

Phytoplankton biomass, dissolved organic matter, and temperature drive respiration in whole lake nutrient additions

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Abstract

Lake respiration is supported by a mixture of autochthonous and allochthonous resources, but the relative significance and interaction of these sources are uncertain across gradients of primary production and organic matter inputs. We manipulated autochthonous resources by adding inorganic nitrogen and phosphorus to two lakes during three summers and monitored a third reference lake. Allochthonous resources were measured as fluorescent dissolved organic matter (FDOM). In the reference and two experimental lakes, daily estimates of respiration were made from continuously deployed oxygen sensors. Daily mean values of temperature and FDOM were determined from high-frequency measurements along with daily measures of chlorophyll *a*, an index of phytoplankton biomass. We analyzed time series of respiration and tested models that used combinations of the independent variables chlorophyll, FDOM, and temperature. The best models included all three of the independent variables. Respiration increased twofold over the temperature range of 14.5–28.6°C. Respiration increased in association with phytoplankton blooms caused by the nutrient additions, but did not track blooms closely, because of large day-to-day variability. Respiration varied positively with FDOM that was primarily allochthonous and differed among lakes and years. We did not detect an interaction between chlorophyll and FDOM despite the large number of observations and range of chlorophyll and FDOM. Hydrologic, climatic, and land use changes are altering temperature and inputs of nutrients and organic matter to lakes. Our results indicate that these changes may lead to linear responses in ecosystem processes like respiration for the wide range of inputs represented in this study.

Respiration is an integrative measure of metabolism and is therefore of fundamental interest in comparing ecosystems as well as assessing contributions to carbon cycling. In lakes across a wide range of conditions, pelagic respiration is related to phytoplankton biomass, total phosphorus, dissolved organic carbon (DOC), and temperature based on historical measures of enclosing plankton in bottles (Pace and Prairie 2005). Using more modern sensor data, Solomon et al. (2013) synthesized continuous measures of dissolved oxygen from 25 lakes and found that mean lake respiration (*R*) was strongly related to mean gross primary production (GPP) but not related to DOC. However, respiration is partially supported by allochthonous organic matter inputs, and in some lakes respiration exceeds GPP indicating the importance of terrestrial organic matter consumption (Cole et al. 2000; Staehr et al. 2012a). An overarching issue is the relationship of respiration to the combined sources for metabolism of autochthonous primary production and allochthonous organic

matter especially in relation to large-scale environmental changes that are altering temperature, internal rates, and external inputs (Solomon et al. 2015).

Plankton and sediment microbes (inclusive of benthic autotrophs and heterotrophs) account for most respiration in lakes (Cole et al. 2006). Using sensors that make frequent measurements, the collective respiration of these groups is measurable as declines in dissolved oxygen during darkness, corrected for air water gas exchange. However, this measure of respiration is complicated by sensor noise, variations in sensor footprint, and spatial and temporal heterogeneity (Van de Bogert et al. 2007, 2012). Day-to-day variation in estimated respiration can be quite large as recognized by many studies making it difficult to discern factors driving respiration (e.g., Staehr and Sand-Jensen 2007; Coloso et al. 2011a).

Furthermore, testing for relationships of respiration to primary production and dissolved organic matter (DOM) is complicated by methods used to measure these variables and by potential interacting effects. For lakes, temperature effects on respiration are well known (Pace and Prairie 2005), may covary with other factors (Yvon-Durocher et al. 2012), and should generally increase due to warming temperatures (O'Reilly et al. 2015; Lathrop et al. 2019). GPP is often

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measured by daytime dissolved oxygen increase plus nighttime decline in oxygen. However, GPP is not independent of respiration which confounds interpretation (Honti and Istvánovics 2019). Assessing the significance of primary production as a driver requires using other approaches such as measurements of additional independent variables like phytoplankton pigments. Lake DOM is often derived mainly from terrestrial organic matter (Wilkinson et al. 2013) and thus can serve as a proxy of allochthonous sources supporting respiration. However, DOM is a combination of pools of varying degradation rates and sources, which complicates using this relatively static variable (Solomon et al. 2013). DOM may also impact primary production through the combined effects of release of limiting nutrients (Vähätalo et al. 2003; Kissman et al. 2013) and absorption of light (Carpenter et al. 1998; Thrane et al. 2014). The potentially interactive effects of shading and nutrient provision are uncertain and may vary across gradients of DOM (Seekell et al. 2015; Solomon et al. 2015).

Whole lake manipulations have the potential to overcome some of the problems summarized above to test the relative importance of controlling factors and their possible interaction. Here, we take advantage of whole lake nutrient additions that caused phytoplankton blooms (Wilkinson et al. 2018) creating variation in this source of respiratory organic carbon. In addition, the experimental lakes used in this study differed in DOM establishing a second source of variation in respiratory carbon. Furthermore, DOM varied among years during the study probably due to winter–spring precipitation and runoff associated with precipitation events (Zwart et al. 2017; Carpenter and Pace 2018). We made continuous observations of phytoplankton pigments, fluorescent DOM (FDOM), temperature, and dissolved oxygen such that daily measures of these postulated drivers could be compared to daily estimates of respiration. We hypothesized that respiration would be driven by variations of phytoplankton biomass, DOM, and temperature. We also evaluated the extent to which there was an interaction between phytoplankton biomass and DOM whereby elevated levels of both stimulate respiration synergistically, or alternatively, elevated levels of DOM suppress respiration through light limitation of primary production.

Methods

Study site and whole lake manipulations

Three co-located lakes at the University of Notre Dame Environmental Research Center in Gogebic County, Michigan were used in this study. Paul, Peter, and Tuesday lakes are extensively described (Carpenter and Kitchell 1993) and have been used in prior whole lake manipulations (Carpenter and Pace 2018). These lakes are oligotrophic to mesotrophic, relatively small (0.9–2.7 ha), and deep (mean depths 3.7–5.7 m) for their size. The lakes do not have perennial inflow or outflow streams and groundwater fluxes are important (Cole and Pace 1998). Peter Lake receives water from a culvert

connecting it to Paul Lake under high flow conditions. Lakes have similar circular morphometry and are steep-sided basins as characteristic of kettle lakes. All lakes have low macrophyte growth. Temperature stratification is strong throughout summer with stable thermoclines that deepen toward the end of the season (August). On warm still days, there is microstratification in the surface mixed layer which breaks down at night (Coloso et al. 2011b). Seasonal temperature patterns are presented in Supporting Information Data S1. Mean chemical and optical conditions during the years of this study, 2013–2015, are presented in Wilkinson et al. (2018). The primary distinction among the study lakes is that Tuesday is surrounded by a bog and has higher DOM concentrations relative to Peter and Paul which have fringing bogs but mainly forested watersheds. Previous studies using both natural abundance and enrichment of stable isotopes indicated that DOM is primarily of terrestrial origin in the study lakes, and that under nutrient additions, the contribution of autochthonous derived organic matter to DOC increased (Bade et al. 2007). Autochthonous organic matter differentially supports bacterial production and respiration relative to allochthonous organic matter (Kritzberg et al. 2004, 2005).

