

*Origin of organic carbon as a driver of variability of carbon
decomposition and storage in lakes*

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Abstract

Lakes are becoming recognized as important carbon sinks, as they store large amounts of carbon relative to their surface area. However, the processes regulating carbon sequestration in lakes are much less well understood than those of other ecosystems. This study explores the influence of the origin of organic matter on decomposition in lakes – specifically that recalcitrant terrestrial carbon may lead to increased carbon storage in lakes. In this study, the rate of DIC increase was measured in 10 lakes across a gradient of terrestrial carbon input. As hypolimnion water color (g440) increased, the rate of DIC build-up in the hypolimnion was also found to increase. However, the rate of DIC build-up did not increase with increasing chlorophyll *a*, suggesting that decomposition in lakes is primarily driven by terrestrial, rather than aquatic carbon inputs.

Introduction

Lakes store a disproportionately large amount of carbon relative to their surface area, and they tend to store more carbon than other kinds of aquatic ecosystems (Dean and Gorham 1998). Globally, lakes cover only 3% of the surface area, but they accumulate about 42 Tg of organic carbon per year, although this is likely an underestimate of modern organic carbon burial (Kalff 2001, Dean and Gorham 1998, Heathcote et al. 2015). In comparison, oceans, which cover over 70% of Earth's surface, only accumulate about 100 Tg of organic carbon per year (Dean and Gorham 1998). Lakes in the Northern Highland Lake District cover only 35% of the surface area, but they store 80% of the carbon (Buffam et al. 2011). Even though lakes store so much carbon, much less is known about carbon sequestration in lakes than in terrestrial ecosystems and oceans, and there are still many unknowns regarding the processes governing carbon sequestration in lakes (Dean and Gorham 1998, Ferland et al. 2014).

There are several possible explanations for why lakes store so much carbon, including the temperature of the sediments and anaerobic versus aerobic decomposition (Gudasz et al. 2011). The influence of the quantity and origin of organic carbon in lakes is also thought to be important, but these factors are not well understood (Gudasz et al. 2011). This study intends to help fill in that gap by focusing on the organic matter quality hypothesis – that lake sediments store large amounts of terrestrial carbon that is difficult to decompose. Terrestrial carbon tends to be recalcitrant, meaning its molecular structure is resistant to decomposition by microbes. Since it has a much higher C:N ratio than algae, terrestrial material is much less labile than algae (Francis et al. 2011). Terrestrial carbon accounts for most of the organic material in northern temperate lakes (Wilkinson et al. 2013). Of the total terrestrial carbon received by northern lakes, up to 15% is sequestered in lake sediments, and up to 45% is evaded to the atmosphere, with the rest eventually going to the oceans (Heathcote et al. 2015). The carbon that is evaded to the atmosphere is mineralized to carbon dioxide and methane by microbes at the sediment interface (Ferland et al. 2014). However, resistance to decomposition leads to much of the terrestrial carbon being efficiently stored in lake sediments (Tranvik et al. 2009). Autochthonous carbon from algae production in lakes is easily decomposable, unlike terrestrial carbon, and the more terrestrial carbon present relative to algal material, the harder all the organic carbon is to decompose (Francis et al. 2011). A 2012 study of decomposition of organic carbon in boreal lakes found that increasing terrestrial carbon input did not increase decomposition by bacteria, which indicates that the terrestrial organic carbon was not being decomposed, but rather it was recalcitrant and contributed to carbon storage in the lakes (Gudasz 2012).

This study intends to address whether the rate of decomposition in lakes is correlated with proxies for terrestrial carbon loading. The lakes at the University of Notre Dame

Environmental Research Center (UNDERC) in the Upper Peninsula of Michigan are well suited for testing this question because there are numerous lakes spanning a gradient of terrestrial carbon input, and they are affected minimally by human development (Wilkinson et al. 2013). Based on the recalcitrance of terrestrial carbon, it was hypothesized that the rate of decomposition in lakes would be negatively correlated with proxies for terrestrial carbon loading, and thus lakes with higher terrestrial carbon input will store more carbon. To test this hypothesis, the rate of DIC increase was measured in 10 lakes along a gradient of terrestrial carbon input. Increasing water color, an index of terrestrial carbon loading, was expected to be higher in lakes with lower rates of decomposition. Similarly, chlorophyll a, an index of algal biomass, was expected to be correlated with increasing rates of decomposition, or DIC build-up, in the lakes.

