

LETTER

Synthesis of a 33-yr series of whole-lake experiments: Effects of nutrients, grazers, and precipitation-driven water color on chlorophyll

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Scientific Significance Statement

Phosphorus inputs and food web structure have strong effects on phytoplankton biomass (chlorophyll) within a few weeks of experimental manipulation of whole lakes. Effects of water color on chlorophyll may be missed in experiments lasting only a few years. Over decades, long-term trends in precipitation drive changes in water color. Responses of chlorophyll to long-term variation in color are comparable in magnitude to effects of experimental manipulations of grazers and phosphorus.

Abstract

We conducted a 33-yr series of whole-lake experiments to measure ecosystem responses to food web structure and nutrient load, compare aquatic and terrestrial carbon flows to consumers, and evaluate indicators of ecosystem resilience. These manipulations showed that chlorophyll responded to nutrient loading and to grazing controlled by a trophic cascade. In this article, we synthesized experimental results using a new analysis of heretofore unrecognized variation in water color, measured as light absorbance at 440 nm. Long-term data revealed fluctuations in precipitation that drive water color variation. We compared effects of nutrient loading, zooplankton biomass, zooplankton body size, and water color on chlorophyll. Water color was an important factor in the chlorophyll response. This driver of the chlorophyll response to manipulation was not resolved until decades of data were available. A long-term context enriched insights from ecosystem experiments and exemplified the complementarity of experimental and long-term approaches in limnology.

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Data Availability Statement: Data are available in the North Temperate Lakes Long-Term Ecological Research database (<http://lter.limnology.wisc.edu/data>) as “cascade project” and at URLs:

<https://lter.limnology.wisc.edu/node/55082/eml> File: ntl17_1_v6.csv.
<https://doi.org/10.6073/pasta/e92243f8bc840d583fdb1d5910691c46>
<https://doi.org/10.6073/pasta/6a658526e313dbcecb0331a1f343c01>
<https://doi.org/10.6073/pasta/8d71e8d3fdec619807e5c05fa1b3eb13>
<https://doi.org/10.6073/pasta/6fc6015c620056034512fde089d50c27>

More detailed information about accessing the data is provided in the Supporting Information found in the online version of this article.

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

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The brown color of lakes is associated with inputs of terrestrial dissolved organic matter (tDOM). The concentrations of tDOM in lakes are typically correlated with light absorbance by a filtered water sample at 440 nm, a measurement referred to as water color (Cuthbert and del Giorgio 1992). Some lakes are “browning” (i.e., increasing in water color) due to increased inputs of tDOM from associated changes in climate, acidification, hydrology, and/or land use (Monteith et al. 2007; Kritzberg 2017; de Wit et al. 2018; Wauthy et al. 2018). Even where there are not directional trends in water color, variability in color can have important impacts on light transmission, primary production, ecosystem metabolism, nutrient availability, and predator-prey interactions (Carpenter et al. 1998; Williamson et al. 1999; Webster et al. 2008; Batt et al. 2015; Zwart et al. 2016; Weidel et al. 2017). Thus, water color is an important feature of lakes in general and specifically in relation to large scale drivers of environmental change (Fergus et al. 2016). Importantly, water color changes in lakes are largely driven by changes occurring in watershed inputs to lakes (Dillon and Molot 1997).

Current and future changes in water color will alter lake ecosystems and in this context, ecosystem experiments are a potentially powerful method for learning about controls, processes, and dynamics of these changes on intact ecosystems (Likens 1985). Prior whole-lake studies using diverse approaches have shown that phytoplankton biomass is affected by nutrients, especially phosphorus (P), and food-web structure. A series of whole-lake experiments at the Canadian Experimental Lakes Area established the effects of P on phytoplankton biomass (Schindler 1974, 2006). Our own whole-lake manipulations of food web structure have shown that trophic cascades affect dominance by large-bodied cladoceran grazers and phytoplankton biomass (Carpenter and Kitchell 1993). Further whole-lake experiments showed that effects of trophic cascades from fish to zooplankton to phytoplankton are comparable in strength to those of P inputs (Carpenter et al. 2001).

In this study, we use existing data from a series of whole-lake experiments to investigate the significance of water color in regulating phytoplankton biomass. The ecosystem experiments were designed to address the roles of nutrients and trophic cascades on phytoplankton biomass, pathways of carbon flow to consumers in lakes, effects of shading on phytoplankton and carbon sources to consumers, and resilience indicators of shifts between alternative states in lakes (Carpenter and Kitchell 1993; Carpenter et al. 2001, 2005, 2011; Pace et al. 2004, 2017; Wilkinson et al. 2014, 2018; Batt et al. 2015). Over the 33-yr duration of these experiments, precipitation varied roughly fourfold over time. This variation in precipitation was associated with roughly threefold variation in water color measured as light absorbance at 440 nm (g_{440}). This article evaluates the long-term role of this unexpected variation in water color in the responses of phytoplankton to experimental manipulation, and synthesizes the

joint effects of P load, trophic cascades as indexed by cladoceran body size, and color on phytoplankton biomass.

Materials and methods

Study sites and manipulations

We conducted multiple whole-ecosystem manipulations over varying sampling periods in Crampton, Long, Paul, Peter, Tuesday, and Ward lakes. The lakes were located within 6 km of one another at the University of Notre Dame Environmental Research Center (89° 32'W 46° 13'N) near Land O'Lakes, Wisconsin. Manipulations included fish additions, fish removals, nutrient additions, and tracer stable isotope additions (Table 1). This article analyzes data from the summer stratified season, about 15 weekly samples per year for lakes that were sampled in years before, during, and after manipulations, except for Ward Lake which was sampled before and during manipulation (Supporting Information).

Crampton Lake was sampled from 2003 to 2006. In 2005, Crampton was labeled with $\text{NaH}^{13}\text{CO}_3$ to study carbon flow through the food web (Pace et al. 2007).

