelihood ratio tests. The treatment effect was significant in all cases (all likelihood ratio tests $p < 0.001$).

The *p* values from the Welch *t*-test for all metabolism variables were extremely low (all *p* values $< 1e^{-6}$), and we therefore argue autocorrelation in our data could not bias the Welch *t*-test to such a degree and alter the significance of our results (e.g., our chance of committing a type I error is low). Thus, for sake of clarity and consistency, in the results we report the Welch *t*-test as explicit treatment of autocorrelation did not change our interpretation of the Welch *t*-test.

Results

Our whole-lake manipulation successfully increased DOC in the treatment basin compared with the reference basin (4.2 mg L^{-1} increase; Fig. 2 and Table 1). The increase in DOC attenuated light more rapidly as k_D increased 1.05 m^{-1}. The whole-lake water temperature was significantly lower by 0.53°C and the thermocline and mixed layer depth became shallower by 0.30 and 0.13 m, respectively, as would be expected based on an increased k_D (Fig. 3). Despite the increased light attenuation, the average light climate in the epilimnion was not significantly reduced in the treatment basin (reduced by only 0.71 μmol PAR m^{-2} s^{-1}; Fig. 3). Additionally, we observed an increase in TP in the treatment basin compared with the reference basin (increased by 2.85 μg L^{-1}; Fig. 3). There were changes in several other limnological variables, including total nitrogen, iron, pH, chlorophyll *a*, and water color, which we report in supplementary text (Table S1).

Table 1. Summary table of variable means and standard deviation in parenthesis for treatment and reference basins both pre- and post-manipulation. Also reported are the statistical results for the Welch t-test for each variable (*see* Statistical analysis section for description of Welch t-test), where n pre and n post are the number of paired observations in the pre- and post-manipulation time periods, respectively.

Variable	Treatment Pre-manipulation	Reference Pre-manipulation	Treatment Post-manipulation	Reference Post-manipulation	n Pre	n Post	t	df	p
DOC (mg C L^{-1})	8.01 (1.68)	8.11 (1.47)	10.60 (1.34)	6.54 (1.09)	28	31	−9.57	55.70	<0.001
k_D (m^{-1})	1.82 (0.44)	1.64 (0.27)	2.86 (1.00)	1.62 (0.37)	28	30	−5.42	45.30	<0.001
Whole-lake temperature (°C)	14.97 (2.12)	15.44 (2.35)	12.60 (2.19)	13.58 (2.54)	29	31	4.17	55.65	<0.001
Thermocline depth (m)	2.40 (0.21)	2.42 (0.21)	1.89 (0.68)	2.21 (0.67)	7507	35,180	77.94	34,629.05	<0.001
Mixed layer depth (m)	0.28 (0.28)	0.34 (0.36)	0.61 (0.75)	0.80 (0.81)	7507	35,180	31.10	20,097.00	<0.001
Light climate (μmol PAR m^{-2} s^{-1})	456.53 (225.32)	459.63 (159.71)	322.90 (184.97)	326.72 (183.80)	49	244	0.03	52.32	0.98
TP (μg P L^{-1})	16.65 (5.45)	17.60 (6.50)	15.94 (4.45)	13.75 (4.54)	25	26	−2.40	42.15	0.035
R (mg O$_2$ L^{-1} day^{-1})	0.70 (0.44)	0.75 (0.64)	1.27 (0.82)	0.78 (0.46)	179	288	−7.98	399.19	<0.001
GPP (mg O$_2$ L^{-1} day^{-1})	0.57 (0.31)	0.62 (0.44)	0.71 (0.59)	0.45 (0.29)	179	288	−6.60	403.67	<0.001
NEP (mg O$_2$ L^{-1} day^{-1})	−0.13 (0.41)	−0.13 (0.44)	−0.56 (0.60)	−0.33 (0.38)	179	288	5.26	399.91	<0.001
R Areal (mg O$_2$ m^{-2} day^{-1})	1044.79 (781.30)	1128.55 (1034.10)	1940.40 (1558.15)	1301.13 (905.68)	179	288	−6.40	404.58	<0.001
GPP Areal (mg O$_2$ m^{-2} day^{-1})	844.40 (532.83)	989.57 (766.72)	1125.16 (1130.52)	779.06 (606.14)	179	288	−6.07	404.20	<0.001
NEP Areal (mg O$_2$ m^{-2} day^{-1})	−200.38 (661.75)	−138.98 (647.09)	−815.24 (1067.52)	−522.07 (575.61)	179	288	3.54	404.95	0.009

