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From Land to Lake: Contrasting Microbial Processes Across a Great Lakes Gradient of Organic Carbon and Inorganic Nutrient Inventories

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From Land to Lake: Contrasting Microbial Processes Across a Great Lakes Gradient of Organic

Carbon and Inorganic Nutrient Inventories.

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I would like to dedicate this thesis to my loving wife, Terry Murphy, whose support is never-

ending and to my mother, Marianne Strickland, a constant ray of love.
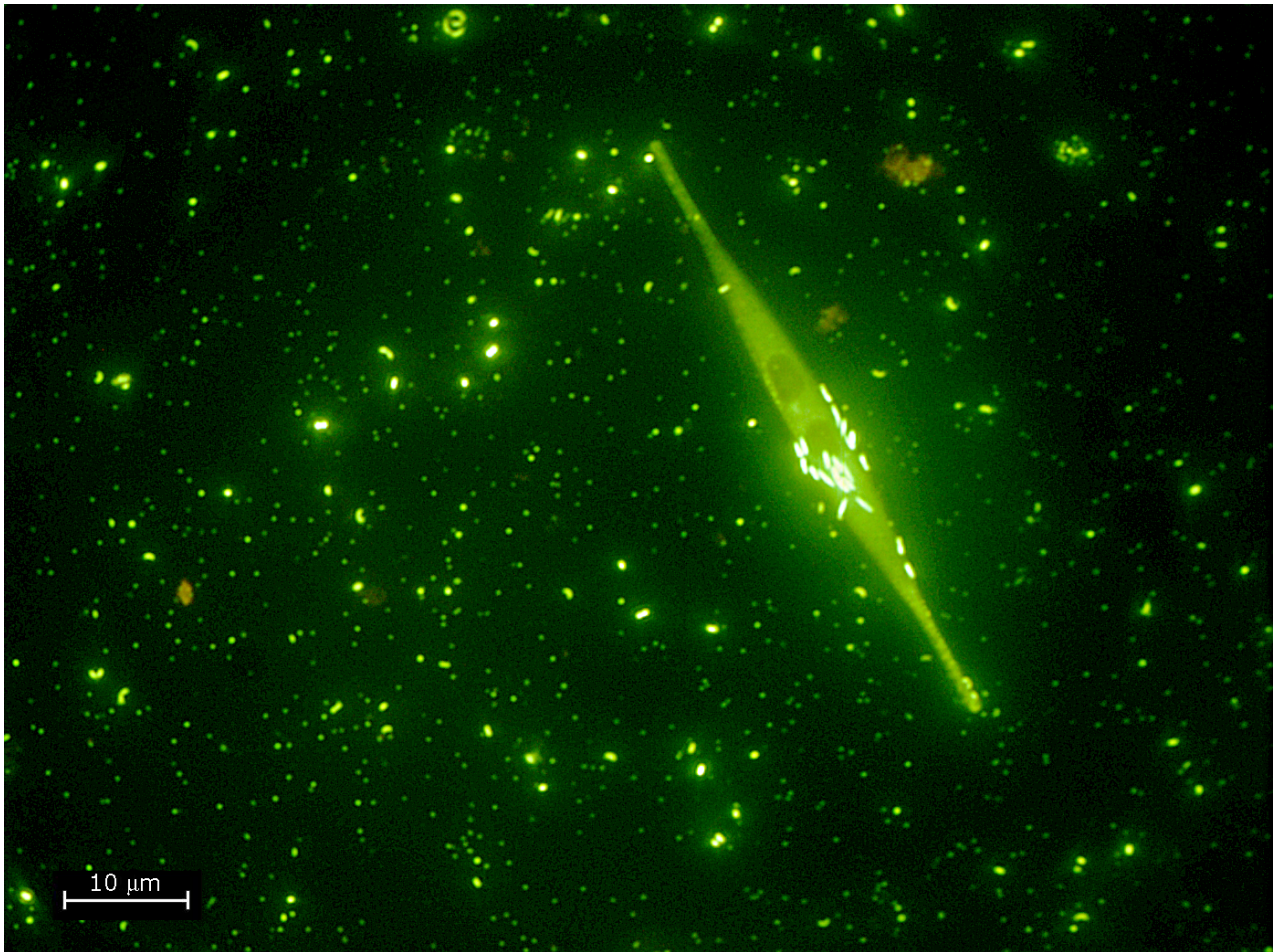
Photosynthesis and respiration fuel the cycle of life in the biosphere. Like stars in the sky, tiny,

but abundant microbial plankton link the planet's geosphere and atmosphere to the food webs in

the hydrosphere through their collective daily activities.