Methods

Study Site

Ten lakes on the property of the University of Notre Dame Environmental Research Center (UNDERC) were sampled. UNDERC is located in the Northern Highlands Lake District in Wisconsin and the Upper Peninsula of Michigan. The lakes sampled were Peter, Paul, Tuesday, Hummingbird, East Long, West Long, Crampton, Tender Bog, Morris, and Cranberry. These lakes were chosen to span a gradient of terrestrial carbon input, from lakes with a high terrestrial carbon to aquatic carbon input ratio to lakes with a low terrestrial carbon to aquatic carbon input ratio. Except for Peter, Paul, and Tuesday, each lake was sampled four times between May 24 and July 13, 2016. Peter, Paul, and Tuesday were sampled every week between May 16 and July 13, 2016.

Dissolved Inorganic Carbon

Water samples were taken from the hypolimnion of each lake using a Van Dorn at approximately 2 m above the sediment at the deepest point in the lake, which was determined using bathymetric maps. Samples were taken at two depths in Peter, Paul, and Tuesday – one from approximately 2 m above the sediment and one from 4 m above the sediment. The samples were taken from the hypolimnion because very little exchange occurs in the hypolimnion with the atmosphere during the period of summer stratification, allowing CO₂ to build up rather than equilibrate with the atmosphere (Cole and Pace 1998). The water samples were analyzed using a Shimadzu GC-8A gas chromatograph with TCD. 60 mL syringes were filled with hypolimnion water to 30 mL, and all air bubbles were removed. Then the syringes were emptied down to 10 mL, and 200 µL of 2N H₂SO₄ and 20 mL of helium were added to each syringe in order to convert all inorganic carbon species to gaseous CO₂. The syringes were then shaken for 1 minute in order to equilibrate the CO₂ with the inert helium headspace. 10 mL of equilibrated headspace was then injected into the gas chromatograph and CO₂ concentration was measured.

Limnological Variables

Chlorophyll *a* (an indicator of algal biomass) and water color (a proxy for terrestrial material concentrations) of the hypolimnion water were also measured (Wilkinson et al. 2013). Hypolimnion water was filtered through Whatman 47 mm GF/F filters to collect algal particles containing chlorophyll *a*. The filters were frozen for at least 24 hours, extracted in 25 mL of methanol for 24 hours, and then analyzed fluorometrically. Hypolimnion water filtered through ashed Whatman GF/F filters was used to measure hypolimnion water color of each lake as absorbance at 440 nm, measured with a spectrophotometer. On the final sampling date, the

spectral slope (Helms et al. 2008) was measured in each lake. Spectral slope is an index of dissolved organic matter molecular weight, which is related to its lability (Helms et al. 2008).

Several additional variables were measured in each lake for each time point. A light profile was taken using a LiCor LI-1000 meter with a spherical sensor to approximate the depth where 1% of the surface light penetrates the water. A dissolved oxygen (DO) and temperature profile of the full depth of each lake was taken using a YSI Professional Plus Multiparameter Handheld meter. Hypolimnion water for pH analysis was collected in air tight BOD bottles to prevent carbon dioxide evasion (Wilkinson et al. 2013) and was measured using the slow equilibration technique for soft waters developed by Stauffer (1990).