Long Lake was sampled from 1988 to 1999 (Carpenter et al. 2001) and again in 2003–2006. In 1991, the lake was divided into separate East and West basins with curtains, leaving a shallow central basin. The East and West basins were subjected to experimental nutrient enrichments from 1993 to 1997. The western curtain was removed in September 1997, after data collection was complete for that year. East Long Lake was labeled with $\text{NaH}^{13}\text{CO}_3$ to study carbon flow through the food web. The eastern curtain was removed in 2003.

Paul Lake served as a reference lake throughout the project (Carpenter and Kitchell 1993) and was also labeled with $\text{NaH}^{13}\text{CO}_3$ in 2001 (Carpenter et al. 2005).

Peter and Tuesday lakes were used in a reciprocal food web transfer experiment in 1984–1986 (Carpenter et al. 1987; Carpenter and Kitchell 1993). Fifty eight kilograms of piscivorous largemouth bass were moved from Peter to Tuesday, and 56 kg of planktivorous minnows were moved from Tuesday to Peter. The introduced bass were removed from Tuesday Lake in September 1986 and replaced with the lake's original minnow species in May 2007.

Following this initial experiment, Peter Lake was managed as a planktivore-dominated lake through 2007 (Carpenter and Kitchell 1993; Carpenter et al. 2001). In 1993–1997, Peter Lake was enriched with nutrients as part of the aforementioned experiment with East and West basins of Long Lake (Carpenter et al. 2001). Peter Lake was labeled with $\text{NaH}^{13}\text{CO}_3$ to study carbon flow in 2001, 2002, and 2012 (Carpenter et al. 2005; Wilkinson et al. 2014). In 2002, the lake was also enriched with nutrients to assess their effects on carbon flow. From 2008 to 2011, largemouth bass were added gradually to Peter Lake to evaluate statistical indicators of ecosystem resilience (Carpenter et al. 2011). Resilience indicators were also

Table 1. Characteristics and histories of measurements and manipulations for the study lakes that were used in this article. For details of measurements and manipulations, see Carpenter and Kitchell (1993), Carpenter et al. (2001), Pace et al. (2004), Carpenter et al. (2005), Pace et al. (2007), Carpenter et al. (2011), Batt et al. (2015), Pace et al. (2017), Wilkinson et al. (2014), and Wilkinson et al. (2018).

Variable	Crampton	East Long	Paul	Peter	Tuesday	Ward	West Long
Area (ha)	25.9	2.3	1.7	2.6	0.91	1.9	3.4
Mean depth (m)	5.0	4.4	3.7	5.7	4.0	1.2	4.8
Years of data	2003–2007	1989–1999 2004 2006	1984–1999 2001–2016	1984–1999 2001–2016	1984–1990 2002 2010; 2012 2012–2016	2010; 2012	1989–1999 2003–2006
Curtain present		5/1991–2003					5/1991–9/1996
¹³ C labeling	2005	1999	2001	2001; 2002; 2012	2002		
P and N additions		1991–1996		1991–1996; 2002; 2013–2015	2013–2015		1991–1996
Experimental darkening						2012	
Rotenone treatment				5/1991	9/1986		
Planktivore addition				1985; 1988–1990; 1991; 2008	1987		
Planktivore removal					1985		
Piscivore addition				2008–2011	1985		
Piscivore removal				1985			

the focus of a nutrient enrichment experiments in Peter and Tuesday lakes from 2013 to 2015 (Pace et al. 2017; Wilkinson et al. 2018).

In addition to the experiments described above, Tuesday Lake was labeled with $\text{NaH}^{13}\text{CO}_3$ in 2002 to assess carbon flow in the food web of a relatively stained lake (Carpenter et al. 2005).

Ward Lake was sampled in 2010 and 2012 (Batt et al. 2012, 2015). In 2012, Ward Lake was darkened experimentally with Aquashade® to evaluate the effects of shading on ecosystem metabolism and carbon flow to consumers. Unlike natural dissolved organic carbon (DOC), Aquashade® is not available as a carbon source to microbes.

Time series data

The lakes were sampled weekly during the summer stratified season, roughly mid-May to mid-September (Supporting Information). Methods for measuring profiles of temperature, irradiance, zooplankton biomass, zooplankton body size, and fluorometric chlorophyll concentration corrected for pheophytin are reported in Carpenter and Kitchell (1993) and an online methods manual (linked to data in Supporting Information). K_d , the vertical light attenuation coefficient for photosynthetically active radiation, was calculated in m^{-1} for the epilimnion using irradiance profiles (Kirk 1994). Light attenuation not due to chlorophyll, K_r , was calculated for the epilimnion as $K_r = K_d - K_{chl} \cdot [\text{Chl}]$ where K_{chl} is the extinction coefficient for chlorophyll measured directly in these lakes ($0.0177 \text{ m}^2 \text{ mg}^{-1}$, (Carpenter et al. 1998)) and $[\text{Chl}]$ is the epilimnion concentration of chlorophyll in mg m^{-3} .

Weekly measurements of color began in 1996. Color was determined from integrated mixed layer water samples. Water was filtered (GF/F glass fiber filters) and absorbance measured at 440 nm with a 10 cm cuvette using reverse-osmosis water as a blank. Color is expressed in units of m^{-1} and denoted as g440.

Statistical analysis

A regression model was developed to estimate g440 in the experimental and reference lakes for 1984–1995 using precipitation and limnological data that were measured in all lakes and all years, and g440 data measured in all lakes in 1996–2016. The variables used for the regression were precipitation for the water year (01 October to 30 September) because concentrations of tDOM in lakes integrate watershed processes over multimonthly or longer time frames (Cardille et al. 2009). Regressions also included weekly measurements of K_r , depth of the thermocline, and temperature of the epilimnion. Lake-specific intercepts were included for each lake using dummy variables (0, 1). We included only dummy variables that improved AIC (Akaike's Information Criterion). We found that square-root transformation was necessary to approximate normally distributed residuals for regressions to predict g440 (Supporting Information). Normal probability plots and collinearity statistics were examined for each regression (Supporting Information).