ACKNOWLEDGEMENTS

ABSTRACT

Freshwater ecosystems have strong linkages to the terrestrial landscapes that surround them, and contributions of carbon and inorganic nutrients from soil, vegetation and anthropogenic sources subsidize autochthonous water body productivity to varying degrees. Abundant freshwater phytoplankton and bacterioplankton are key to linking the planet's geosphere and atmosphere to the food webs in the hydrosphere through their growth and respiration. Rich resources that move through land margin waterways make them active sites for cycling organic carbon and thus important, but understudied, contributors to global climate. During 2010-2011, we examined seasonal changes in carbon and nutrient inventories, plankton community composition and metabolism along a land-to-lake gradient in a major West Michigan watershed at four interconnected habitats ranging from a small creek to offshore Lake Michigan. In all seasons Lake Michigan had significantly lower concentrations of CDOM and DOC than any of the other sites. Lake levels of NO_3 were not significantly lower than tributaries other than Cedar Creek, and SRP was not measurable in any of the sites other than Cedar Creek. Bacterial production as % of GPP revealed a distinct land-to-lake gradient from an average of 448% in Cedar creek to 5% in Lake Michigan. Microbial activity in Cedar Creek (bacterial production 3-93 μg C/L/d, and plankton respiration 9-193 μg C/L/d) was generally higher than all other sites. Muskegon Lake dominated GPP among the sites reaching a peak of >1000 μg carbon/L/d during a large fall *Microcystis* bloom. Offshore Lake Michigan had less variation in GPP and R than the other sites with GPP:R ratio close to 1 in all seasons but spring. Metabolism appears to be substantially subsidized by terrigenous inputs in the creek/river ecosystem with heterotrophy dominant over autotrophy. Autotrophy was maximized in the coastal/estuary, whereas both

autotrophy and heterotrophy were minimal but in near-balance in offshore waters receiving little subsidy from the land. Along this land-to-lake gradient terrestrial subsidies combined with a host of other factors making conditions "just right" for a hot-spot to emerge, highlighting Muskegon Lake estuary a "Goldilocks Zone" of net biological productivity.

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LIST OF ABBREVIATIONS

ANOVA – One-way analysis of variance

APHA – American Public Health Association

APR – April

BGE – Bacterial growth efficiency

BOD – Biological oxygen demand

BP – Bacterial Production

C – Carbon

CC – Cedar Creek

CDOM – Colored dissolved organic matter

Chl *a* – Chlorophyll *a*

CO_2 – Carbon dioxide

DEC – December

DOC – Dissolved organic carbon

DOM – Dissolved organic matter

DON – Dissolved organic nitrogen

fg – Femtograms (10^{-15} grams)

GF/F – Glass microfiber filter

GLERL – Great Lakes Environmental Research Laboratory

GPP – Gross primary production

[^{3}H]leucine – leucine radiolabeled with tritium

HCl – Hydrochloric acid

HSD – Honest significant difference

JUL – July

KHP – Potassium hydrogen phthalate

MI – Lake Michigan

MSGC – Michigan Space Grant Consortium

MU – Muskegon Lake

NH_3 – Ammonia

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NOAA – National Oceanic and Atmospheric Administration

NPP – Net primary production

OD – Optical density

Pg – Petagrams (10^{15} grams)