Data Analysis

The rate of decomposition in each lake was calculated as the increase in DIC concentration in the hypolimnion per unit time, specifically the amount produced per day. This is the slope of a linear regression of DIC concentration versus day of year. Regression analyses were then performed of the rates of DIC production versus the proxies for terrestrial carbon loading, which indicate any relationship between the rate of decomposition in the lakes and terrestrial carbon loading. One DIC concentration point from Paul (9m), day 180, was omitted from the linear regression due to sampling error. Linear regressions were also done to compare DIC concentration with hypolimnion water color (absorbance at 440 nm), hypolimnion chlorophyll *a*, hypolimnion pH, and the light extinction coefficient, to determine if there is a relationship between the amount of DIC in the hypolimnion of a lake and any of those variables. Further linear regressions were performed comparing each of the hypolimnetic variables measured with each other and with their epilimnetic counterparts to determine if the water chemistry of the two layers were related.

Results

Eight of the ten lakes sampled showed an increase in DIC concentration in the hypolimnion over eight weeks (Figure 1). DIC concentration decreased only in Tender Bog and East Long. In Peter, Paul, and Tuesday, the DIC concentrations increased at a greater rate at the shallower hypolimnion depth than at the lower depth.

DIC concentration in the hypolimnion increased with increased hypolimnion water color (g440) ($p = 0.011$), hypolimnion chlorophyll *a* ($p < 0.001$), and hypolimnion pH ($p = 0.037$) (Figure 2). Although there was a strong pattern between DIC concentration and the light extinction coefficient, the relationship was not statistically significant ($p=0.052$). Hypolimnion DIC concentration also correlated positively with surface water color (g440) ($p = 0.038$) (Table 1). Further, there were positive correlations between hypolimnetic and epilimnetic pH ($p < 0.001$) and water color (g440) ($p < 0.001$) (Table 1). There were positive and negative correlations, respectively, between hypolimnion pH and hypolimnion chlorophyll *a* ($p < 0.001$) and between hypolimnion pH and hypolimnion water color ($p = 0.01$).

As hypolimnion water color (g440) increased, the rate of DIC build-up in the hypolimnion decreased ($p = 0.049$) (Figure 3). The rate of DIC build-up in the hypolimnion increased as hypolimnion pH increased ($p = 0.020$) (Figure 3). However, hypolimnion chlorophyll *a* was not significantly correlated with the rate of DIC build-up in the hypolimnion ($p = 0.056$). The rate of DIC build-up in the hypolimnion was also unrelated to the light extinction coefficient ($p = 0.84$), the maximum depth of the lake ($p = 0.79$), and the surface area of the lake ($p = 0.24$) (Table 2).

Discussion

As expected, there was a build-up of DIC in the hypolimnions of most of the lakes over the sampling period (Figure 1). This result is consistent with the lack of mixing of hypolimnion water with epilimnion water, which traps the DIC produced by decomposition in the hypolimnion, since solutes and gases do not permeate the thermocline of stratified lakes (Wilkinson et al. 2014, Cole and Pace 1998). Surprisingly, two of the lakes sampled, Tender Bog and East Long, showed a decrease in DIC concentration in the hypolimnion over the sampling period. This unexpected phenomenon could have several explanations. There could have been some mixing in Tender Bog and East Long, which would allow the DIC to escape the hypolimnion. It is also possible that Tender Bog was not fully stratified or had only recently stratified at the beginning of the sampling period. In Peter, Paul, and Tuesday, there were different rates of DIC build-up at different depths in the hypolimnion, which indicates that there is heterogeneity within the hypolimnion, which could also be part of the decrease in DIC concentration observed in two lakes. In Peter, Paul, and Tuesday, there was a greater rate of DIC build-up in the hypolimnion at the shallower point than the deeper point, which could be caused by slightly higher temperatures at the shallower depth.

The increase in DIC concentrations observed with increased water color and chlorophyll *a* is likely due to a greater amount of carbon available for decomposition in lakes with darker water color and more chlorophyll *a*, since water color indicates terrestrial carbon, and chlorophyll *a* indicates algal biomass. Simply, lakes with more carbon input, terrestrial or aquatic, have greater DIC concentrations. There is also an increase in DIC concentration with

increased pH, which is linked to a positive correlation between pH and chlorophyll *a* (Table 1). In these lakes, pH is driven by metabolism in these poorly buffered systems, such that the more photosynthesis occurs, the higher the pH will be because of the drawdown of CO₂, removing carbonic acid from the system. Similarly, an increase in respiration from the input of terrestrial material decreases the pH by adding CO₂ to the lake, which explains the negative correlation between water color and pH. The relationship between epilimnetic and hypolimnetic water color (g440) is particularly interesting (Table 1). The hypolimnion water color is consistently about 7m⁻¹ darker than the epilimnion water color. This phenomenon could be caused by photobleaching in the epilimnion. Sunlight causes photobleaching or photodegradation of dissolved organic matter, which can accelerate mineralization and lighten the color of the water (Laurion et al. 2000).