Ecosystem respiration increased in the treatment basin as a result of the DOC manipulation (increased 0.537 mg O$_2$ L^{-1} day^{-1}). GPP also increased in the treatment basin (increased 0.314 mg O$_2$ L^{-1} day^{-1}), however, not nearly as much as R, resulting in a more negative NEP in the treatment basin (decreased 0.237 mg O$_2$ L^{-1} day^{-1}) (Fig. 4). All metabolic results were also significant when quantified on an areal basis (Table 1).

We also compared the treatment basin metabolism estimates to an alternative reference lake, Paul Lake. Ecosystem respiration and GPP significantly increased in the treatment basin (increased 0.597 mg O$_2$ L^{-1} day^{-1} and 0.162 mg O$_2$ L^{-1} day^{-1} for R and GPP, respectively), while NEP significantly decreased in the treatment basin (decreased −0.244 mg O$_2$ L^{-1} day^{-1}). This indicates that the increase in GPP and R and decrease in NEP is significant in our treatment basin and does not result from a decline in DOC in the reference basin. For all subsequent analyses, we use our reference lake, West Long, for interpretation of the results as supplementary data (e.g., allochthonous DOC loading) was not available for Paul Lake. For Welch t-test results, see the Supplementary Material.

Background respiration increased across the experimental manipulation (increase of 0.137 mg O$_2$ L^{-1} day^{-1}), however, this increase was not significant as basin differences in background respiration had overlapping 95% confidence intervals between the pre- and post-manipulation periods. Background respiration was always higher in the treatment basin, and also exhibited strong patterns with annual precipitation as wetter years (post-manipulation) had higher background respiration in both basins (Fig. 5).