We used AR(1) time series regressions with 1-week time steps to evaluate the effects of P load, grazers, and g440 on two chlorophyll response variates: areal chlorophyll integrated through the photic zone to 10% light depth, and

volumetric concentration of chlorophyll in the epilimnion. Estimated g440 from the above model was used for all lakes and years in the chlorophyll models. We fit 1-week projections of log10-transformed chlorophyll to current values of log10-transformed chlorophyll, cladoceran body length (mean weighted by biomass per individual), crustacean or cladoceran biomass (chosen using AIC), estimated g440, and precipitation. These four independent variates were used to compare the effects of P load, grazing, and color on chlorophyll. We included dummy variables (0, 1) for individual lakes only if they improved AIC. We limited our analyses to these relatively simple models based on our experimental design and the potential role of g440. After fitting the models, we examined residuals using normal probability plots, autocorrelation functions, collinearity statistics, and correlations with interaction terms and other potential drivers. All statistics were calculated in R 3.5.1 using the base and *olsrr* packages (Hebbali 2018; R Core Team 2018).

Results

Precipitation and color

After curtain installation in 1991, East Long Lake attained extreme values of g440 well beyond the range of values observed in other lakes, and responded differently to precipitation and nutrient enrichment (Christensen et al. 1996). Because this artificial rise in g440 would bias the regression model for other lakes (Supporting Information), we did not consider East Long Lake in our overall regression model. The unique behavior of East Long Lake was discussed by others (Christensen et al. 1996; Carpenter et al. 1998) and is subject of further experiments (Zwart et al. 2016).

Total water-year precipitation was lowest for 2007 (i.e., 01 October 2006 to 30 September 2007) and highest for 2016 during the study period (Supporting Information Fig. S1). Precipitation during the 6-month period of highest biological activity, April to September, was also minimal in 2007 and maximal in 2016. The precipitation time series had three relatively distinct periods. Precipitation was stable from 1984 to 1999, low from 2000 to 2009 where only 3 yr had a total precipitation > 600 mm, and high from 2013 to 2016 where several years had total precipitation > 1000 mm (Supporting Information Fig. S1).

Observed light absorbance by dissolved organic matter, g440, varied widely among lakes (Supporting Information Fig. S2). In some lakes, g440 also varied among years. Crampton, Paul, and Peter lakes had consistent levels of g440, except for elevated levels in Peter Lake in 2013 and 2014, years of relatively high precipitation and experimental P enrichment. Tuesday, Ward, and West Long lakes have higher and more variable levels of g440. Like Peter Lake, Tuesday Lake also had unusually high levels of g440 in 2013 and 2014, years of high precipitation and experimental nutrient input. West Long

Lake's g440 was variable and had no obvious association with removal of the east curtain in 2003 or experimental P addition in 1996 and 1997. In Ward Lake (sampled only in 2010 and 2012) g440 was slightly elevated in 2012, the year that the lake water was experimentally darkened with a commercially available dye. This change was likely due to light absorption by the dye and reduced photodegradation of naturally occurring organic matter.

Annual precipitation from April to September was associated with g440, with apparent differences in y -axis intercept among the lakes, for the four lakes with relatively long time series (Paul, Peter, Tuesday, and West Long) (Fig. 1A). The model for weekly data explained more than 93% of the variance in square-root transformed g440 (Fig. 1B, Supporting Information Table S2). Precipitation in April–September and P load had positive effects on g440. Depth of the thermocline was inversely related to g440 (Houser 2006). Thermocline depth was moderately collinear with other predictors but nonetheless improved AIC. Light extinction corrected for chlorophyll was positively associated with g440. This association is expected because the two variates measure similar features of lake water. Temperature of the epilimnion had a weak positive link to g440. Each lake had a unique intercept relative to Crampton Lake. Lake effects were collinear (Supporting Information Table S2) because individual experiments used subsets of the lakes (Table 1). Nonetheless, accounting for lake-specific intercepts improved AIC. Predictions and observations of the square root of g440 clustered along the 1 : 1 line (Fig. 1B, see Supporting Information for method to transform predictions to original units).

Chlorophyll response

Among all lakes, areal chlorophyll ranged from about 2–150 mg m⁻² in weekly samples over the period 1984–2016 (Supporting Information Fig. S4). Variability is evident over time in each lake, and among lakes. More detailed analysis, however, shows that much of this variability is attributable to manipulations, changes in color, or lake characteristics represented by lake-specific intercept terms.

Areal chlorophyll was strongly responsive to all independent variates (Supporting Information Table S3). Removal of any single independent variate increased AIC. Effects of P load were positive. Effects of cladoceran length and crustacean biomass were negative and positive, respectively (Carpenter and Kitchell 1993; Carpenter et al. 2001). The effect of g440 was negative. Paul Lake had a unique intercept.

One-step ahead predictions were directly correlated with observations ($R^2 = 0.54$, Fig. 2A). Residuals showed no apparent nonlinearities or differences among lakes. Residuals were uncorrelated with thermocline depth, epilimnion temperature, K_d, and K_d corrected for absorbance by chlorophyll. The six pairwise interaction terms for the four model effects were not correlated significantly with the residuals.