We added inorganic nitrogen and phosphorus to Peter and Tuesday lakes in 2013, 2014, and 2015 with Paul Lake serving as an unfertilized reference. Stock solutions of ammonium nitrate and phosphoric acid were mixed with lake water and pumped from a moving boat daily to achieve a targeted loading rate. N : P molar ratios were 15 for the added nutrients except in 2013 when a lower ratio was used (see Wilkinson et al. 2018). Daily loads were ramped up weekly in 2013 with an overall mean loading rate of $2.6 \text{ mg P m}^{-2} \text{ d}^{-1}$. Daily loads were at a constant rate of $3 \text{ mg P m}^{-2} \text{ d}^{-1}$ in 2014 and 2015. With one exception, we added nutrients from the beginning of June to near the end of August (> 80 d). The exception was Peter Lake in 2015 when nutrients were added for only 28 d with the duration of this manipulation related to testing the use of early warning indicators (Pace et al. 2017).

Continuous, daily, and weekly measurements

We deployed an instrumented raft in each lake with submerged sensors (0.75 m depth) and data loggers (sondes) to make continuous measurements. A weather station was deployed on the raft on Peter Lake to measure wind speed and direction as well as air temperature. We used Hydrolab DS5X as our primary sondes and YSI 6600V2-4 as backup sondes to measure temperature, dissolved oxygen, and phycocyanin (relative fluorescence) at 5-min intervals. The fluorescence measured by the phycocyanin probe was strongly related to direct measures of phycocyanin (Pace et al. 2017) and consistent with a limited number of microscopic estimates of cyanobacterial biomass (Wilkinson et al. 2018). Brief interruptions in the primary sonde occurred due to the need for calibration and cleaning as well as occasional malfunction (e.g., debris on a sensor). These gaps were filled using data

from the backup sonde by interpolations using a bivariate autoregressive state-space model fitted simultaneously to the time series from both sensors (MARSS package, version 3.9, R version 3.1.30). Fluorescent DOM (FDOM) was measured using WET Labs ECO fluorometers (excitation\emission: 370 and 460 nm). Fluorescence measurements from these instruments were corrected using coincident temperature measurements and lab calibrations conducted across a range of temperatures (Watras et al. 2011). Gaps in FDOM records were filled by interpolation with a few longer periods of missing data filled by establishing lake-year-specific relationships between light absorption and FDOM.

We measured chlorophyll *a* (Chl *a*) concentrations, an index of phytoplankton biomass, daily. Surface water samples from 0.5 m at the deepest point of each lake were returned to the lab and filtered under low vacuum through 47-mm glass fiber (GF/F) filters. The filters were frozen and pigments subsequently extracted by soaking filters in methanol prior to analysis of Chl *a* by fluorometry using standard methods that correct for pheopigments (Holm-Hansen 1978).

The concentration of DOC and the absorption of light primarily due to chromophoric DOM were measured weekly. Surface mixed layer samples were filtered through precombusted GF/F filters. A 20-mL portion of the filtrate was added to a 20-mL glass scintillation vial with plastic cone caps and preserved with 200 μL of a 1 mol L^{-1} H_2SO_4 solution. These samples were analyzed with a Shimadzu 5050 TOC (total organic carbon) analyzer to determine the nonpurgeable DOC (Findlay et al. 2010). A second 60-mL portion of the filtrate from the original water samples was refrigerated and subsequently analyzed spectrophotometrically by measuring light absorption in a 10-cm path-length cell at 440 nm (Cuthbert and del Giorgio 1992). These measures, often called water color, are hereafter referred to as a_{440} .

Respiration

We used the overnight decline of dissolved oxygen measured at 5-min intervals between 22 : 00 and 4 : 00 h to estimate respiration using the bookkeeping method with correction for gas exchange with the atmosphere (Cole et al. 2000; Winslow et al. 2016). By restricting the measurement period to hours of darkness, this approach has the advantage of avoiding assumptions associated with net daytime increases in oxygen concentration due to simultaneous photosynthesis and respiration. The approach also avoids estimating respiration from two separate time periods—midnight to dawn and sunset to midnight—which is a method commonly applied when determining both primary production and respiration from oxygen dynamics (Winslow et al. 2016). Our approach has the disadvantage of only using a portion of the daily oxygen data, but given our focus on respiration, a consistent time period where respiration is the dominant process provided a daily estimate with fewer assumptions.

Daily estimates were made by using the general model previously applied to our study lakes by Cole et al. (2000) where during the dark:

$$\Delta\text{DO} = R + D. \quad (1)$$

ΔDO is the change in dissolved oxygen over the measurement time interval (5 min). R is the respiratory consumption of oxygen ($\text{mg O}_2 \text{ L}^{-1}$) and is a negative value (see below). D is the exchange with the atmosphere ($\text{mg O}_2 \text{ L}^{-1}$) which can either be positive (net flux to the atmosphere) or negative (net flux to the lake). The change in oxygen (ΔDO) was measured directly. D was estimated using a method that accounts for both exchange due to wind and convection (Read et al. 2012):

$$D = k(\text{DO}_t - \text{DO}_{\text{sat}}) / z_{\text{mix},t}, \quad (2)$$

where k is the gas exchange coefficient of DO, DO_t is the measured value of oxygen at time t , DO_{sat} is the saturation concentration of oxygen at t , and $z_{\text{mix},t}$ is the mixed layer depth at t .

Equation 1 was solved for R at 5-min intervals with these values summed over the 6-h dark period and prorated to a daily value ($\text{mg O}_2 \text{ L}^{-1} \text{ d}^{-1}$).