Discussion

DOC has strong physical, chemical, and biological effects, and therefore impacts all aspects of a lake's ecology (Solomon et al. 2015). In light of the recent phenomenon of global browning, studying how lakes will change under elevated DOC concentration scenarios is imperative. However, our current expectations for lake responses to widespread lake browning rely on substituting space for time (e.g., Hanson et al. 2003) or modeling (Read and Rose 2013), as adequate whole-ecosystem studies are lacking. To date there have been four whole-lake studies on the effects of increased DOC concentration on lake ecosystem processes. However, two of the studies involved extreme and rapid changes in DOC concentrations unlike those that have been observed in most natural systems (Sadro and Melack 2012; Brothers et al. 2014), another study added DOC in the form of sucrose, which is much more labile and less chromophoric than natural DOC (Blomqvist et al. 2001), and the fourth study unexpectedly observed an increase in DOC concentration that confounded a nutrient and food-web structure manipulation experiment (Christensen et al. 1996). In this study, we induced an increase in DOC concentration, using
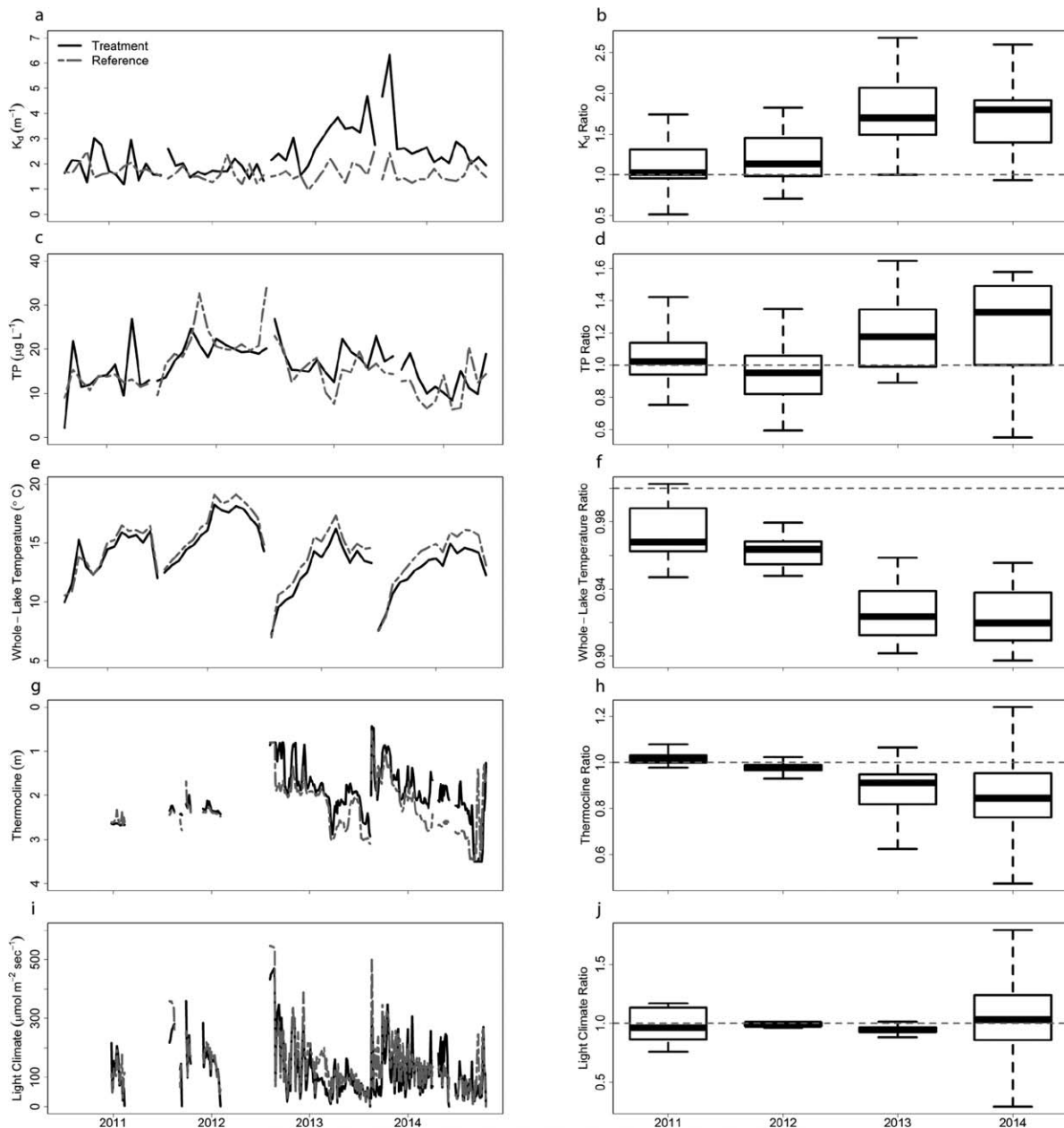
Fig. 3. Time series of (A) light attenuation (K_d), (C) total phosphorus (TP), (E) whole-lake temperature, (G) thermocline depth, and (I) light climate in the epilimnion for both the treatment and reference basins over the 4-year experiment. Periods of each year during which time observations of the lake were not made (October–April) are omitted from these plots. Annual distributions of weekly ratios (treatment : reference) of all physiochemical parameters are presented to the right of each time series and are formatted as in Fig. 2.

a natural source, by a magnitude consistent with observed long-term trends (Monteith et al. 2007) in one basin of an experimental lake to examine the physiochemical and basal metabolic responses of a lake under elevated DOC concentration.

Metabolic responses

We unexpectedly observed an increase in GPP as a result of our whole-lake manipulation. This increase in GPP was likely due to the increase in TP as well as the lack of change