POM – Particulate organic matter

PP – Primary production

R – Respiration

RESP – Respiration

RV – Muskegon River

SEP – September

SRP – Soluble reactive phosphorus

CHAPTER I

<u>Introduction</u>

On the southeastern coast of Lake Michigan a common feature of major tributaries is termination in drowned river mouth lakes, which are important water bodies for nutrient exchange with the Lake Michigan nearshore (Larson et al. 2013). Drowned river mouths act as freshwater estuaries and although they are subject to Lake Michigan water levels and seiche, they are mostly protected from the action of its waves and currents. Rich terrestrial input of nutrients and organics from upstream watersheds have an increased residence time when they reach the estuary, which maximizes productivity before discharge to the nearshore. This unique hydrology forms land margin ecosystems that are hot spots for degradation, sedimentation, and export of organic matter along freshwater tributary systems, and could be an underestimated piece of the carbon cycle puzzle. Modeling studies show that freshwater ecosystems are very important sites of global carbon cycling (Cole et al., 2007; Hall et al., 2016) respiring about half of the terrestrially derived carbon that passes through them and delivering the remainder to oceans. Thus, understanding the range of metabolic processes along the varied waterways of a freshwater land-to-lake gradient is important to fully demonstrating this large contribution to global carbon cycling.

<u>Purpose</u>

Marine and freshwater eukaryotic and prokaryotic phytoplankton provide approximately half of net global photosynthesis (Field et al., 1998) and aquatic heterotrophic bacterioplankton respire about half of the carbon produced (Karl, 2007), but the source and fate of carbon and nutrients in freshwater systems must include the comparatively close linkage to terrestrial

systems. Because aquatic ecosystems are "open" systems to one degree or another, they require a terrestrial source of organic matter to subsidize the metabolic needs of that system (Wetzel 2001). In freshwater low-productivity lakes, such as Lake Michigan, autotrophic and heterotrophic bacteria are key players in ecosystem metabolism, but the lake ecosystem has been in transition in recent history. Two primary reasons for the changes in ecosystem dynamics are human-derived and include Best Management Practices that reduced phosphorus loads and the rapid spread of invasive dreissenid mussels that resulted in benthification and further oligotrophication of the lake (Evans et al., 2011; Cuhel and Aguilar 2013). Primary productivity in Lake Michigan is now similar to oligotrophic Lake Superior (Evans et al., 2011). The phytoplankton community of southern Lake Michigan is reduced and its composition has changed dramatically, especially during early spring mixing (Fahnenstiel et al., 2010). All major phytoplankton groups have declined in abundance with the exception of cyanobacteria and chlorophytes, which affects the broader food-web of the lake and carbon flux through it. Strong autotrophic-heterotrophic coupling is required in oligotrophic systems to regenerate scarce nutrients (Cotner and Biddanda, 2002) and prokaryotic heterotrophic respiration often exceeds primary production, leaving little organic matter for export to benthos or sediments. So the input from high-productivity drowned river mouths and upstream sources of organic and inorganic nutrients are critical factors in the dynamics of this changing ecosystem. Much remains to be described about the composition of land-to-lake planktonic microbial communities and the processes or mechanisms by which they survive and proliferate (Wetzel, 2001; Keogh et al., 2003; Biddanda et al., 2006; Wilhelm et al., 2006; Newton et al., 2011).

Scope

The Laurentian Great Lakes contain about 20% of Earth's fresh surface water (Beeton,

1984) and are a drinking water source for approximately 40 million people (NOAA-GLERL 2016). Keeping the lakes healthy is essential for natural and human wellbeing. Of the five lakes, Lake Michigan is the second largest by volume. Previous estimates suggest that approximately 10% of the metabolism of heterotrophic bacteria in Lake Michigan relies on carbon derived from terrestrial subsidies and about 20% of the lake's primary production relies on riverine loading of phosphorus (Biddanda and Cotner, 2002). The importance of terrestrial subsidies to the Lake Michigan ecosystem makes the impact of microbial metabolism in its feeder tributaries and estuaries an important factor in the lakes overall health. The 7,000 km^2 Muskegon River watershed terminates in Muskegon Lake, a drowned river mouth that feeds rich resources to nearshore, and to a lesser extent offshore, Lake Michigan. Since ongoing changes in this Great Lake ecosystem affect the planktonic microbial communities found there, how Lake Michigan processes compare to those of upstream sources is an important question to address for future reference. In 2014 Weinke et al. summarized the results of a long-term study from Muskegon Lake to offshore Lake Michigan. Much like the thesis results presented here and in the work of others (Bhagat and Ruetz, 2014; Carter et al., 2006; Marko et al., 2013; Ogdahl et al., 2010), the drowned river mouth estuary was a highly productive lake. The productivity systematically decreased along a transect to offshore Lake Michigan, where planktonic production and respiration converged and switched dominance (Figure 1). As the distance from terrestrial subsidies widened, surface waters changed from net autotrophic nearshore waters (carbon dioxide sinks) to net heterotrophic offshore waters (carbon dioxide sources). The scope of this thesis project expands the reach of study area examined by Weinke et al 2014 to include the rates of microbial metabolism and available resources in tributary surface waters feeding Muskegon Lake.