The method to estimate air–water gas exchange (D) required measuring or estimating water temperature, wind speed, shortwave and longwave radiation, relative humidity, barometric pressures, and depth of the mixed layer. We used a thermistor chain with sensors deployed at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, and 5 m to provide a continuous estimate of mixed layer depth. Continuous measures of wind speed and photosynthetically active radiation (PAR) were also made at the lakes. We used the PAR data to estimate shortwave and longwave radiation. We used relative humidity (RH) and barometric pressure (BP) data measured at Sparking Lake as part of the North Temperate Lakes Long Term Ecological Research program. For times when there were no data for Sparking Lake, additional data for RH and BP were derived from high-resolution meteorological data from the airport in Woodruff, Wisconsin and from the National Ecological Observatory Network (NEON) observation tower located at the University of Notre Dame Environmental Research Center. NEON tower data were only available for 2015. The distance of the NEON tower, Sparking Lake, and Woodruff Airport from the study lakes was 3, 31, and 40 km, respectively. Four measured variables (surface water temperature, mixed layer depth, relative humidity, and atmospheric pressure) had missing data after aggregating all above sources, with a total of 0.54% of observations missing (0.028–0.882% depending on variable). Linear interpolation of gaps < 3 h was used to fill 40% of these missing values (18–100% depending variable), and remaining values were filled with either daily or overall means. Details of the calculation of k from physical variable time series described above followed equations presented in Winslow

et al. (2016) available in the LakeMetabolizer R package, which includes the method of Read et al. (2012).

In some cases once accounting for gas exchange, increases in oxygen were observed overnight which resulted in positive values of respiration. Inspection of these time series revealed that positive respiration was associated with high variability of measured oxygen over the night period and was not the result of a single or a few outliers. We included positive values of respiration up to $0.5 \text{ mg O}_2 \text{ L}^{-1} \text{ d}^{-1}$ but removed greater values to reduce the influence of positive values on the analysis. In order to maintain a continuous daily time series for statistical purposes, we filled time series by linear interpolation using R values from the day before and after a large positive value. Overall, we removed and filled 20 values from a total of 894 values. For the final data set 54 values or 6% of the total daily measurements were positive at $\leq 0.5 \text{ mg O}_2 \text{ L}^{-1} \text{ d}^{-1}$.

Statistical analysis

From the above measured properties and processes, we established daily data for four variables that we hypothesized drove variation in respiration. Daily measures of Chl *a* and phycocyanin measured variation in autotrophic biomass of phytoplankton and cyanobacteria, respectively. FDOM measured variation in the dominant allochthonous carbon source derived mainly from watershed inputs to the study lakes (Wilkinson et al. 2013). Temperature varied seasonally with the largest range based on daily means being $14.5\text{--}28.6^\circ\text{C}$ in Peter Lake during 2013. Temperature, phycocyanin, and FDOM were measured at 5-min intervals. Daily data were based on averaging values from the first daily value at 00 : 05 to 24 : 00 h for the respiration estimate that began on that same date at 22 : 00 h.

We used generalized least squares in a time-series regression analysis (Fox and Weisburg 2019) to test for the best models of respiration. This method allows incorporation of serial dependency into models. We applied the *gls* function from the *nlme* package (version 3.1.137, with R version 3.5.3). Independent variables were Chl *a*, phycocyanin, FDOM, and temperature. We tested models that included either chlorophyll or phycocyanin, but we did not examine models that included both variables because of the correlation between these two measures. We examined one-, two-, and three-variable models along with models that considered differences among lakes and years as categorical variables. We also considered a model with an interaction term for chlorophyll and FDOM. Model comparison was based on Akaike's information criterion (AIC) while model fit was based on residual standard error (RSE) and the correlation of observed and predicted values. For the models that included lake and year as categorical variables, we calculated variance inflation factors (VIFs) to further compare models beyond AIC. VIF quantifies multicollinearity by comparing the r^2 of a model with multiple variables to a model with one variable. VIFs for individual predictor variables above

five were considered to have unacceptably high multicollinearity (Ieno and Zuur 2015).

We fit the locally weighted scatterplot smoothing (LOWESS) function in the base-R statistical package (version 3.5.3) to daily respiration values for each lake-year in order to help visualize trends. We used a smoother span of 0.25 which is the proportion of observations that influence the smoothed curve at each value. Shorter spans show more variability and longer spans smooth the data more toward the mean.

Results

Night oxygen dynamics

Oxygen concentrations declined overnight (Fig. 1). Oxygen loss was typically small under unfertilized, low phytoplankton conditions (Fig. 1a) with much larger losses (e.g., > 6% decline in percent saturation) under fertilized, high phytoplankton conditions (Fig. 1b). While oxygen concentration variability was modest on some dates (Fig. 1a,b), variability was quite high on other dates (Figs. 1c,d). As a consequence, daily estimates of R were highly variable over time (described below).

Lake contrasts

Background, natural P-loading rates are $0.1\text{--}0.3 \text{ mg P m}^{-2} \text{ d}^{-1}$ (Carpenter et al. 2001), whereas the daily average of experimental P-loads was $2.6\text{--}3.0 \text{ mg P m}^{-2} \text{ d}^{-1}$ (Table 1). The nutrient additions to the manipulated lakes increased both total phosphorus and total nitrogen substantially relative to the reference lake (Wilkinson et al. 2018). Tuesday Lake had higher DOC concentrations and a440 than Peter and Paul lakes (Table 1). In addition, DOC and a440 were greater in 2013 and 2014 relative to 2015 in all three lakes (Table 1). These high values were above long-term means (Pace et al. 2019) and reflected greater inputs of terrestrial organic matter.

Daily values of FDOM from in situ sensors were consistent with the weekly measurements of a440 and DOC in the sense of the consistent differences among lakes and among years (Fig. 2). Seasonal variation in FDOM was low (Fig. 2) with coefficients of variation (CVs) within lake-years ranging from 0.02 to 0.08.

We observed a few, one-day peaks of chlorophyll in the reference lake whereas concentrations were elevated in the two manipulated lakes (Fig. 3). Periods where chlorophyll concentrations exceeded $20 \mu\text{g L}^{-1}$ over at least a few days were observed: late in 2013 in Tuesday Lake, from day of year 165–185 in Peter Lake 2015, and for final part of the season in Tuesday Lake in 2015 (Fig. 3). The nutrient additions thus created blooms and variable bloom periods in the two manipulated lakes.