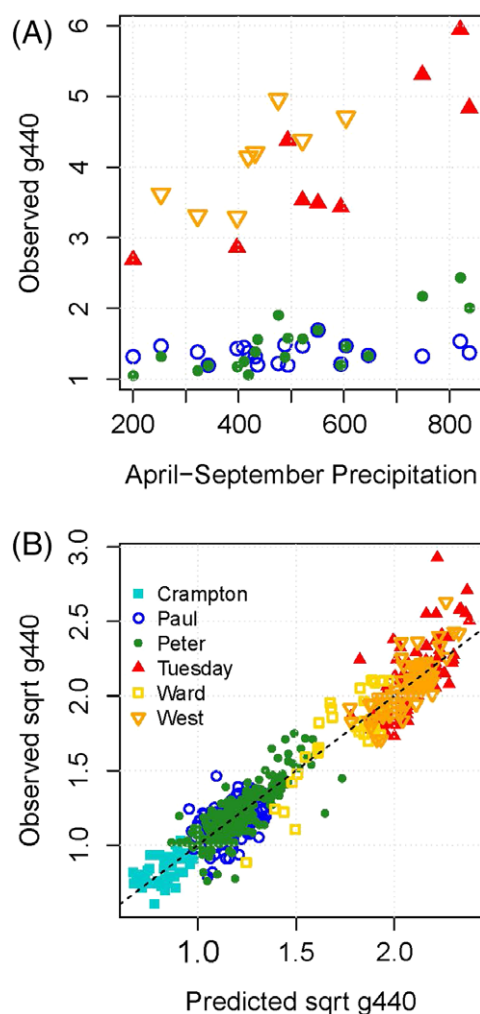


Fig. 1. (A) Precipitation (mm) in April–September vs. g440 for four lakes with relatively long-time series. (B) Predictions vs. observations for the regression model used to fill in missing g440 values (Supporting Information Table S1). Axes are square-root transformed. Dashed black line has intercept 0 and slope 1.

Epilimnetic chlorophyll concentrations ranged from less than 1 mg m^{-3} to over 100 mg m^{-3} in Peter Lake (Supporting Information Fig. S5). Other lakes showed smaller but still notable temporal variability. Much of this variability was attributable to manipulations, color, and intrinsic lake characteristics.

Chlorophyll concentration in the epilimnion was also responsive to all independent variates (Supporting Information Table S4). Removal of any single predictor increased AIC. Effects of P load rate, cladoceran length, and crustacean biomass were consistent with results for areal chlorophyll. However, the effect of g440 was in the opposite direction, or positive for epilimnetic concentration. This suggests that higher water color may concentrate a given mass of chlorophyll in a thinner epilimnion.

One-step ahead predictions of epilimnetic chlorophyll were well-correlated with observations ($R^2 = 0.68$, Fig. 2B). As with

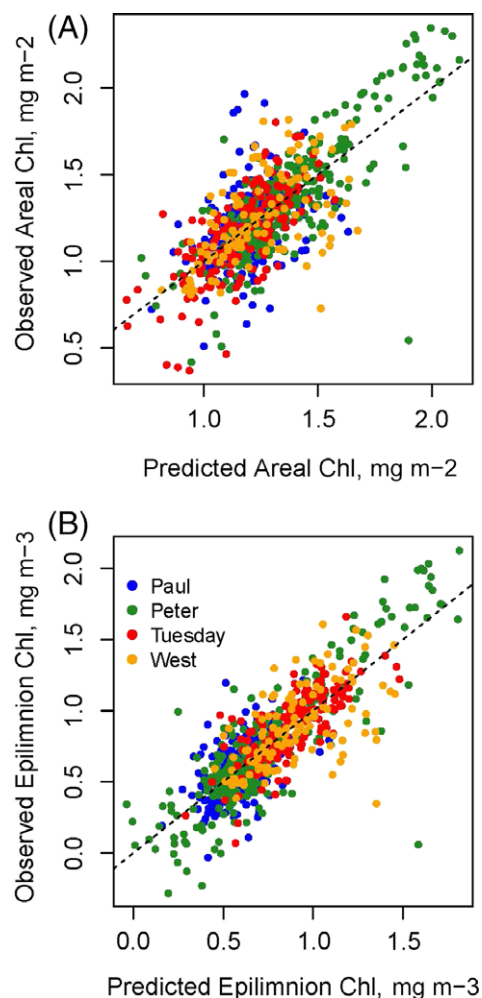


Fig. 2. (A) Predictions vs. observations for the regression model to predict areal photic-zone chlorophyll (mg m^{-2}) in four lakes (Supporting Information Table S2). (B) Predictions vs. observations for the regression model to predict epilimnion chlorophyll concentration (mg m^{-3}) in four lakes (Supporting Information Table S3). In both panels, dashed black line has intercept 0 and slope 1. All axes are log10 transformed.

areal chlorophyll, we detected no correlations of the residuals with thermocline depth, epilimnion temperature, K_d , and K_r . However, two of the two-way interactions were significantly correlated with epilimnetic chlorophyll. P load X crustacean biomass was positively correlated with residuals ($r = 0.0697$, $p = 0.038$). P load X g440 was inversely correlated with residuals ($r = -0.0650$, $p = 0.052$).

Discussion

At the start of these whole-lake experiments, we did not anticipate that they would lead to the 33-yr series of studies presented here. Each experiment, combined with findings of other investigators in other places, prompted new questions and new research. Our manipulations were designed to test the importance of nutrient inputs and trophic cascades as

