The Relationship Between Water Residence Time and Dissolved Organic Carbon Quality and its Impact on Bacterial Respiration

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Abstract

Dissolved organic carbon (DOC) is an important energy source for bacteria within lake ecosystems. Bacterial metabolism and subsequent respiration of DOC is controlled by its quality, as bacteria prefer labile DOC as opposed to recalcitrant DOC. Water residence time affects DOC quality as DOC changes in character over time, becoming more recalcitrant as it ages. I investigated bacterial respiration rates across a gradient of water residence time to determine if DOC quality varies with residence time. I hypothesized that DOC in lakes with long water residence times would result in reduced rates of bacterial respiration, while DOC in lakes with short water residence times and inlets would result in high bacterial respiration rates. To test my hypothesis, I sampled lakes and their corresponding inlets in the Upper Peninsula of Michigan and measured bacterial respiration rates over a three-week period. The results of a regression analysis show that there is a weak positive relationship (linear regression analysis, $R^2 < 0.1$) between the ratio of average inlet bacterial respiration to average lake bacterial respiration and increasing watershed area to lake volume ratio (used as a proxy for water residence time). The results contradict my hypothesis, implying that other factors such as DOC concentration, nutrient availability, and bacterial community composition are more important in controlling bacterial respiration rates than water residence times.

Introduction

Dissolved organic carbon (DOC) is defined as a mixture of carbon compounds that exist in aquatic ecosystems (Attermeyer et al. 2014). DOC is a significant form of carbon that is transferred across terrestrial and aquatic ecosystems (Kolka et al. 2008). Lakes with higher concentrations of DOC have brown-colored water that ranges from light brown to dark brown (Waiser and Robarts 2000). This coloration is due to differences in abundance of particulate

organic matter dissolved in the water, which have high concentrations of humic substances that absorb light (Waiser and Robarts 2000, Pace and Cole 2002). DOC within lakes not only differs in concentration, but quality. Labile DOC refers to low molecular weight dissolved carbon that is highly soluble and easily transformable, while recalcitrant or humic DOC is resistant to being broken down by chemical processes (Hendrickson et al. 2002). DOC quality is mostly determined by its source (Kritzberg et al. 2004). Phytoplankton and algae within the system produce labile autochthonous DOC, while humic allochthonous DOC is terrestrial carbon that enters the system as organic matter (Attermeyer et al. 2014).

In addition to source, the age of DOC can determine its quality, as DOC tends to change in character over time through microbial degradation (Berggren 2009). As DOC ages it becomes recalcitrant, while fresh inputs of DOC are more labile (Berggren 2009). DOC "age" is a function of water residence time. Differences in water residence time can be studied in drainage lakes where water enters the lake through an inlet and exits via an outlet. In lakes, water residence time increases with increasing water volume. Inlets have smaller volumes of water than lakes; therefore, water travels quickly through inlets resulting in short residence times. DOC character varies between a lake and its corresponding inlet due to differences in water residence times (Gergel et al. 1999). Lakes with long water residence times tend to have low DOC concentrations due to low DOC input rates coupled with high photo induced degradation/biological mineralization rates. Inlets and small lakes with large wetland watersheds have short residence times with high DOC inputs that result in high DOC concentrations (Pace and Cole 2002).

DOC serves as a crucial energy source for bacteria within lake ecosystems (Attermeyer et al. 2014). It has been shown in previous studies that bacterial growth and respiration is highly

regulated by the quality, rather than the concentration, of DOC (Xu et al. 2013). High DOC lability is correlated with increased bacterial growth, while low DOC lability is associated with decreased bacterial growth (Xu et al. 2013). As DOC ages, it becomes more recalcitrant, decreasing bacterial respiration (Berggren 2009). It has been shown that water residence time affects DOC quality, which, in turn, affects bacterial respiration. I investigated bacterial respiration rates across a gradient of water residence time because I hypothesized that there are subsequent differences in DOC quality due to shorter or longer residence times. It is important to understand the factors that affect bacterial respiration, as bacteria are the main sinks of DOC in aquatic ecosystems (Rich et al. 1996). Without these sinks, DOC would accumulate, sharply reducing productivity, and leading to the creation of dystrophic lakes. Changing levels of DOC as a result of climate change have the potential to drastically affect bacterial respiration, which has important implications for the aquatic carbon cycle (Xu et al. 2013, Attermeyer et al. 2014). I hypothesized that DOC in lakes with long water residence times would result in reduced rates of bacterial respiration, while DOC in lakes with short water residence times and inlets would result in high bacterial respiration rates.