Across a gradient of increasing water color (a proxy for terrestrial carbon input), there was a decrease in the rate of DIC build-up in the hypolimnion (Figure 3). However, there was no similar correlation between chlorophyll *a* (a proxy for algal biomass) and rate of DIC build-up. These results suggest that the rate of DIC build-up, or decomposition, in lakes is driven primarily by terrestrial rather than aquatic carbon inputs. This higher rate of decomposition in lakes with higher terrestrial carbon input indicates that lakes with higher terrestrial carbon input store more carbon. There was also an increase in the rate of DIC build-up in the hypolimnion across a gradient of increasing pH, as expected given the relationship between high pH and high rates of photosynthesis and respiration. There was also no relationship between the rate of DIC build-up and the morphology of the lake (maximum depth and surface area). This result is in contrast to the findings of Ferland et al. (2014), which found that carbon burial efficiency is strongly related

to lake morphology. However, this difference probably reflects the differences in the small, forest sheltered kettle lakes at UNDERC versus the large, boreal reservoirs in Quebec.

These results give us insight into the processes that lead to carbon burial in lakes, which is important to understand because of global climate change. Global climate change will cause changes in precipitation, temperature, and seasonality, which will likely lead to stronger, more prolonged stratification in lakes (Tranvik et al. 2009). The resulting extended period of anoxia in the hypolimnion of lakes will lead to reduced mineralization of terrestrial organic carbon and therefore increased carbon sequestration (Tranvik et al. 2009). However, the prolonged anoxia could also increase methane emissions from lakes (Tranvik et al. 2009). A better understanding of the factors that contribute to variability in carbon storage in lakes, like the importance of terrestrial carbon input, will help us to adapt to a changing climate.

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Figures and Tables

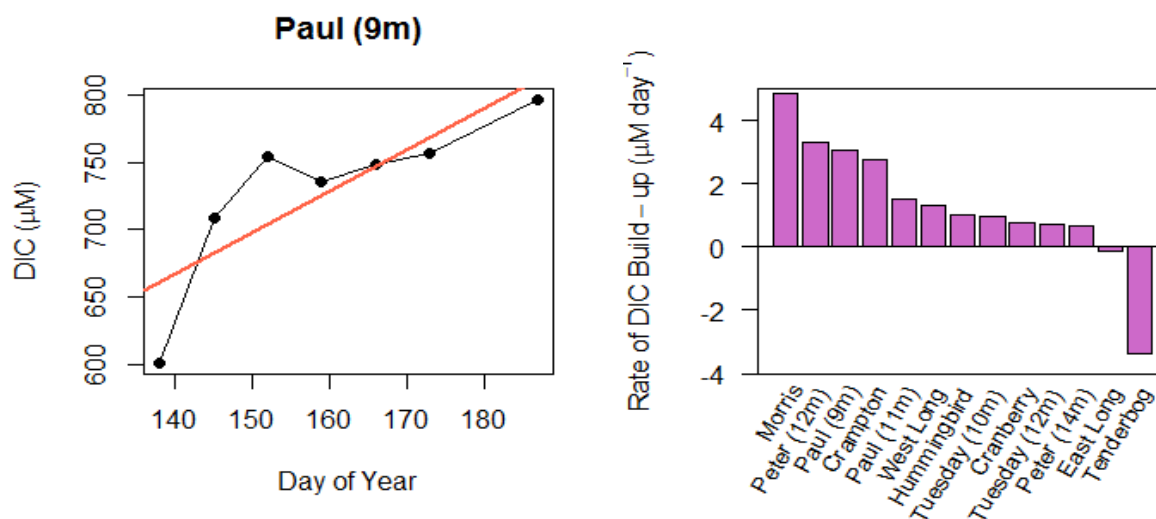


Figure 1. (a) The build-up of DIC in the hypolimnion of Paul at a depth of 9 m over eight weeks. The red line is the linear regression model ($DIC = 3.06 * DOY + 238.8$, $R^2 = 0.69$, p -value = 0.021, $F_{1,7} = 11$). (b) The rate of DIC build-up for all lakes and depths sampled.