<u>Assumptions</u>

The purpose of this thesis study was to examine changes in carbon flux and gross microbial community composition along a land-to-lake gradient in a major West Michigan watershed. Our project study area included four interconnected sites in the watershed where surface water was sampled in each season between May 2010 and April 2011. Distinctly different ecosystems characterized the sites, which included Cedar Creek, Muskegon River, Muskegon Lake and offshore Lake Michigan. We assumed that the spatial and temporal framework would highlight an array of differences within and between the varied sampling sites.

<u>Hypothesis</u>

Our objective was to describe concurrent seasonal changes in biogeochemical inventories, ecosystem production-respiration processes and associated phytoplankton and bacterioplankton communities along a land-to-lake gradient to test the hypotheses that: 1) microbial composition varies systematically from highly productive riverine waters to oligotrophic pelagic lake waters, and 2) seasonal variations in microbial populations reflect changes in terrigenous subsidies and temperature in a Great Lakes watershed.

<u>Significance</u>

Although an ecologically important feature of any watershed, differences in microbial community composition and concurrent ecosystem metabolic processes across a land-to-lake environmental gradient is still not well described in the literature. The biogeochemical processes and the environmental conditions that are associated with land-to-lake gradients are locally relevant to restoration and future management of large lake ecosystems (Allen et al., 2013; Larson et al., 2013) and, on a larger scale, to global carbon cycling (Cole et al., 2007; McClain et al., 2003; Weinke et al., 2014). The knowledge gained from this research project will be a useful

reference source for ongoing studies in this watershed and an important comparative source for

any biogeochemical land-margin study of tributaries and their receiving waters.

FIGURE CAPTION AND FIGURE

Figure 1. Conceptual diagram of systematic variability in planktonic gross production, respiration and net production along a land-to-lake gradient in aquatic ecosystems. Axes scales are relative, and the grey horizontal line near the bottom serves as the "zero carbon balance" reference line.
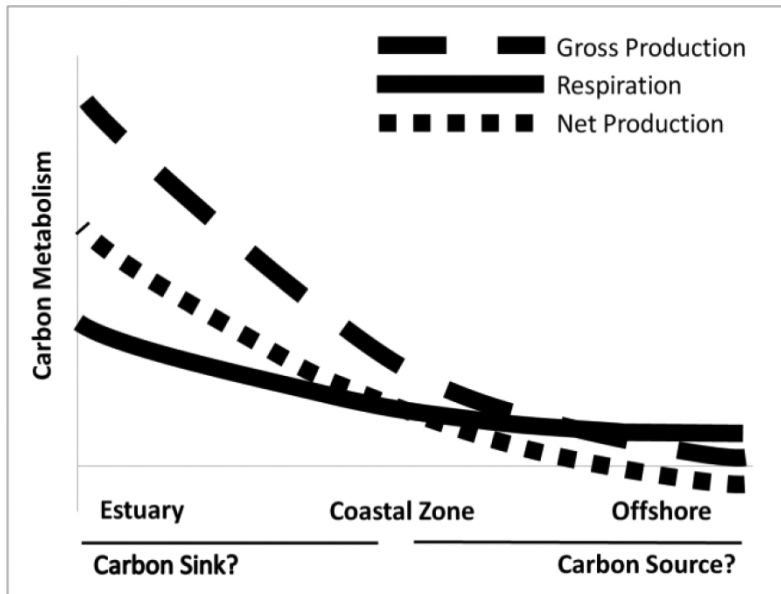
DEFINITIONS

allochthonous – Not indigenous to the system being studied

ANOVA – One-way analysis of variance

APHA – American Public Health Association

APR – April

autochthonous – Native to or produced within a system

BGE – Bacterial growth efficiency

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CHAPTER II

From Land to Lake: Contrasting Microbial Processes Across a Great Lakes Gradient of Organic

Carbon and Inorganic Nutrient Inventories.