The dynamics of phycocyanin (not presented) were similar to chlorophyll (Wilkinson et al. 2018). Large peaks of phycocyanin co-occurred with large peaks in chlorophyll except in two cases. There was a period of relatively high phycocyanin

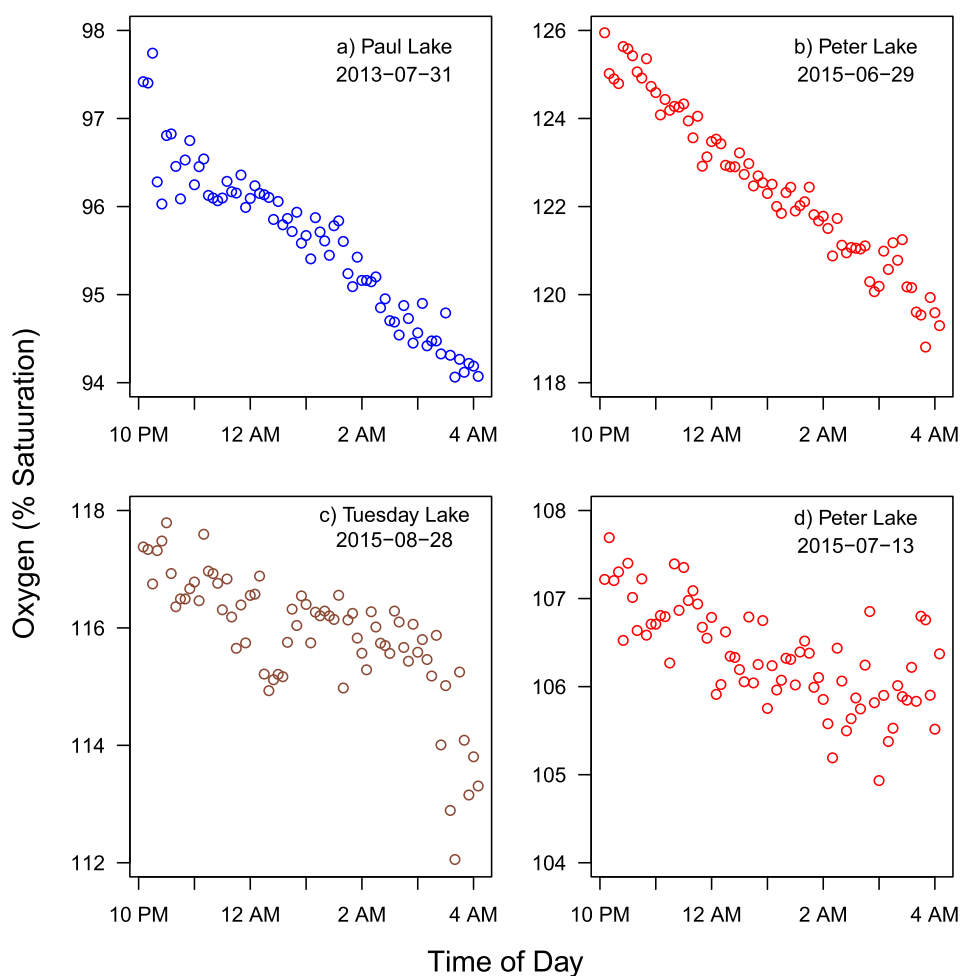


Fig. 1. Examples of overnight dissolved oxygen (DO) declines as percent saturation for (a) Paul Lake, 31 July 2013 (DO range: 8.28–8.09 mg L⁻¹), (b) Peter Lake, 29 June 2015 (DO range: 10.27–9.81 mg L⁻¹), (c) Tuesday Lake, 28 August 2015 (DO range: 10.38–9.82 mg L⁻¹), (d) Peter Lake, 13 July 2015 (DO range: 8.60–8.44 mg L⁻¹). Note that the dates indicated in the figure are for the start time.

Table 1. Conditions and treatments for each lake and year. Paul Lake was an unmanipulated reference. Peter and Tuesday lakes received inorganic nutrient additions each year. P and N loads represent the daily average nutrient additions to Peter and Tuesday lakes over the duration described in the text. DOC and a₄₄₀ are means and standard deviations (parentheses) of weekly ($n = 14\text{--}16$ per year) observations taken from the mixed layer of each lake.

Lake	Year	P-load (mg P m ⁻² d ⁻¹)	N-load (mg N m ⁻² d ⁻¹)	a ₄₄₀ (m ⁻¹)	DOC (mg C L ⁻¹)
Paul	2013	NA	NA	1.5 ± 0.2	4.5 ± 1.0
	2014	NA	NA	1.3 ± 0.2	4.3 ± 0.4
	2015	NA	NA	1.3 ± 0.1	3.9 ± 0.1
Peter	2013	2.6	9.9	2.4 ± 0.5	5.9 ± 0.4
	2014	3.0	20.3	2.2 ± 0.3	5.9 ± 0.5
	2015	3.0	20.3	1.6 ± 0.2	5.8 ± 0.7
Tuesday	2013	2.6	9.9	6.0 ± 0.9	12.8 ± 3.4
	2014	3.0	20.3	5.3 ± 1.0	12.3 ± 4.7
	2015	3.0	20.3	4.4 ± 0.3	10.3 ± 1.0

NA, no addition.