Table 1. Regression analyses of hypolimnetic DIC and hypolimnetic and epilimnetic limnological variables.

Independent Variable	Dependent Variable	Equation	r^2	p -value
Depth	DIC	$DIC = -30.4 * Z_{Hypo} + 1118.2$	0.062	0.023
Hypo Water Color (g440)	DIC	$DIC = 24.2 * Hypo_g440 + 459.9$	0.21	0.011
Hypo Chlorophyll <i>a</i>	DIC	$DIC = 20.4 * Hypo_Chl + 529.2$	0.41	<0.001
Hypo pH	DIC	$DIC = 263.8 * Hypo_pH + (-670.3)$	0.14	0.037
Light Extinction Coefficient	DIC	$DIC = 132.6 * kd + 557.3$	0.12	0.052
Surface Water Color (g440)	DIC	$DIC = 25.1 * Surf_g440 + 588.6$	0.16	0.038
Surface pH	DIC	$DIC = 117.6 * Surf_pH + 149.4$	0.06	0.22
Surface Chlorophyll <i>a</i>	DIC	$DIC = 1.4 * Surf_Chl + 791.5$	0.0021	0.85
Hypo Water Color (g440)	Light Extinction Coefficient	$kd = 0.069 * Hypo_g440 + 0.79$	0.32	0.0012
Hypo Water Color (g440)	Hypo Chlorophyll <i>a</i>	$Hypo_Chl = -0.077 * Hypo_g440 + 15.2$	0.0021	0.82
Hypo pH	Hypo Water Color (g440)	$Hypo_pH = -6.1 * Hypo_g440 + 49.3$	0.21	0.010
Hypo pH	Hypo Chlorophyll <i>a</i>	$Hypo_Chl = 16.2 * Hypo_pH + (-76.4)$	0.54	<0.001
Hypo pH	Light Extinction Coefficient	$kd = -0.41 * Hypo_pH + 4.1$	0.063	0.17
Hypo Chlorophyll <i>a</i>	Light Extinction Coefficient	$kd = 0.012 * Hypo_Chl + 1.7$	0.027	0.40
Surface Chlorophyll <i>a</i>	Hypo Chlorophyll <i>a</i>	$Hypo_Chl = 0.082 * Surf_Chl + 14.1$	0.0070	0.73
Surface pH	Hypo pH	$Hypo_pH = 0.68 * Surf_pH + 1.7$	0.93	< 0.001
Surface Water Color (g440)	Hypo Water Color (g440)	$Hypo_g440 = 1.0 * Surf_g440 + 6.9$	0.68	<0.001