in light climate across the experimental manipulation. TP has been commonly used as a conservative indicator of the often-limiting nutrient for primary productivity in lakes (Schindler 1977), and there have been numerous spatial surveys showing positive correlation between TP and GPP (e.g., Smith 1979; Wetzel 2001; Hanson et al. 2003; Solomon et al. 2013). Furthermore, based on ecological stoichiometry, we expected that an increase in phosphorus would result in an increase in carbon fixed by phytoplankton. Assuming a
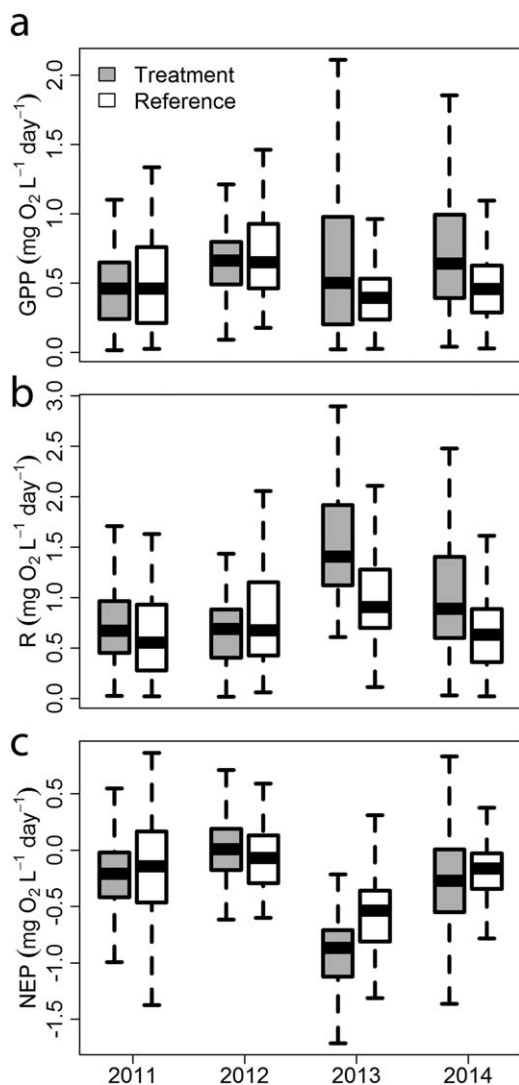
Fig. 4. Annual distribution of daily gross primary production (GPP, panel A), respiration (R, panel B), and net ecosystem production (NEP, panel C) for both the treatment and reference basins. Box plots show the 5th, 25th, 50th, 75th, and 95th percentiles.

The increase in phosphorus due to our experimental manipulation was likely not an experimental artifact but rather an often overlooked elemental flux occurring in concert with the global browning phenomenon (Kopáček et al. 2015). One of the most highly cited explanations for lake browning has been the recovery from sulfate deposition and acidification (Monteith et al. 2007). As soil pH increases from decreased sulfate deposition and acidification, dissolved organic matter becomes more soluble and is released into stream and lake water. Dissolved organic matter consists of nutrients as well as carbon (Lennon and Pfaff 2005) and, thus, limiting nutrients such as phosphorus are likely more readily transported into lake water under this mechanism of global browning. There are additional hypothesized mechanisms leading to lake browning, such as hydrologic changes, which was the mechanism we used in our whole-lake experiment. As there is often DOC and TP co-export from the terrestrial landscape (e.g., Dillon and Molot 1997; Dillon and Molot 2005), increased stream water and solute load to a lake will likely result in increased DOC and TP concentrations.

The lack of change in the average epilimnetic light climate in the treatment relative to the reference basin also must have played a role in enabling the unexpected increase in GPP in the treatment basin. Both the intensity of light attenuation and depth of the surface mixed layer determine the amount of light exposure for phytoplankton (Eq. 1). Elevated k_D or a deeper z_{mix} or both occurring jointly will reduce the amount of light exposure to epilimnetic phytoplankton. In our whole-lake experiment, we observed an increase in k_D but a shallower z_{mix}, which resulted in essentially no change in epilimnetic light climate ($< 1\%$ reduction in light climate). Counterintuitively, epilimnetic phytoplankton in lakes subject to elevated DOC do not necessarily experience lower light levels because z_{mix} may become shallower due to a change in the vertical structure of heat from the increased k_D (Read and Rose 2013). However, lake size likely modifies the relationship
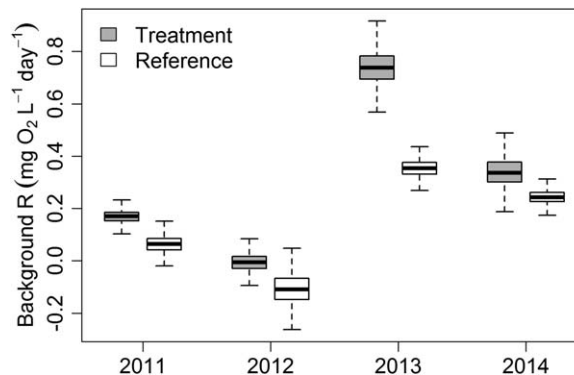