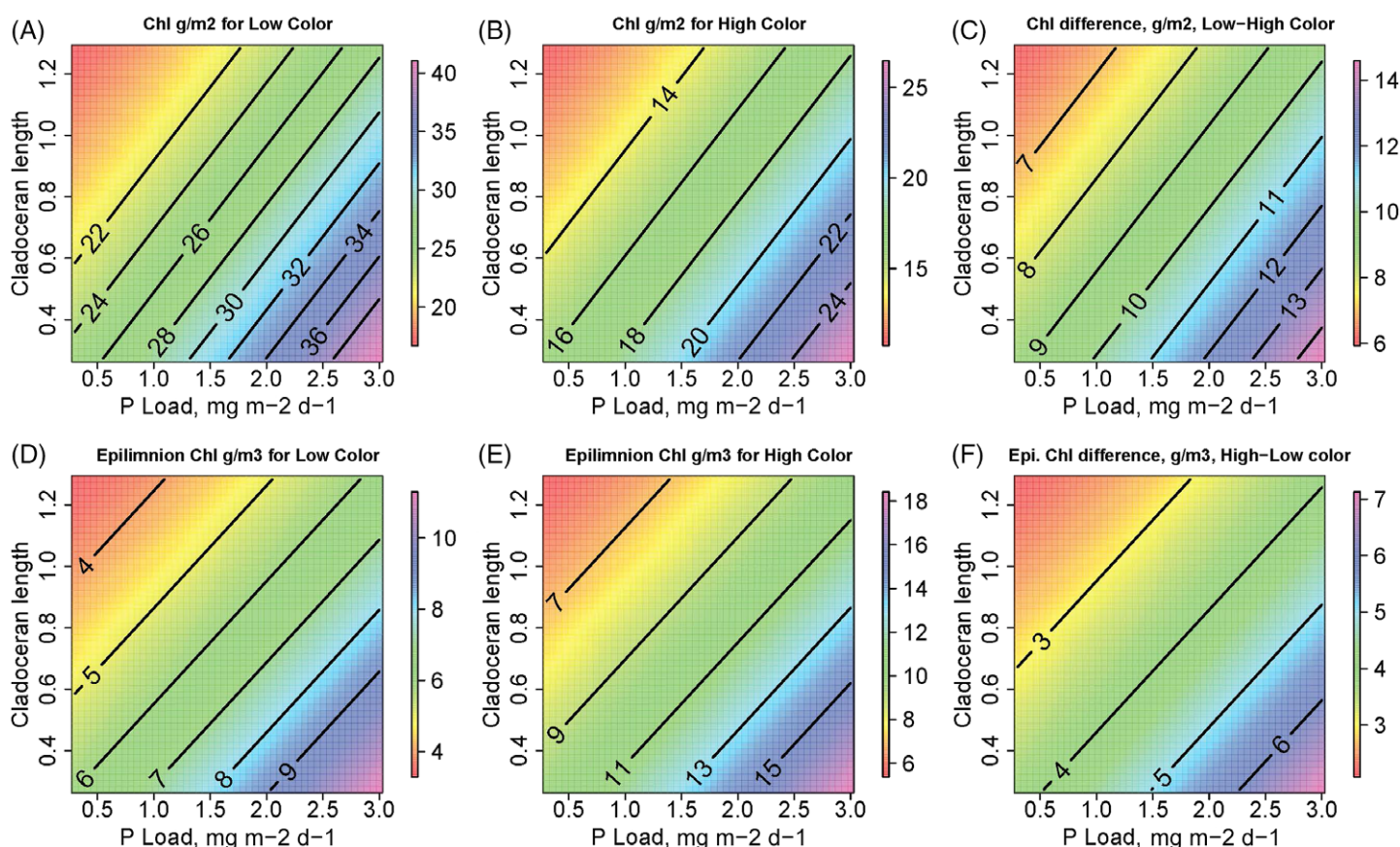


Fig. 3. Each panel shows a response surface at steady state (Supporting Information) of log₁₀ areal chlorophyll (top row, mg m⁻²) or log₁₀ epilimnetic chlorophyll (bottom row, mg m⁻³) vs. identical gradients of P load (mg m⁻² d⁻¹) and cladoceran length (mm). Note on each panel the diagonal lines are chlorophyll in back-transformed units to mass/area or mass/volume. The first column is computed for low g₄₄₀ (10th percentile), the middle column is computed for high g₄₄₀ (90th percentile), and the third column shows the difference. Note that the order of subtraction is always the surface with higher values minus the surface for lower values, and this is reversed for areal vs. epilimnetic chlorophyll.

controls on lake productivity, examine the importance of allochthonous organic matter in supporting consumers, and evaluate indicators of ecosystem resilience.

We were surprised many times in the course of this research (Carpenter and Kitchell 1993; Carpenter et al. 2010). The long-term variation in precipitation and color represents another unanticipated outcome. Annual precipitation is a dominant factor in DOC loading, retention, and potentially also in many lakes (Björnerås et al. 2017; de Wit et al. 2018). Nonetheless, studies of only a few years may not discern the role of colored organic matter in responses of phytoplankton to nutrient or food web manipulations. This was the case for our individual whole-lake experiments. An exception is the time series analyses for 1991–1995 in four lakes (East and West Long, Paul and Peter) showing that large increases in DOC concentration due to isolating East Long Lake with a curtain had significant effects on the responses to nutrient and food web manipulation (Carpenter et al. 1998). Because of this finding, we made occasional color measurements in 1994–1995 and added weekly color measurements to the slate of routine observations starting in 1996.

Increases in g₄₄₀ had different effects on areal and volumetric chlorophyll. g₄₄₀ was inversely related to areal chlorophyll integrated over the photic zone (Supporting Information Table S2). Shading likely accounts for the decrease in areal chlorophyll as g₄₄₀ increases. Areal gross primary production was negatively related to color in a survey of Norwegian lakes (Thrane et al. 2014) and in a manipulation that increased DOC (Zwart et al. 2016). In contrast with areal chlorophyll, epilimnetic concentrations were positively correlated with increasing g₄₄₀ in our study (Supporting Information Table S3). This increase in epilimnetic chlorophyll, combined with a decrease in photic zone areal chlorophyll, suggests that shading by g₄₄₀ compresses phytoplankton in a narrower epilimnion with higher concentrations of nutrients, similar to results from a large comparative study of lakes (Nürnberg and Shaw 1998). The positive effect of g₄₄₀ on epilimnetic chlorophyll appears to emerge in studies of multiple lakes with a broader range of g₄₄₀, morphometry, and other characteristics than is found in single lake studies.

Water color measures light absorbance by DOC, and in our study lakes DOC is mainly of terrestrial origin (Bade et al. 2007;

Wilkinson et al. 2013). Color might also have been enhanced by precipitation-driven inputs of iron into the lakes. Iron has a positive effect on water color and changing iron concentrations are associated with the phenomenon of inland water browning (Dillon and Molot 2005; Kritzberg and Ekström 2012; Weyhenmeyer et al. 2014). Increases or decreases in color might represent a change in terrestrial energetic subsidies for consumers but these interactions are potentially complex and context dependent (Solomon et al. 2015). Our models of chlorophyll do not address these complex interactions except that no significant color \times grazer interactions were observed suggesting shading was the main effect of color.