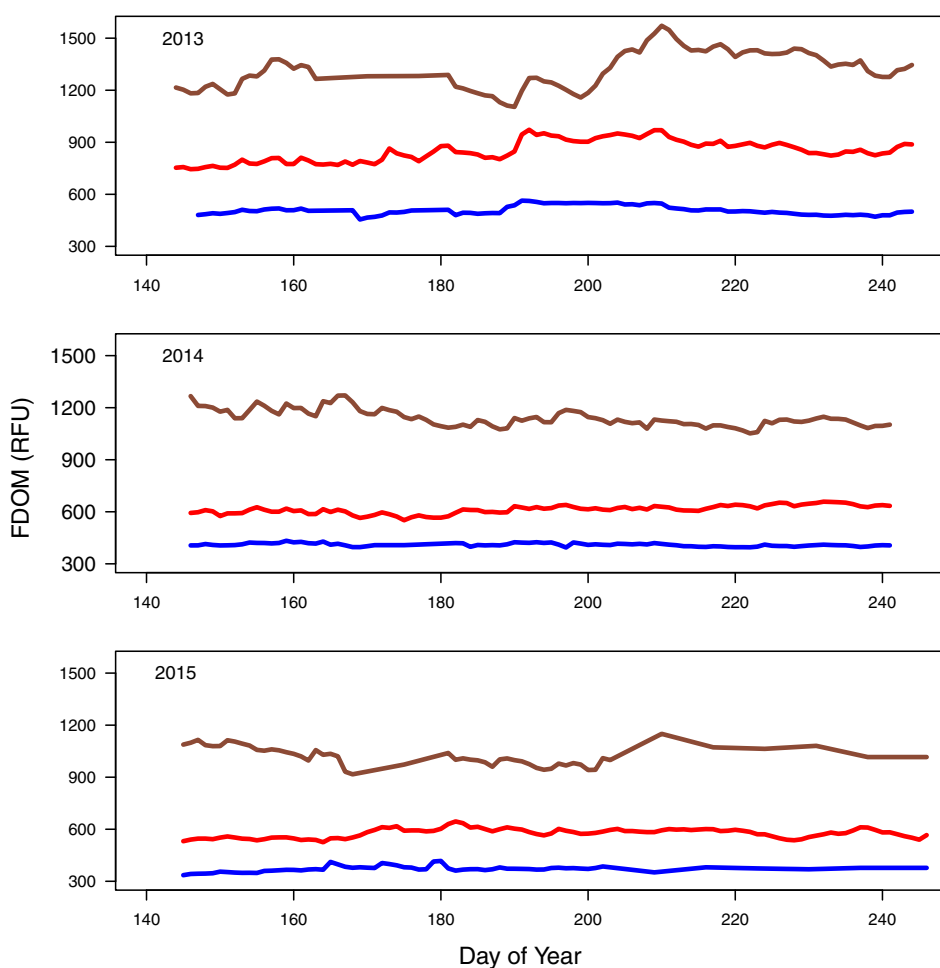


Fig. 2. Daily FDOM (relative fluorescence units = RFU) in Tuesday (brown lines), Peter (red lines), and Paul (blue lines) lakes vs. day of year for 2013, 2014, and 2015.

during 2014 in Peter Lake with surface scums noted, but chlorophyll concentrations remained below $20 \mu\text{g L}^{-1}$. The phytoplankton bloom as measured by chlorophyll in Tuesday Lake during 2013 (Fig. 3) was not associated with high levels of phycocyanin. Algal counts from this bloom period indicated the algal community was dominated by dinoflagellates and chrysophytes (Wilkinson et al. 2018).

There was an increase in FDOM in Tuesday Lake during 2013 at the same time phytoplankton increased but increases in FDOM were not observed in Tuesday during the 2015 bloom (Figs. 2, 3). FDOM was relatively invariant in Peter Lake and did not vary in concert with blooms (Figs. 2, 3).

Respiration

Daily respiration was variable with LOWESS fits to the data indicating little seasonal trend in the reference lake in all 3 yr (Fig. 4) with the exception that respiration rates tended to be higher (i.e., more negative, see Eq. 1) during mid-summer with warmer water temperatures. Respiration tended to increase across the season in the experimental lakes particularly in

Tuesday Lake which also tended to have the highest rates. The effect of the bloom in Peter Lake during 2015 that peaked near day of year 180 was also reflected in overall greater respiration, albeit with high variability, based on the LOWESS fit (Fig. 4).

Statistical models of respiration

We applied an autoregressive term of lag 1 to all models after exploring different approaches to accounting for serial dependency. The autocorrelation and partial autocorrelation functions degraded slowly to zero over a large number of lag terms (Supporting Information Data S1). Autocorrelation, however, was also relatively low even in the strongest lag ($t = 1$) with values typically < 0.2 . Thus, day-to-day variability was largely uncorrelated and using a model with lag 1 was judged sufficient compared to models with additional terms. This view was supported by inspection of residuals for models reported below.

Models using phycocyanin had higher AICs than equivalent (in terms of variables included) models using chlorophyll. We, therefore, only present results for models with

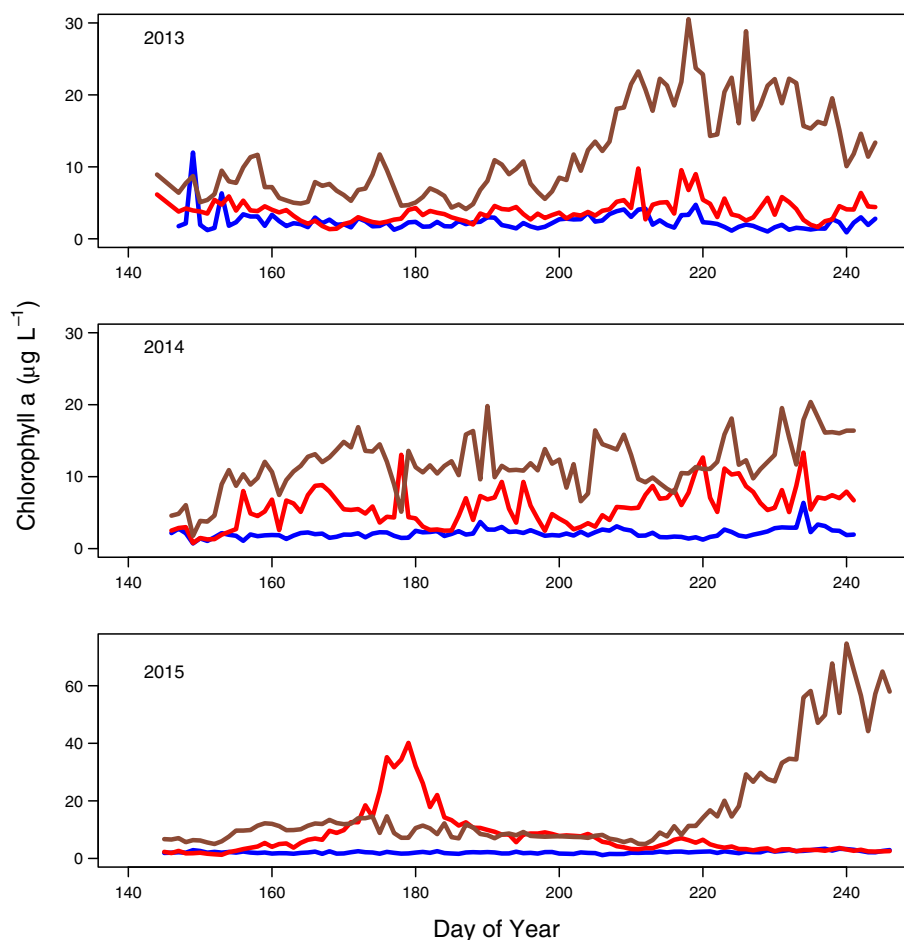


Fig. 3. Daily chlorophyll *a* in Tuesday (brown lines), Peter (red lines), and Paul (blue lines) lakes vs. day of year for 2013, 2014, and 2015.

chlorophyll. We compared one-, two-, and three-variable models of respiration using the possible combinations of chlorophyll, FDOM, and temperature. All the one- and two-variable models had substantially higher AICs than the three-variable model (Table 2). The minimum difference for one and two-variable models in AIC compared with the three-variable model was 17. The RSE of the three-variable model was 0.660 and the correlation (r) between predicted and observed values was 0.406. Given that average respiration was $-0.912 \text{ mg O}_2 \text{ L}^{-1} \text{ d}^{-1}$ for all the data, the RSEs were high and the relationship between observations and predictions was not strong. This residual noise reflects the large day-to-day variation in respiration (Fig. 4).