Materials and Methods

Sampling Protocol

Water samples were collected from 12 lakes and corresponding inlets in Vilas County, Wisconsin and Gogebic County, Michigan over a two week period in mid June, 2015 (Appendix A). The lakes were selected using drainage ratio data collected using ArcGIS software by S. Jones. Drainage ratios are defined as watershed area to lake volume (WALV), which was used as a proxy for residence time due to the difficulty in measuring residence time in natural lakes. The lakes selected ranged from short residence times to long residence times with drainage ratios of

567.152955 to 0.3370324 (Table 1). Integrated water samples of the permanently mixed layer (PML) were collected from the center of the lakes, respectively, using a Van Dorn sampler. Water was collected from the inlets using a surface grab with sample bottles. All water samples were stored in a refrigerator until they were filtered.

Experimental Design

The PML and inlet samples from each lake were initially filtered through a 153 μm filter to remove any large particles. The samples were then vacuum filtered into a volumetric flask through a 0.20 μm filter to remove bacteria. Three replicates were used for each lake and each inlet sampled. Each replicate consisted of 150 mL of filtered water in a 200 mL glass serum bottle. Sample bottles were inoculated with 1 mL of unfiltered water to ensure that relatively equal amounts of bacteria were present in each replicate. The bottles were then corked with rubber stoppers and crimped with aluminum caps so that the headspace of the bottles remained isolated from the outside air. The water collected from the last four lakes and inlets sampled, (LI, HH, LN, NR; Appendix A), was placed in 150 mL glass serum bottles. Ratios were used to determine that 113.5 mL of water in 150 mL bottles is proportionate to 151 mL of water in 200 mL bottles. The bottles were stored in the dark to eliminate the potential for photosynthesis.

Gas Chromatography

Gas chromatography was used to measure the carbon dioxide concentrations in the bottles. Carbon dioxide concentrations were measured every two days after they were initially bottled, from 6/09/15 to 6/23/15, using an Agilent 6890N Network Gas Chromatograph. The bioassay data was collected using Galaxie Chromatography Data System Software by Varian. From 6/09/15 to 6/21/15, 5 mL of headspace air was extracted from the bottles for sampling. From 6/22/15 to 7/07/15, 8 mL of headspace air was extracted from the bottles and 8 mL of lab

air was injected back into the bottles after sampling. After two weeks, from 6/23/15 to 7/01/15, the bottles were sampled every four days instead of every two days. Final sampling of the bottles took place six days after the previous sampling. The bioassay data was exported in summary reports after each run and carbon dioxide concentrations were calculated from the resulting data using R software. Bacterial respiration was then calculated as a production rate over two day, four day, and six day intervals using the carbon dioxide concentrations for each bottle. Some replicates had negative concentrations due to sampling errors in the gas chromatography. The replicates with negative concentrations were not used to calculate bacterial respiration.

Statistical Analysis

The calculated bacterial respiration rates were graphed and fit with an exponential curve because respiration follows an exponential trend. Some of the respiration rates were negative either due to gas chromatography sampling errors or calculation errors. A constant was added to all of the respiration rates to make all of the values positive without changing the slopes of the exponential curves. The slope of the exponential curve was used to determine bacterial respiration rate per replicate for the bioassay. The slopes of the replicates per lake and inlet were averaged to determine the bacterial respiration rate for each lake and inlet. I used the ratio of average inlet bacterial respiration rate to average lake bacterial respiration rate as a response variable to determine the impact of drainage ratio (water residence time) of lake respiration. Inlet to lake bacterial respiration ratios were calculated to standardize bacterial respiration across the lakes due to the fact that all lakes get carbon of different quality from their inlet. A regression analysis was used to determine the statistical relationship between the bacterial respiration rates and residence time.