We computed response surfaces of chlorophyll to P load and cladoceran length at contrasting levels of g440 to illustrate the patterns found in the fitted models (Fig. 3). Nutrient input caused increases in chlorophyll and biomass of crustacean zooplankton, as occurred in many ecosystem experiments (Schindler 1974, 2006). Fish manipulations that decreased planktivory caused increases in body size of cladoceran zooplankton and decreases in chlorophyll. In contrast, fish manipulations to increase planktivory caused decreases in body size of cladoceran zooplankton and increases in chlorophyll. Similar responses to fish predation occurred other whole-lake food web manipulations (Shapiro and Wright 1984; Hansson et al. 1998; Lathrop et al. 2002; Jeppesen et al. 2007; Bernes et al. 2015).

Two of the factor interactions are notable. P load and crustacean biomass have a synergistic association with epilimnetic chlorophyll. This synergy suggests that P load intensified a positive feedback between phytoplankton and herbivores. Such a synergy is consistent with nutrient recycling by zooplankton which is well documented in these lakes (Bergquist and Carpenter 1986; Elser et al. 1988). Water color and P load together had an inverse statistical interaction with epilimnetic chlorophyll. This interaction is consistent with observations that the magnitude of chlorophyll response to P load was lower in years when g440 was relatively high (Pace et al. In press).

In these relatively unproductive lakes in a forested landscape, precipitation and landscape characteristics determine natural inputs of color and nutrients. Higher g440 decreases chlorophyll and dampens its growth response to P load. Food-web structure and grazing affect loss rates of chlorophyll. Years of higher precipitation have higher color, lower areal chlorophyll, and weaker responses of epilimnetic chlorophyll to P loading. Years of drought would show the reverse response (Webster et al. 1996). However, effects of precipitation on chlorophyll may be strongly positive in more productive lakes of agricultural watersheds due to runoff of manure or P-rich soil (Hamilton 2012; Lathrop and Carpenter 2014; Motew et al. 2018).

Ecosystem experiments, long-term studies, comparisons across a range of ecosystems, and theories together build knowledge of ecosystem processes (Carpenter 1998). In ecosystem experiments, deliberate manipulations cause the

observed changes and sharpen inference. However, ecosystem experiments usually span only a few years and a few sites, or lakes. To broaden perspectives in time and space, ecologists turn to long-term and comparative studies, respectively (Weathers et al. 2013). The set of ecosystem experiments described here approaches the duration in time of some long-term studies. Over the 33-yr span of the experiments, we observed broad changes in precipitation which substantially altered water color and thereby affected the light environment for phytoplankton growth. Water color was a significant factor for interpreting the whole-lake experiments. Thus, the long-term context of the experiments became a crucial factor for their interpretation. The insights that emerged show the value of embedding ecosystem experiments in a matrix of long-term ecological research.

References

- Bade, D. L., S. R. Carpenter, J. J. Cole, M. L. Pace, E. Kritzberg, M. C. Vande Bogert, R. M. Cory, and D. M. McKnight. 2007. Sources and fates of dissolved organic carbon in lakes as determined by whole-lake carbon isotope additions. *Biogeochemistry* **84**: 115–129. <https://doi.org/10.1007/s10533-006-9013-y>.
- Batt, R. D., S. R. Carpenter, J. J. Cole, M. L. Pace, T. J. Cline, R. A. Johnson, and D. A. Seekell. 2012. Resources supporting the food web of a naturally productive lake. *Limnol. Oceanogr.* **57**: 1443–1452. <https://doi.org/10.4319/lo.2012.57.5.1443>.
- Batt, R. D., R. D. Batt, S. R. Carpenter, J. J. Cole, M. L. Pace, R. A. Johnson, J. T. Kurtzweil, and G. M. Wilkinson. 2015. Altered energy flow in the food web of an experimentally darkened lake. *Ecosphere* **6**. <https://doi.org/10.1890/ES14-00241.1>.
- Bergquist, A. M., and S. R. Carpenter. 1986. Limnetic herbivory: Effects on phytoplankton populations and primary production. *Ecology* **67**: 1351–1360. <https://doi.org/10.2307/1938691>.
- Bernes, C., S. R. Carpenter, A. Gårdmark, P. Larsson, L. Persson, C. Skov, J. D. M. Speed, and E. Van Donk. 2015. What is the influence of a reduction of planktivorous and benthivorous fish on water quality in temperate eutrophic lakes? A systematic review. *Environ. Evid.* **4**: 7. <https://doi.org/10.1186/2047-2382-4-1>.
- Björnerås, C., and others. 2017. Widespread increases in iron concentration in European and North American freshwaters. *Global Biogeochem. Cycles* **31**: 1488–1500. <https://doi.org/10.1002/2017GB005749>.
- Cardille, J. A., S. R. Carpenter, J. A. Foley, P. C. Hanson, M. G. Turner, and J. A. Vano. 2009. Climate change and lakes: Estimating sensitivities of water and carbon budgets. *J. Geophys. Res. Biogeosci.* **114**: G03011. <https://doi.org/10.1029/2008jg000891>.
- Carpenter, S. R. 1998. The need for large-scale experiments to assess and predict the response of ecosystems to