Fig. 5. Box plots of annual background respiration for both the treatment and reference basins. Box plots show the 5th, 25th, 50th, 75th, and 75th percentiles of 1000 bootstrapped estimates of this quantity for each basin-year combination.

steady state system (*see* Methods for description), a Redfield ratio of 106 : 1 (C : P), and a 1 : 1 respiratory quotient (CO$_2$: O$_2$), we expected an increase of 2.85 μg P L^{-1} to result in a GPP increase of 0.31 mg O$_2$ L^{-1} day^{-1}. This was remarkably close to our observed increase in GPP (0.314 mg O$_2$ L^{-1} day^{-1}), and suggests the modest increase in TP was sufficient to explain the unexpected increase in GPP. Interestingly, the average increase in external P loading to the treatment basin (0.117 μg P L^{-1} day^{-1}) could not be used to explain the increase in GPP using the Redfield ratio and 1 : 1 respiratory quotient, highlighting the likely importance of rapid epilimnetic phosphorus cycling for support of GPP within the treatment basin.

between elevated DOC and light climate due to differences in the dominant source of turbulence in the epilimnion. As shown previously, wind shear contributes most to epilimnetic turbulence for larger lakes (surface area > 0.5 km^2) while convection is the dominant source of turbulence for smaller lakes (Read et al. 2012). Wind shear mixing in large lakes can sustain a deep z_{mix} even when penetration of solar radiation is reduced, indicating that larger lakes subject to elevated DOC may exhibit a reduction in epilimnitic light climate as k_D would increase while z_{mix} changes minimally. Conversely, smaller lakes subject to elevated DOC may even exhibit a slight increase in epilimnetic light climate as solar radiation and molecular diffusion of heat drive z_{mix} in small lakes. Thus, z_{mix} may become shallower more rapidly than the increase in k_D. Based on a lake size relationship from Read et al. (2012), we expected convection rather than wind shear to be the larger source of epilimnetic turbulence to our experimental lake; therefore, our DOC manipulation had a large effect on the vertical heat structure of our lake, and was likely the reason why epilimnetic light climate did not change despite the increased k_D.

The respiration increase exceeded the GPP increase in the treatment basin, thus, we observed a large decrease in epilimnetic NEP in response to elevated DOC. The observed decrease in NEP can be explained by the excess DOC loaded to the treatment basin relative to the reference basin. During the post-manipulation years, the treatment basin received on average 0.10 mg C L^{-1} day^{-1} more DOC compared with the reference basin. As we estimated that only 10% of terrestrial DOC loaded to the treatment basin was exported, we expected the excess carbon load to have a large impact on heterotrophic respiration. Indeed, using a 1 : 1 respiratory quotient, the average decrease in NEP was 0.089 mg C L^{-1} day^{-1}, or nearly 90% of the excess allochthonous DOC loaded to the treatment basin. This result indicates that changes in lake heterotrophy under global browning scenarios may be predictable if there are well-constrained estimates of allochthonous DOC loading.

With an increase in allochthonous DOC loading to the treatment basin, we expected an increase in respiration of the slow-to-degrade carbon pool (background respiration), as allochthonous DOC has typically been thought to be recalcitrant in nature. However, we observed only a slight, nonsignificant increase in background respiration due to our experimental manipulation. Background respiration increased by 0.137 mg O$_2$ L^{-1} day^{-1}, equating to 0.051 mg C L^{-1} day^{-1}, or about 50% of the decrease in NEP or increase in allochthonous carbon load. This indicates that the respiration by heterotrophs of the slow-to-degrade carbon pool can only account for about one half of the observed excess respiration over primary production. This means that either: (1) there was a significant amount of allochthonous carbon that is being respired quickly (within a few days or weeks) once entering the lake, (2) there was a priming effect from the increase in GPP, or (3) there was fast