Results

There was a weak positive correlation between the ratio of high average inlet bacterial respiration to low average lake bacterial respiration and increasing watershed area to lake volume ratio ($y = 0.0018x + 1.7971$, p value = 0.5285; Figure 1). Drainage ratios of the lakes ranged from 0.3370324 to 567.152955 and ratios of average inlet bacterial respiration to average lake bacterial respiration ranged from 0.4576 to 5.7044 (Table 1) As water residence time decreased, corresponding to an increasing drainage ratio value, the ratio of average inlet bacterial respiration to average lake bacterial respiration tended to increase. In lakes with shorter water residence times, bacterial respiration in the inlet greatly exceeded bacterial respiration in the lake. In lakes with longer residence times, bacterial respiration in the lake matched or exceeded bacterial respiration in the inlet. Overall, there is no significant relationship between bacterial respiration and water residence time (linear regression analysis, p value > 0.1; Figure 1).

Discussion

I used bioassays to determine the relative bacterial respiration rates of inlets and lakes across a gradient of drainage ratios (water residence time) to determine the impact that lake residence time has on bacterial respiration. Lakes with shorter water residence times had low rates of bacterial respiration, while lakes with longer water residence times had higher rates of bacterial respiration. Although the results of the regression analysis demonstrate that the relationship between the ratio of high average inlet bacterial respiration to low average lake bacterial respiration and shorter water residence time is extremely weak, the relationship contradicts my original hypothesis that lakes with shorter residence times would have higher rates of bacterial respiration. I saw an increasing relationship between drainage ratio and bacterial respiration rates when I expected to see a decreasing relationship because I predicted

that lakes with shorter residence times would have characteristics similar to that of inlets. Inlets have large inputs of allochthonous DOC and extremely short residence times due to their small water volume (Gergel et al. 1999). The DOC in inlets is very labile, as fresh inputs are constantly cycled through the system. Labile DOC is easily decomposed and therefore preferentially metabolized by bacteria, resulting in higher respiration rates (Xu et al. 2013, Kritzberg et al. 2004). Since smaller lakes have shorter residence times similar to the residence times of inlets, I expected that they would also have frequent inputs of allochthonous, labile DOC that would contribute to high bacterial respiration rates.

It is clear that factors beyond the relationship between water residence time and DOC quality control bacterial respiration in lakes. Lakes with longer water residence times tend to be clearer because they have lower inputs of allochthonous DOC. Clear lakes have more light penetration, which corresponds with greater numbers of phytoplankton (Storch and Saunders 1976). Phytoplankton are primary producers that produce autochthonous DOC, which is more labile than allochthonous DOC (Kritzberg et al. 2004). Large phytoplankton communities in clear lakes with long water residence times that produce autochthonous DOC that is preferentially consumed by bacteria might offer one explanation as to why lakes with longer residence times have high rates of bacterial respiration.

Bioavailable nutrients are another factor that strongly influence bacterial respiration rates in lakes that were not tested in this study. Bacteria require large amounts of nitrogen and phosphorus relative to carbon; therefore, bacterial metabolism of DOC is highly regulated by nutrient availability (Smith and Prairie 2004). Previous studies have shown that bacterial growth and metabolism is mainly dependent on phosphorus availability rather than DOC concentration or nitrogen availability (Smith and Prairie 2004). An increase in phosphorus and other

bioavailable nutrients increases primary production as well as the input rate and quality of DOC, which causes increases in bacterial growth efficiency (del Giorgio and Cole 1998, Xu et al. 2013). The interdependent relationship of nutrient availability and DOC quality suggests that both factors are crucial in regulating bacterial respiration in lakes. It has been shown that bacterial respiration also increases with increasing concentrations of glucose along with phosphorus, suggesting that bacteria prefer to metabolize the most accessible source of energy to avoid energetically costly processes and maximize their energy flow (Smith and Prairie 2004). Glucose is secreted by phytoplankton (Moshiri et al. 1979), which are present in greater numbers in clear, large lakes with longer residence times (Storch and Saunders 1976). The effect of glucose concentrations on bacterial respiration rates might offer another explanation as to why bacterial respiration rates were higher in lakes with longer residence times.