- perturbation, New York, New York, p. 287–312. In M. L. Pace, and P. M. Groffman [eds], *Successes, limitations and frontiers in ecosystem science*. Springer.
- Carpenter, S. R., and others. 1987. Regulation of lake primary productivity by food web structure. *Ecology* **68**: 1863–1876. <https://doi.org/10.2307/1939878>.
- Carpenter, S. R., and J. F. Kitchell [eds]. 1993. *Trophic cascades in lakes*. Cambridge, U.K.: Cambridge Univ. Press.
- Carpenter, S. R., J. J. Cole, J. F. Kitchell, and M. L. Pace. 1998. Impact of dissolved organic carbon, phosphorus and grazing on phytoplankton biomass and production in lakes. *Limnol. Oceanogr.* **43**: 73–80. <https://doi.org/10.4319/lo.1998.43.1.0073>.
- Carpenter, S. R., and others. 2001. Trophic cascades, nutrients and lake productivity: Whole-lake experiments. *Ecol. Monogr.* **71**: 163–186. [https://doi.org/10.1890/0012-9615\(2001\)071\[0163:TCNALP\]2.0.CO;2](https://doi.org/10.1890/0012-9615(2001)071[0163:TCNALP]2.0.CO;2).
- Carpenter, S. R., and others. 2005. Ecosystem subsidies: Terrestrial support of aquatic food webs from ^{13}C addition to contrasting lakes. *Ecology* **86**: 2737–2750. <https://doi.org/10.1890/04-1282>.
- Carpenter, S. R., J. J. Cole, J. F. Kitchell, and M. L. Pace. 2010. Trophic cascades in lakes: Lessons and prospects, p. 55–69. In J. Terborgh, and J. Estes [eds], *Trophic cascades*. Washington, DC.: Island Press.
- Carpenter, S. R., and others. 2011. Early warnings of regime shifts: A whole-ecosystem experiment. *Science* **332**: 1079–1082. <https://doi.org/10.1126/science.1203672>.
- Christensen, D. L., and others. 1996. Pelagic responses to changes in dissolved organic carbon following division of a seepage lake. *Limnol. Oceanogr.* **41**: 553–559. <https://doi.org/10.4319/lo.1996.41.3.0553>.
- Cuthbert, I. D., and P. del Giorgio. 1992. Toward a standard method of measuring color in freshwater. *Limnol. Oceanogr.* **37**: 1319–1326. <https://doi.org/10.4319/lo.1992.37.6.1319>.
- de Wit, H. A., R.-. M. Couture, L. Jackson-Blake, M. N. Futter, S. Valinia, K. Austnes, J.-. L. Guerrero, and Y. Lin. 2018. Pipes or chimneys? For carbon cycling in small boreal lakes, precipitation matters most. *Limnol. Oceanogr.: Lett.* **3**: 275–284. <https://doi.org/10.1002/lo2.10077>.
- Dillon, P. J., and L. A. Molot. 1997. Effect of landscape form on export of dissolved organic carbon, iron, and phosphorus from forested stream catchments. *Water Resour. Res.* **33**: 2591–2600. <https://doi.org/10.1029/97WR01921>.
- Dillon, P. J., and L. A. Molot. 2005. Long-term trends in catchment export and lake retention of dissolved organic carbon, dissolved organic nitrogen, total iron, and total phosphorus: The Dorset, Ontario, study, 1978–1998. *J. Geophys. Res. Biogeosci.* **110**: 2591–2600. <https://doi.org/10.1029/2004JG000003>.
- Elser, J. J., M. M. Elser, N. A. MacKay, and S. R. Carpenter. 1988. Zooplankton-mediated transitions between N- and P-limited algal growth. *Limnol. Oceanogr.* **33**: 1–14. <https://doi.org/10.4319/lo.1988.33.1.0001>.
- Fergus, C. E., A. O. Finley, P. A. Soranno, and T. Wagner. 2016. Spatial variation in nutrient and water color effects on lake chlorophyll at macroscales. *PLoS One* **11**: e0164592. <https://doi.org/10.1371/journal.pone.0164592>.
- Hamilton, S. K. 2012. Biogeochemical time lags may delay responses of streams to ecological restoration. *Freshw. Biol.* **57**: 43–57. <https://doi.org/10.1111/j.1365-2427.2011.02685.x>.
- Hansson, L.-A., and others. 1998. Biomanipulation as an application of food-chain theory: Constraints, synthesis, and recommendations for temperate lakes. *Ecosystems* **1**: 558–574. <https://doi.org/10.1007/s100219900051>.
- Hebbali, A. 2018. olsrr: Tools for building OLS regression models. R package version 0.5.1. Available from <https://CRAN.R-project.org/package=olsrr>. Accessed 08-25-2018.
- Houser, J. N. 2006. Water color affects the stratification, surface temperature, heat content, and mean epilimnetic irradiance of small lakes. *Can. J. Fish. Aquat. Sci.* **63**: 2447–2455. <https://doi.org/10.1139/f06-131>.
- Jeppesen, E., and others. 2007. Restoration of shallow lakes by nutrient control and biomanipulation—the successful strategy varies with lake size and climate. *Hydrobiologia* **581**: 269–285. <https://doi.org/10.1007/s10750-006-0507-3>.
- Kirk, J. T. O. 1994. *Light and photosynthesis in aquatic systems*, 2nd ed. Cambridge, U.K.: Cambridge Univ. Press.
- Kritzberg, E. S. 2017. Centennial-long trends of lake browning show major effect of afforestation. *Limnol. Oceanogr.: Lett.* **2**: 105–112. <https://doi.org/10.1002/lo2.10041>.
- Kritzberg, E. S., and S. M. Ekström. 2012. Increasing iron concentrations in surface waters – a factor behind brownification? *Biogeosciences* **9**: 1465–1478. <https://doi.org/10.5194/bg-9-1465-2012>.
- Lathrop, R. C., and others. 2002. Stocking piscivores to improve fishing and water clarity: A synthesis of the Lake Mendota biomanipulation project. *Freshw. Biol.* **47**: 2410–2424. <https://doi.org/10.1046/j.1365-2427.2002.01011.x>.
- Lathrop, R. C., and S. R. Carpenter. 2014. Water quality implications from three decades of phosphorus loads and trophic dynamics in the Yahara chain of lakes. *Inland Waters* **4**: 1–14. <https://doi.org/10.5268/IW-4.1.680>.
- Likens, G. E. 1985. An experimental approach for the study of ecosystems: The fifth Tansley lecture. *J. Ecol.* **73**: 381–396. <https://doi.org/10.2307/2260481>.
- Monteith, D. T., and others. 2007. Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature* **450**: 537–540. <https://doi.org/10.1038/nature06316>.
- Motew, M., E. G. Booth, S. R. Carpenter, X. Chen, and C. J. Kucharik. 2018. The synergistic effect of manure supply and extreme precipitation on surface water quality. *Environ. Res. Lett.* **13**: 044016. <https://doi.org/10.1088/1748-9326/aaade6>.
- Nürnberg, G. K., and M. Shaw. 1998. Productivity of clear and humic lakes: Nutrients, phytoplankton, bacteria. *Hydrobiologia* **382**: 97–112. <https://doi.org/10.1023/A:1003445406964>.