Three-variable models that included lake and year as categorical variables (hereafter “factors”) were explored to establish the impact of the different lakes and years. These models tended to have lower AICs, slightly lower RSEs, and a higher correlation of predicted vs. observed. The best factor model based on lowest AIC included “lake” and the delta AIC for this model relative to the three-variable model was 10.

Parameter values for the three-variable model of respiration (without factors) were all negative and highly significant (Table 3). Hence higher chlorophyll concentrations, greater

FDOM, and higher water temperatures were all associated with higher rates of respiration (meaning here more negative R , Eq. 1) consistent with our hypothesis.

The three-variable model with lake as a factor points to a problem of separating differences in lakes and FDOM in this study (Table 3). The parameter values of chlorophyll and temperature are highly significant and negative, while the parameter values of FDOM and lakes (as factors) are not significant. A reasonable interpretation is that the variability accounted for in respiration by FDOM in the simpler three-variable model is split mainly among FDOM and the factor terms for lakes in the more complex model. This model had high VIFs for FDOM (8.2) and the lake factor term (9.0) which was also the case for the model that included both lake and year as factors ($\text{VIF}_{\text{FDOM}} = 31$, $\text{VIF}_{\text{lake}} = 28$). Hence, the factor models, while minimizing AIC, were also problematic.

Another way to test the importance of FDOM to respiration is to examine a model based only on the reference lake, where FDOM varied (among years) while variation in chlorophyll was low (Figs. 2 and 3). A three-variable model for this data was similar to the model for all lakes except chlorophyll was not significant. In a two-variable model that excluded

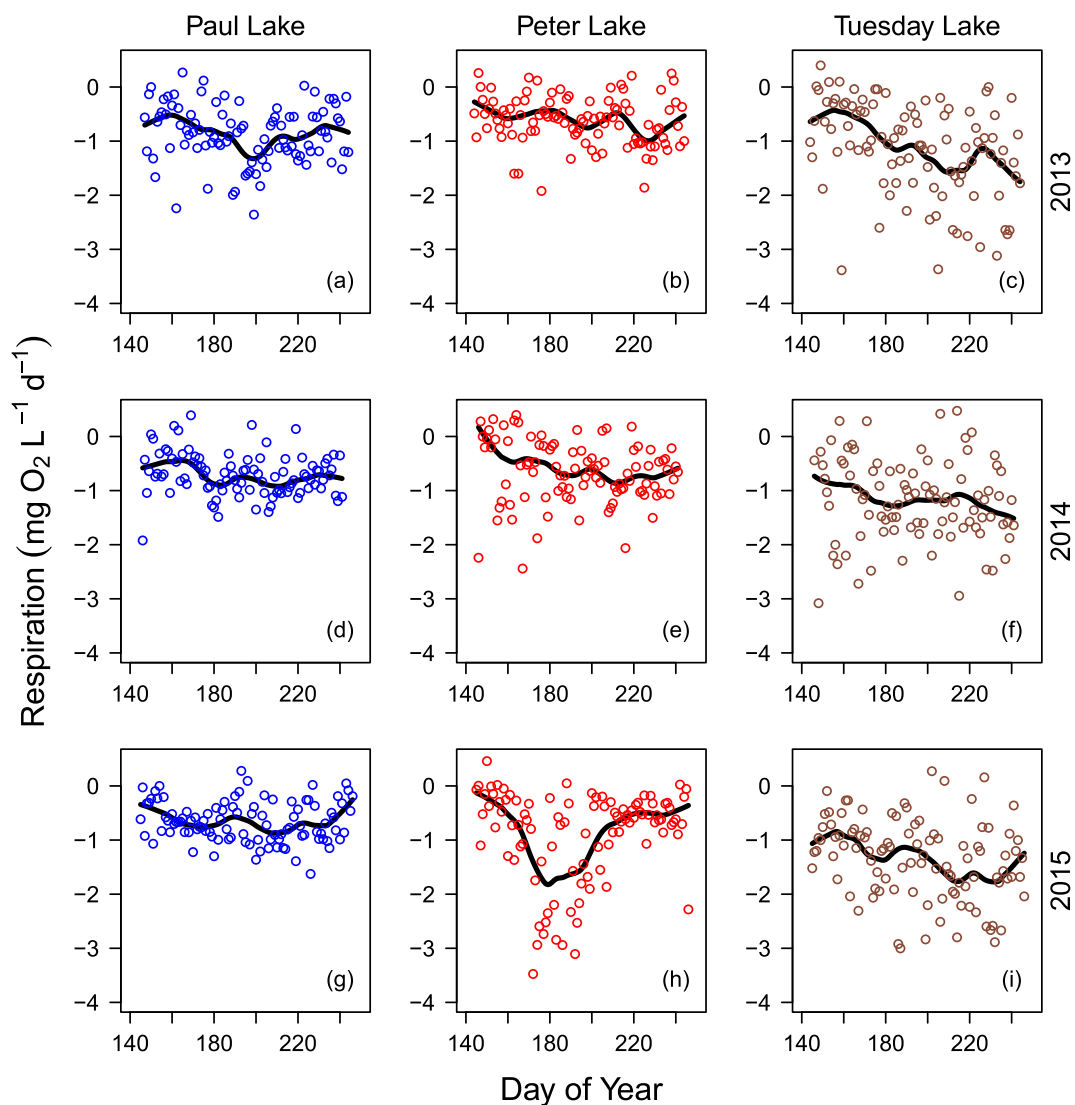


Fig. 4. Respiration vs. day of year (DOY) in (a) Paul Lake (blue points) 2013, (b) Peter Lake (red points), 2013, (c) Tuesday Lake (brown points) 2013, (d) Paul 2014, (e) Peter 2014, (f) Tuesday 2014, (g) Paul 2015, (h) Peter 2015, (i) Tuesday 2015. Black lines are LOWESS fits to the data (see text). Two outliers where respiration was more negative than $-5 \text{ mg O}_2 \text{ L}^{-1} \text{ d}^{-1}$ are not shown (Tuesday Lake, DOYs 147 and 175 in 2014).

chlorophyll and used only Paul Lake data, FDOM ($t = -4.34$) and temperature ($t = -5.30$) were highly significant (Supporting Information Data S1).