Bacterial respiration in lakes is likely governed by a number of different factors, of which I hypothesized that residence time was one. This study was limited by time constraints that reduced the number of lakes sampled and corresponding breadth of water residence times. Time constraints also limited the amount of time over which bacterial respiration could be measured. Future studies plan to examine a greater range of residence times in addition to more extensive monitoring of bacterial respiration rates. Continuing the investigation of the relationship between bacterial respiration and water residence time will shed more light on the results of this study and the effect of residence time on lake ecosystems. Additional studies may seek to understand the roles of DOC concentration, nutrient availability, and bacterial community composition in bacterial respiration and how these factors correlate with residence times in lakes. It is important to understand the relationship between DOC quality and bacterial respiration in lake ecosystems. Shifting levels of DOC inputs due to climate change and human activities have the potential to

drastically alter bacterial respiration rates. The production of carbon dioxide by bacteria in lakes has significant implications for the aquatic carbon cycle (Xu et al. 2013), which further contribute to the changing climate. As global temperatures continue to rise, an all-encompassing understanding of the carbon cycle is imperative.

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Tables

Table 1

Lake	Drainage Ratio (Watershed Area/Lake Volume)	Corresponding Water Residence Time
Little Oxbow	567.152955	Shortest
Little Horsehead	71.6215377	
North Crab	59.9910694	
Horsehead	12.9105489	
Lake of the Hills	11.7235384	
Helen	6.56651605	
Palmer	3.54995648	
Langford	1.14956029	
Escanaba	1.04242961	
Bay	0.67590375	
Jute	0.57865637	
Ike Walton	0.3370324	Longest

Drainage ratios of lakes sampled and corresponding water residence time estimates.