ABSTRACT

Freshwater aquatic ecosystems receive carbon and nutrients from within the system as well as from the terrestrial environment in varying proportions. During 2010-2011, we examined seasonal changes in carbon and nutrient inventories, plankton community composition and metabolism along a land-to-lake gradient in a major West Michigan watershed at four interconnected habitats ranging from a small creek to offshore Lake Michigan. In all seasons Lake Michigan had significantly lower concentrations of CDOM and DOC than any of the other sites. Lake levels of NO_3 were not significantly lower than tributaries other than Cedar Creek, and SRP was not measurable in any of the sites other than Cedar Creek. Bacterial production as % of GPP revealed a distinct land-to-lake gradient from an average of 448% in Cedar creek to 5% in Lake Michigan. Microbial activity in Cedar Creek (bacterial production 3-93 μg C/L/d, and plankton respiration 9-193 μg C/L/d) was generally higher than all other sites. Muskegon Lake dominated GPP among the sites reaching a peak of >1000 μg carbon/L/d during a large fall *Microcystis* bloom. Offshore Lake Michigan had less variation in GPP and R than the other sites with GPP:R ratio close to 1 in all seasons but spring. Aquatic metabolism appears to be substantially subsidized by terrigenous inputs in the creek/river ecosystem with heterotrophy dominant over autotrophy. Autotrophy was maximized in the coastal/estuary "Goldilocks Zone" with longer residence times, whereas both autotrophy and heterotrophy were minimal but in near-balance in offshore waters receiving little subsidy from the land.

Keywords: Estuarine Gradient, Lake Michigan, Microbial plankton, Metabolism, Carbon balance

INTRODUCTION

Primary production and respiration fuel the cycle of life in the biosphere linked to movement of many elements through Earth's geochemical cycles. On a global basis, phytoplankton, including photosynthetic bacterioplankton, carry out close to half of net photosynthesis (Field et al., 1998) and aquatic heterotrophic bacterioplankton respire about half of this carbon (Cole et al., 1988; Karl, 2007). Planktonic metabolism thus tightly links the planet's atmosphere as well as the hydrosphere to the aquatic microbial community. There is also a strong terrestrial link to aquatic productivity. Approximately 20% of marine net primary production occurs in coastal zones even though coastal zones represents only 10% of total ocean area (Schlesinger and Berhardt, 2013). Aquatic production and respiration are both higher closer to land due to terrestrial inputs of organics and nutrients and where larger eukaryotic organisms often play a bigger role in community metabolism (Cotner and Biddanda, 2002). However, in waters farther from land margins, such as the vast pelagic waters that cover some 70% of Earth's surface, production and respiration by autotrophic and heterotrophic prokaryotic bacterioplankton dominate carbon flux (del Giorgio et al., 1997; Karl, 1999). Here bacterioplankton substrate, dissolved organic carbon (DOC), makes up one of the largest reservoirs of carbon in the biosphere – comparable to carbon in the atmosphere and on land (Hedges and Oades, 1997). Photosynthetic microbes contribute most of the aquatic organic matter and heterotrophic microbes degrade and recycle it (del Giorgio and Williams, 2005). Collectively, microbial activity regulates environmental redox states, nutrient cycling, and gases relevant to global climate – making microorganisms the major movers of energy and materials in the aquatic world and beyond (Falkowski et al., 2008).

The Laurentian Great Lakes contain about 20% of Earth's fresh surface water (Beeton,