A model that included an interaction term for chlorophyll and FDOM did not have a lower AIC than the simpler three-variable model without interaction. Furthermore, the interaction term was positive but not significant ($t = 1.12$, $p = 0.218$) suggesting little evidence for a positive nonlinear effect of chlorophyll and FDOM and no evidence for a negative nonlinear effect (Supporting Information Data S1).

Discussion

By varying primary production against differing DOM concentrations, our whole-lake manipulations confirmed the

importance of both autochthonous and allochthonous sources in supporting respiration. More importantly, we did not find evidence that these sources act synergistically in either a positive or negative direction. Models incorporating daily data for chlorophyll, FDOM, and temperature had the lowest AICs. Using “lakes” and/or “years” as categorical variables produced models with lower AICs but with some nonsignificant driver variables and high multicollinearity.

DOM is not completely derived from allochthonous sources especially under nutrient enrichment as we have documented in prior whole lake C-13 additions (Bade et al. 2007). However, the DOM in the three study lakes is largely terrestrial, likely because algal-derived DOM turns over rapidly, and with one exception we did not observe increases in FDOM or in weekly observations of DOC in association

Table 2. Comparison of one-, two-, and three-variable models for respiration as well as three-variable models with either “lake,” “year,” or “lake and year” as categorical variable (i.e., factors). Models are compared based on the difference (Δ) in Akaike information criteria (AIC) with three-variable model of chlorophyll + FDOM + temperature. Residual standard error (RSE) and correlation (r) of predicted vs. observed are also presented.

Model	Δ AIC	RSE	r
<i>One variable</i>			
Chlorophyll	42	0.683	0.326
FDOM	54	0.691	0.295
Temperature	90	0.714	0.157
<i>Two variables</i>			
Chlorophyll + FDOM	27	0.674	0.360
Chlorophyll + temperature	17	0.670	0.376
FDOM + temperature	35	0.679	0.341
<i>Three variables</i>			
Chlorophyll + FDOM + temperature	0	0.660	0.406
<i>Three variables with factors</i>			
Chlorophyll + FDOM + temperature + Lake	-10	0.654	0.428
Chlorophyll + FDOM + temperature + year	0	0.659	0.412
Chlorophyll + FDOM + temperature + Lake + year	-8	0.653	0.429

with phytoplankton blooms. We interpret increased respiration in relation to higher concentrations of DOM as mainly an effect of allochthonous inputs.

Despite a large number of observations under varying chlorophyll concentrations and differing FDOM levels, there was not an interactive effect of these variables on respiration. FDOM reduces primary production through light limitation and by inference this effect would lower respiration by autotrophs, but this mechanism did not emerge as an interactive effect. FDOM also may be a source of nutrients (Vähätalo et al. 2003) increasing phytoplankton production and respiration, and FDOM stimulates respiration (Brighenti et al. 2018).

These mechanisms might explain the lack of an interaction. Another possibility is that interactive effects were not detected due to the high variability in daily respiration such there was too much error to detect an interaction. Nevertheless the statistical models based on a large number of observations emphasize the direct importance of algal biomass, DOM, and temperature in determining respiration consistent with prior studies (Staeher et al. 2010; Coloso et al. 2011a).

As in other studies, we observed high day-to-day variation in estimates of respiration based on overnight declines in oxygen (Staeher and Sand-Jensen 2007; Coloso et al. 2011a). Some of this variation could be attributed to measurement error, but previous studies indicate coefficients of variation for replicate respiration measurements of oxygen concentration are < 10% (Van de Bogert et al. 2007). Prior studies have documented significant spatial heterogeneity of oxygen dynamics in lake surface water (Van de Bogert et al. 2007, 2012), and water movements are the likely cause of the large day-to-day variation in respiration (Brothers et al. 2017). Because our focus was on measuring bloom dynamics for the assessment of early warnings (Wilkinson et al. 2018), we did not deploy multiple sondes in different locations or over depth to account for horizontal (Van de Bogert et al. 2012) or vertical (Staeher et al. 2012b) variation, and this is a limitation on our analysis. Aside from a higher density of spatially distributed oxygen measurements, it remains unclear how to develop a better approach to modeling and measuring the dynamics of oxygen. Differences in respiration measures are related in part to sediment respiration which may have varied in relation to lake size (smaller lakes have greater sediment area to volume), and in any case, differentially influence a centrally located sonde as inferred in prior studies (Van de Bogert et al. 2007; Coloso et al. 2011a). Variability in the oxygen consumption process is also likely related to variable sensor footprints, variations in mixing depths (Supporting Information Data S1), subdaily dynamics, heterogeneous sediments, and plankton patchiness and migrations (Coloso et al. 2011b; Sadro

Table 3. Summary of two models for respiration with the lowest AIC for the two types of three-variable models. The first column indicates the models and coefficient names and subsequent columns are parameter values, parameter standard errors (SE), t -statistics, and probability (P) that the parameter estimate is different from zero.

	Value	SE	t	p
<i>Three-variable model</i>				
Chlorophyll	-0.0213	0.00338	-6.32	<0.0001
FDOM	-0.000397	0.0000896	-4.43	<0.0001
Temperature	-0.0587	0.0106	-5.51	<0.0001
<i>Three-variable model with lake factors</i>				
Chlorophyll	-0.0196	0.00347	-5.65	<0.0001
FDOM	0.000115	0.000217	-0.53	0.60
Temperature	-0.058	0.0104	-5.59	<0.0001
Peter	0.111	0.0831	1.34	0.18
Tuesday	-0.217	0.178	-1.22	0.22

et al. 2014; Bogard et al. 2019; Mantikci et al. 2020). Fully accounting for additional habitats in lakes is also important to assess respiration for the entire lake system as revealed by recent studies (Vesterinen et al. 2016; Giling et al. 2017). While processes affecting oxygen (e.g., production, respiration, photolysis) are known, understanding and measuring the oxygen sources and sinks and the hydrodynamic integration of these processes limits respiration measurements at the whole lake scale.