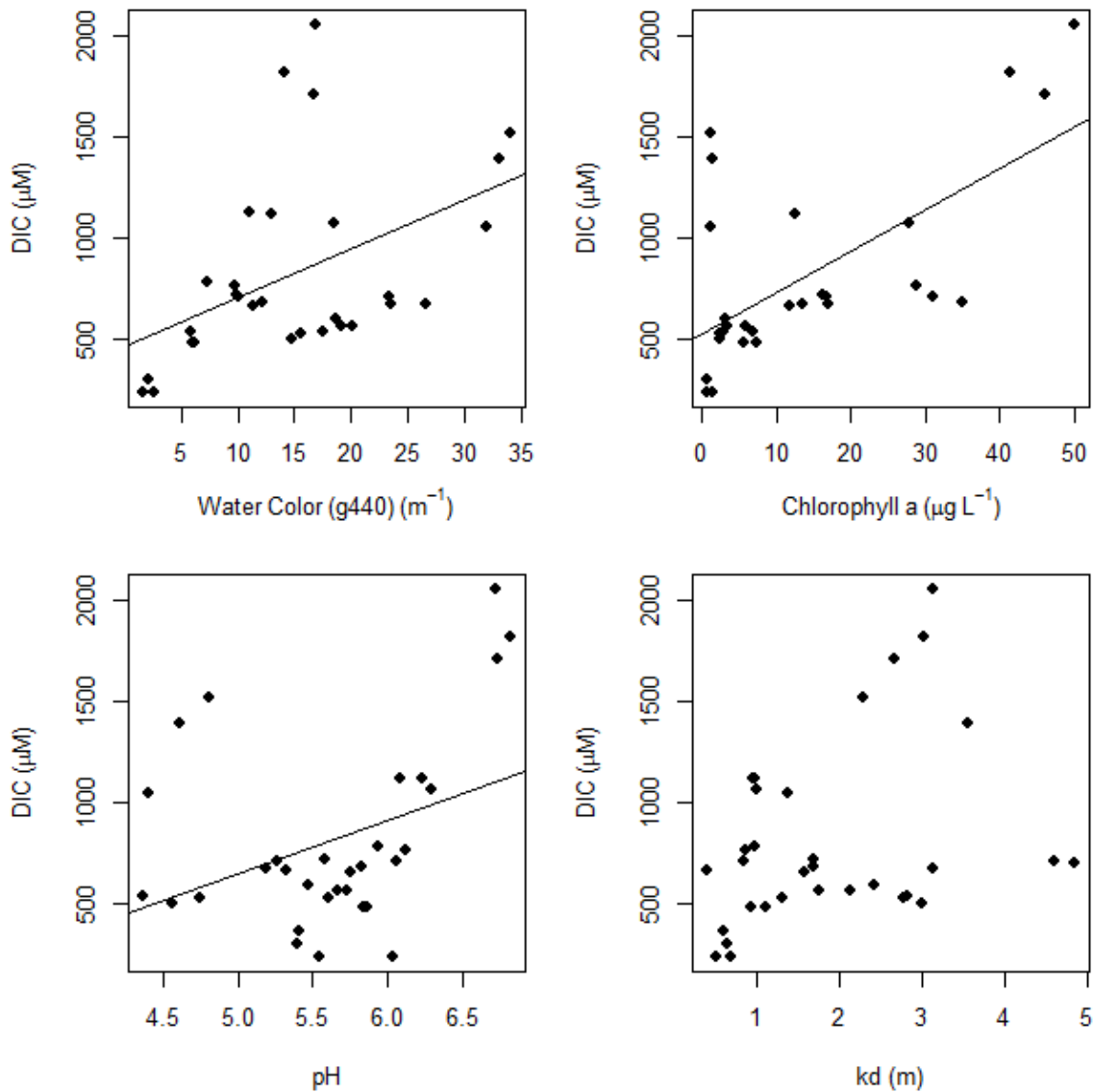


Figure 2. DIC concentration plotted against hypolimnion water color (g440), hypolimnion chlorophyll *a*, hypolimnion pH, and the light extinction coefficient (*kd*). (a) Linear regression model: $\text{DIC} = 24.2 * \text{Hypo_g440} + 459.9$, $R^2 = 0.21$, $p\text{-value} = 0.011$, $F_{1,28} = 7.5$. (b) Linear regression model: $\text{DIC} = 20.4 * \text{Hypo_Chl} + 529.2$, $R^2 = 0.41$, $p\text{-value} < 0.001$, $F_{1,26} = 18.4$. (c) Linear regression model: $\text{DIC} = 263.8 * \text{Hypo_pH} + (-670.3)$, $R^2 = 0.14$, $p\text{-value} = 0.037$, $F_{1,29} = 4.8$. (d) Linear regression model: $\text{DIC} = 132.6 * kd + 557.3$, $R^2 = 0.12$, $p\text{-value} = 0.052$, $F_{1,30} = 4.1$.