photo-oxidation or photochemically mediated bacterial oxidation of the newly transported carbon, or all three processes occurred at once. Previous work has shown that dissolved labile low-molecular weight allochthonous carbon can support as much as 95% of bacterial production in some lakes (Berggren et al. 2010), and the priming effect can lead to fast mineralization of terrestrial carbon (Guenet et al. 2010, 2014; Bianchi et al. 2015), however, see Bengtsson et al. (2014) and Catalán et al. (2015). Additionally, photo-oxidation or photochemically mediated bacterial oxidation can sometimes account for a majority of DOC mineralization, especially in newly transported carbon (Cory et al. 2013, 2014). Although we cannot distinguish between these three mechanisms with our results, it seems clear that some or perhaps even a majority of the observed decrease in NEP was a result of a relatively quick turnover of allochthonous carbon.

Whole-ecosystem experiments often reveal processes that cannot be captured using bottle or mesocosm experiments (Schindler 1998). For example, bottle incubations are the basis for estimating turnover rates of DOC in lakes, typically reported as 0.005 day^{-1} for north-temperate lakes (Hanson et al. 2011). However, if we use this rate multiplied by the increase in DOC concentration across the experiment (increase of 4.2 mg C L^{-1}), we would expect only a 0.021 mg C L^{-1} day^{-1} decrease in NEP due to our experimental manipulation, which would vastly underestimate our observed decrease in NEP (0.089 mg C L^{-1} day^{-1}) or increase in allochthonous DOC load (0.10 mg C L^{-1} day^{-1}). These results add further support to the existence of a relatively labile allochthonous carbon pool respired quickly once transported to the lake, and also echoes the need to constrain lake organic carbon budgets to inform estimates of in-lake process rates (Hanson et al. 2014).

Physiochemical responses

The increase in DOC concentration caused dramatic changes in the temperature regime of our treatment basin, as we observed a decrease in the thermocline and mixed layer depths. Vertical structure of heat in small lakes ($<$ 0.5 km^2) is typically driven by molecular diffusion and the attenuation of light (Read and Rose 2013), and as small lakes dominate globally (Downing et al. 2006), the recent browning phenomenon likely has strongly affected the temperature regime in many lakes worldwide. Thermocline and z_{mix} control habitat use for phytoplankton, zoobenthos, and fish, and as such, may adversely affect consumer productivity in many lakes due to a reduction in suitable habitat as shown in previous studies (Kelly et al. 2014; Craig et al. 2015).

Whole-lake temperature significantly decreased due to reduced heat penetration resulting from experimentally elevated DOC concentrations. This decrease was similar in magnitude to modeled expectations (Read and Rose 2013), and has important implications for metabolic rates at all trophic levels, from bacterial mineralization of sedimented carbon

(Gudasz et al. 2010) to fish growth (Blair et al. 2013). Non-linear relationships of whole-lake temperature in response to climatic warming are likely to occur under global browning. For example, modeling results showed that clear lakes to be more sensitive to changes in air temperature, indicating that DOC may buffer lakes to the effects of climatic warming (Read and Rose 2013).

Conclusion

Results from this whole-lake experimental increase in DOC demonstrate that relying solely on cross-system comparative studies, bottle incubations, and mesocosm experiments may drastically mislead expectations of important ecosystem responses to global environmental change. Contrary to expectations gleaned from cross-system comparative studies, we observed an increase in GPP as a result of our experimental manipulation, because limiting nutrient concentrations increased and the average epilimnetic light climate did not change. This increase in GPP emphasizes the importance of coupled elemental cycles, the often over-looked co-export of carbon and limiting nutrients from watersheds to lakes, and the effect of lake size on hydrophysics under global browning scenarios. The more negative NEP resulting from the increased DOC demonstrates that global browning likely has had a large influence on lake carbon flux to the atmosphere, and that a significant portion of allochthonous carbon may be relatively labile and quickly oxidized once transported to lakes. Relying on bottle incubations would exclude important processes such as continuous flux of newly transported terrestrial carbon, a priming effect from increased GPP, and photochemically mediated bacterial oxidation, which can lead to an expected DOC turnover rate nearly one-half of what was detected at the ecosystem scale.

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