We used a model of gas exchange that represents physical processes influencing exchange in small lakes (Read et al. 2012). This model, in theory, improved our measures of gas exchange, but a simpler model that only calculates gas exchange from wind-driven turbulence gave similar respiration estimates (data not presented). Gas exchange in the study lakes was a relatively small flux compared to oxygen consumption due to respiration. For example for the oxygen decline in Paul Lake (31 July 2013) presented in Fig. 1, about 10% of the oxygen flux was due to air–water exchange with the balance due to respiration. The more realistic model of gas exchange did not significantly change our estimates of respiration and applying the model required incorporating inputs of a number of additional variables (e.g., barometric pressure, relative humidity). Broader comparisons of gas-exchange among lakes indicate that model choice can significantly affect estimates of respiration (Dugan et al. 2016) but that was not case for the similar lakes considered in our study.

The whole lake nutrient additions caused phytoplankton blooms, and respiration tended to increase during blooms. For example in Peter Lake in 2015 there was a phytoplankton bloom where chlorophyll concentrations were $> 10 \mu\text{g L}^{-1}$ from 20 June (day of year 171) to 08 July (day of year 189). Respiration before the bloom varied from near 0 to $-1 \text{ mg O}_2 \text{ L}^{-1} \text{ d}^{-1}$, whereas during the bloom, respiration varied from approximately -1 to $-3 \text{ mg O}_2 \text{ L}^{-1} \text{ d}^{-1}$ and remained elevated but variable for a period after the bloom (Fig. 4). In this manipulation nutrient input was halted as the bloom first developed (Pace et al. 2017). Hence, there were no additional blooms. Respiration in August 2015 returned to pre-bloom levels varying, as before the bloom, between 0 and $-1 \text{ mg O}_2 \text{ L}^{-1} \text{ d}^{-1}$. Overall, respiration was affected by blooms but was not as dynamic as chlorophyll concentrations, and respiration did not strongly track blooms at least partly due to the obscuring effects of large day-to-day variability.

DOC and a_{440} were elevated in the lakes in 2013 and 2014 and more similar to data from previous years in 2015 (Pace et al. 2019). FDOM was also elevated in the first 2 yr (i.e., 2013, 2014) relative to 2015. For example, mean FDOM was 508, 409, and 372 relative fluorescence units in Paul Lake in 2013, 2014, and 2015, respectively. The effect of elevated FDOM is evident for Paul Lake where mean R was highest in 2013 ($-0.87 \text{ mg O}_2 \text{ L}^{-1} \text{ d}^{-1}$) and lowest in 2015 ($-0.66 \text{ mg O}_2 \text{ L}^{-1} \text{ d}^{-1}$). The 2015 mean was closer to a seasonal mean previously measured for the same lake of

$-0.5 \text{ mg O}_2 \text{ L}^{-1} \text{ d}^{-1}$ (Coloso et al. 2011b). FDOM had a strong effect on respiration but because we did not manipulate FDOM, there is a possibility that these effects are due to other differences among lakes and years. Zwart et al. (2016) observed strong direct effects on respiration in a whole lake manipulation that increased DOC by 4.2 mg C L^{-1} relative to a reference basin. Respiration increased substantially and changes in respiration were much greater than changes in GPP likely due to increased allochthonous loading of labile organic carbon created by the manipulation (Zwart et al. 2016). We observed a similar strong positive effect of FDOM on respiration in the reference lake where there was no change in chlorophyll. Thus, our study in addition to other whole lake manipulations (Zwart et al. 2016) indicates a consistent positive effect of DOM on respiration.

The coefficients describing the relationship of temperature to respiration (Table 2) enable estimating how respiration changes with an increase in 10°C (i.e., Q10). The measured temperature range in this study was from 14.5°C to 28.6°C . Using the three-variable model with chlorophyll and CDOM set at their means and without an autoregressive term, the calculated Q10 value was 2.0. This value falls within the range of Q10s summarized by Cardoso et al. (2014) for data mainly from temperate and boreal lakes inclusive of benthic and planktonic respiration where reported Q10 values were in the range 1.3–3.7 (excluding two outliers). Our estimated Q10 value gives confidence that the temperature parameter values (all near -0.058 , see Table 3) were reasonable estimates of the effect of temperature on respiration.

Our study covered a 10-fold range of nutrient loading rates, a 100-fold range in phytoplankton biomass based on daily Chl *a* concentrations, and a 3-fold range of average DOC concentrations. These ranges encompass most lakes excepting oligotrophic, clear-water lakes, highly colored brown-water lakes, and highly eutrophic, green-water lakes. Yet, respiration varied more modestly (Fig. 4), perhaps because the benthic component of respiration responded little to the temporary changes in phytoplankton. We cannot separate the planktonic and benthic contributions to respiration in this study but note that our prior carbon flux models indicate benthic respiration is 24–42% of the total for these lakes (Cole et al. 2006). Limited change or possibly a decline in benthic respiration due to lower periphyton productivity (Vadeboncoeur et al. 2001) could have contributed to the modest increases in respiration observed in these lake manipulations.

Our ecosystem manipulations indicate positive and non-interacting responses of lake respiration to manipulations of nutrients and increases in DOM. Across a range of oligotrophic to highly eutrophic lakes, the importance of FDOM as well as interactions with primary production are less likely to be the main drivers as nutrient inputs dominate responses. Hence, if there are nonlinearities in how respiration responds to changes in driving variables, we hypothesize these are only likely at higher levels of FDOM (DOC concentrations

> 13 mg C L⁻¹, a440 > 6 m⁻¹, i.e., greater than Tuesday Lake values) where FDOM more strongly inhibits primary production. Seekell et al. (2015) observed effects of FDOM on primary production at a lower threshold of DOC concentrations. However, a higher threshold for respiration (relative to primary production) is possible if increases in heterotrophic respiration of allochthonous carbon occur across gradients of FDOM that compensate for a possible inhibition of autotrophic respiration. Hydrological, climate, and land-use changes (Monteith et al. 2007; Weyhenmeyer et al. 2014; Kritzberg 2017) are causing changes in FDOM and nutrient inputs. For lakes that fall within the ranges we studied (especially in regions with little urbanization or agriculture), changes in inputs may lead to predictable responses in ecosystem processes like respiration.

Data availability statement

Data for the daily measures of chlorophyll, temperature, FDOM, and respiration presented in this paper are available in the North Temperate Lakes Long-Term Ecological Research data portal (<https://doi.org/10.6073/pasta/9991295661980ff90924f9746b5f42ce>).

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Conflict of Interest

None declared.

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