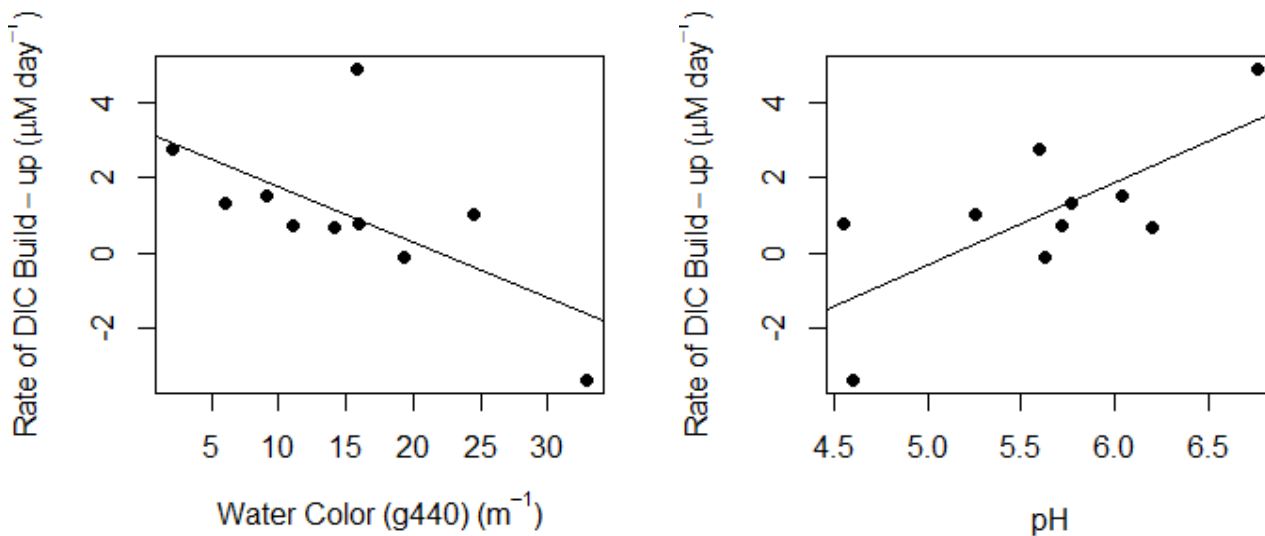


Figure 3. (a) The rate of build-up of DIC in the hypolimnion of all lakes compared with hypolimnion water color (g440) (Rate = $-0.15 * \text{Hypo_g440} + 3.22$, $R^2 = 0.40$, p-value = 0.049, $F_{1,8} = 5.4$) (b) The rate of build-up of DIC in the hypolimnion of all lakes compared with hypolimnion pH (Rate = $2.20 * \text{Hypo_pH} + (-11.3)$, $R^2 = 0.51$, p-value = 0.020, $F_{1,8} = 8.4$).

Table 2. Regression analyses of the rate of DIC build-up in the hypolimnion and limnological variables

<i>Independent Variable</i>	<i>Dependent Variable</i>	<i>Equation</i>	<i>r²</i>	<i>p-value</i>
Hypo Chlorophyll <i>a</i>	Rate of DIC Build-up	Rate = $0.0875 * \text{Hypo_Chl} + (-0.2829)$	0.38	0.056
Hypo Water Color (g440)	Rate of DIC Build-up	Rate = $-0.15 * \text{Hypo_g440} + 3.22$	0.40	0.049
Hypo pH	Rate of DIC Build-up	Rate = $2.20 * \text{Hypo_pH} + (-11.3)$	0.51	0.020
Light Extinction Coefficient	Rate of DIC Build-up	Rate = $-0.18 * \text{kd} + 1.33$	0.0057	0.84
Maximum Depth	Rate of DIC Build-up	Rate = $-0.056 * \text{Z_max} + 1.80$	0.011	0.79
Surface Area	Rate of DIC Build-up	Rate = $0.12 * \text{Surface Area} + 0.52$	0.19	0.24