- Pace, M. L., and others. 2004. Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. *Nature* **427**: 240–243. <https://doi.org/10.1038/nature02227>.
- Pace, M. L., and others. 2007. Does terrestrial organic carbon subsidize the planktonic food web in a clear-water lake? *Limnol. Oceanogr.* **52**: 2177–2189. <https://doi.org/10.4319/lo.2007.52.5.2177>.
- Pace, M. L., R. D. Batt, C. D. Buelo, S. R. Carpenter, J. J. Cole, J. T. Kurtzweil, and G. M. Wilkinson. 2017. Reversal of a cyanobacterial bloom in response to early warnings. *Proc. Natl. Acad. Sci. USA* **114**: 352–357. <https://doi.org/10.1073/pnas.1612424114>.
- Pace, M. L., S. R. Carpenter, and G. M. Wilkinson. 2018. Long term studies and reproducibility: Lessons from whole-lake experiments. *Limnol. Oceanogr.* **63**. <https://doi.org/10.1002/lno.11012>.
- R Core Team. 2018. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Schindler, D. W. 1974. Eutrophication and recovery in experimental lakes: Implications for lake management. *Science* **184**: 897–899. <https://doi.org/10.1126/science.184.4139.897>.
- Schindler, D. W. 2006. Recent advances in the understanding and management of eutrophication. *Limnol. Oceanogr.* **51**: 356–363. https://doi.org/10.4319/lo.2006.51.1_part_2.0356.
- Shapiro, J., and D. I. Wright. 1984. Lake restoration by biomanipulation: Round Lake, Minnesota, the first two years. *Freshw. Biol.* **14**: 371–383.
- Solomon, C. T., and others. 2015. Ecosystem consequences of changing inputs of terrestrial dissolved organic matter to lakes: Current knowledge and future challenges. *Ecosystems* **18**: 376–389. <https://doi.org/10.1007/s10021-015-9848-y>.
- Thrane, J.-E., D. O. Hessen, and T. Andersen. 2014. The absorption of light in lakes: Negative impact of dissolved organic carbon on primary productivity. *Ecosystems* **17**: 1040–1052. <https://doi.org/10.1007/s10021-014-9776-2>.
- Wauthy, M., M. Rautio, K. S. Christoffersen, L. Forsström, I. Laurion, H. L. Mariash, S. Peura, and W. F. Vincent. 2018. Increasing dominance of terrigenous organic matter in circumpolar freshwaters due to permafrost thaw. *Limnol. Oceanogr.* **3**: 186–198. <https://doi.org/10.1002/lol2.10063>.
- Weathers, K. C., D. L. Strayer, and G. E. Likens. 2013. *Fundamentals of ecosystem science*. Waltham, Massachusetts, USA: Elsevier Academic Press.
- Webster, K. E., T. K. Kratz, C. J. Bowser, J. J. Magnuson, and W. J. Rose. 1996. The influence of landscape position on lake chemical responses to drought in northern Wisconsin. *Limnol. Oceanogr.* **41**: 977–984. <https://doi.org/10.4319/lo.1996.41.5.0977>.
- Webster, K. E., and others. 2008. An empirical evaluation of the nutrient-color paradigm for lakes. *Limnol. Oceanogr.* **53**: 1137–1148. <https://doi.org/10.4319/lo.2008.53.3.1137>.
- Weidel, B. C., K. Baglini, S. E. Jones, P. T. Kelly, C. T. Solomon, and J. A. Zwart. 2017. Light climate and dissolved organic carbon concentration influence species-specific changes in fish zooplanktivory. *Inland Waters* **7**: 210–217. <https://doi.org/10.1080/20442041.2017.1329121>.
- Weyhenmeyer, G. A., Y. Prairie, and L. J. Tranvik. 2014. Browning of boreal fresh waters coupled to carbon-iron interactions along the aquatic continuum. *PLoS One* **9**: e88104. <https://doi.org/10.1371/journal.pone.0088104>.
- Wilkinson, G. M., M. L. Pace, and J. J. Cole. 2013. Terrestrial dominance of organic matter in north temperate lakes. *Global Biogeochem. Cycles* **27**: 43–51. <https://doi.org/10.1029/2012GB004453>.
- Wilkinson, G. M., S. R. Carpenter, J. J. Cole, and M. L. Pace. 2014. Use of deep autochthonous resources by zooplankton: Results of a metalimnetic addition of ^{13}C to a small lake. *Limnol. Oceanogr.* **59**: 986–996. <https://doi.org/10.4319/lo.2014.59.3.0986>.
- Wilkinson, G. M., G. M. Wilkinson, S. R. Carpenter, J. J. Cole, M. L. Pace, R. D. Batt, C. D. Buelo, and J. T. Kurtzweil. 2018. Early warning signals precede cyanobacterial blooms in multiple whole-lake experiments. *Ecol. Monogr.* **88**: 188–203. <https://doi.org/10.1002/ecm.1286>.
- Williamson, C. E., D. P. Morris, M. L. Pace, and O. G. Olson. 1999. Dissolved organic carbon and nutrients as regulators of lake ecosystems: Resurrection of a more integrated paradigm. *Limnol. Oceanogr.* **44**: 795–803. https://doi.org/10.4319/lo.1999.44.3_part_2.0795.
- Zwart, J. A., N. Craig, P. T. Kelly, S. D. Sebestyen, C. T. Solomon, B. C. Weidel, and S. E. Jones. 2016. Metabolic and physiochemical responses to a whole-lake experimental increase in dissolved organic carbon in a north-temperate lake. *Limnol. Oceanogr.* **61**: 723–734. <https://doi.org/10.1002/lno.10248>.

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