Figures

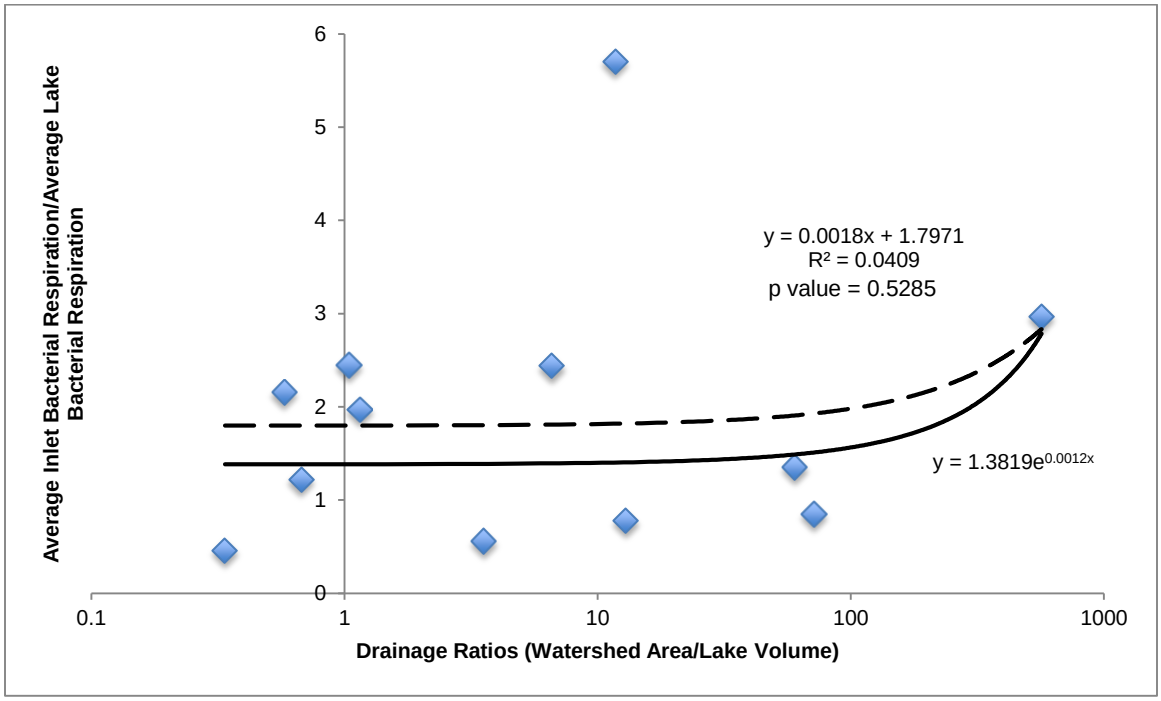
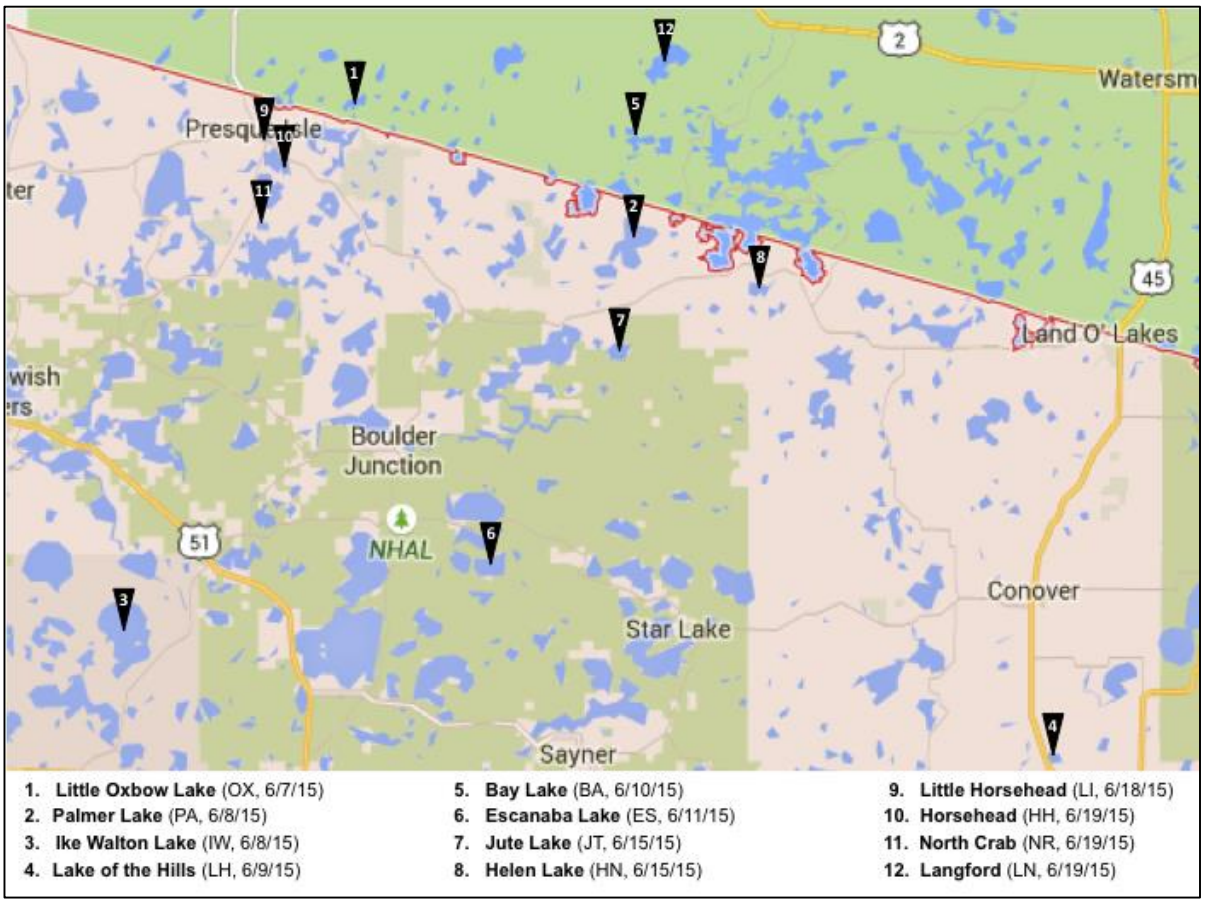


Figure 1. Relationship between ratio of average inlet bacterial respiration to average lake bacterial respiration and drainage ratio (water residence time). The results of the regression analysis demonstrate that lakes with shorter water residence times tended to have lower rates of bacterial respiration, while lakes with longer water residence times usually had higher rates of bacterial respiration although the relationship is weak ($R^2 < 0.1$).

Appendices

Appendix A



Map of lakes sampled in Vilas County, WI and Gogebic County, MI. Lakes are ordered by sample date.