1984) and Lake Michigan basin is the second largest, by volume (~4,900 km^3), of these five Great Lakes. The Straits of Mackinac provide a major waterway between Lake Michigan and Lake Huron, a hydrological connection that equilibrates lake levels and combines the two basins to form the largest freshwater lake, by surface area, in the world. Much remains to be revealed about the composition of the microbial community in these important freshwater systems (Keough et al., 2003; Wilhelm et al., 2006) and about how ongoing ecosystem changes (Scavia et al., 2014), especially those caused by dreissenid mussels in the Lake Michigan basin, affect lake planktonic microbial communities and food web structure (Allan et al., 2013; Cuhel and Aguilar, 2013; Evans et al., 2011; Fahnenstiel et al., 2010; Hecky et al., 2004; Turschak et al., 2014). In low-productivity lakes, such as Lake Michigan, autotrophic and heterotrophic bacteria play key roles in ecosystem metabolism (Fahnenstiel and Scavia, 1987; Scavia and Laird, 1987). It's commonly accepted that strong coupling between autotrophic and heterotrophic processes is required to regenerate scarce nutrients when bacterioplankton respiration is equal to or greater than primary production, resulting in little organic matter left over for support of higher trophic levels and export to sediments (del Giorgio et al., 1997). On the other hand, in high-productivity lakes and rivers, where larger eukaryotic autotrophs and phagotrophic metazoans utilize a rich supply of inorganic and particulate organic nutrients, autotrophic-heterotrophic coupling is weak (leading to increased export by sedimentation or riverine discharge). The heterotrophic microbial community shifts along the gradient from domination by osmotrophs to domination by phagotrophs, and moves from dissolved organic matter (DOM) in the oligotrophic system to particulate organic matter (POM) in the eutrophic system as the primary carbon source (Cotner and Biddanda, 2002; Wetzel, 2001).

Freshwater aquatic ecosystems receive organic carbon from primary production

occurring within the system (autochthonous) as well as from the terrestrial environment (allochthonous). It is estimated that allochthonous contributions of organic carbon provide for approximately 10% of the metabolism of heterotrophic bacteria in Lake Michigan and about 20% of the lake's primary production relies on riverine loading of phosphorus (Biddanda and Cotner, 2002). These and other recent findings support the idea of terrestrial materials substantially subsidizing the aquatic ecosystem (Dagg and Breed, 2003; Gergel et al., 1999; Karlsson et al., 2002; Lennon and Pfaff, 2005; Pace and Cole, 1996; Prairie and Kalff, 1986; Smith et al., 2003) and are conceptually analogous to "outwelling", where highly productive estuaries or mixing zones subsidize coastal ecosystems by discharging surplus nutrients and organic matter (Larson et al., 2013; Odum and Barrett, 2005). However prior to discharge, rivers and estuaries actively process terrigenous nutrients and carbon during transport to receiving waters (Marko et al., 2013). Productivities peak in many estuaries and nearshore coastal zones around the world as exemplified by the Mississippi River estuary and the dramatic increase in phytoplankton growth and primary production measured from point of discharge to near- and mid-field plume (Dagg and Breed, 2003). In fact, land margin coastal ecosystems are recognized as key hotspots with hot moments in the global carbon cycle (Cole et al., 2007; McClain et al., 2003; Weinke et al., 2014). Cole and others argue that freshwater ecosystems are not merely "passive pipes", but are highly "reactive sites" of global carbon cycling. Lakes and rivers, which cover about 1% of the planet's surface, receive an estimated ~ 2.4 Pg/year of carbon exported from terrestrial sources. Of that carbon, resident heterotrophs respire ~ 1.1 Pg and ~ 0.4 Pg is buried in freshwater sediments (an amount comparable with carbon buried annually in all of Earth's oceans), thus only half of this terrestrially derived carbon ever reaches the oceans (Cole et al., 2007; Tranvik et al., 2009). These findings emphasize the reactive role of inland waters in

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the global carbon cycle – in terms of globally significant respiration as well as carbon sequestration.

Lake Michigan is a critical ecological and economic resource in the region, but a variety of environmental stressors are degrading it on many fronts. Increased understanding of tributary influence on Lake Michigan's seasonal cycles is crucial to the lake's future health. Riverine discharge and other energy subsidies from the nearshore zone affect production, respiration and energy pathways in Lake Michigan (Johengen et al., 2008; Turschak et al., 2014), and it follows that there may be important links between environmental gradients, ecosystem metabolism and microbial community composition. In this study we examined seasonal changes in biogeochemical inventories, microbial community metabolism and the general composition of the phytoplankton and bacterioplankton communities along a land-to-lake gradient in a major western Michigan watershed. Our objective was to describe concurrent seasonal changes in environmental gradients, ecosystem production-respiration processes and broad categories of associated microbes (such as autotrophs and heterotrophs) along the sub-ecosystems of a Lake Michigan watershed. We tested the hypotheses that: 1) nutrient and carbon inventories decrease systematically from highly productive riverine waters to oligotrophic pelagic offshore lake waters, and 2) seasonal variations in community metabolism reflect changes in phytoplankton and bacterioplankton abundance in this Great Lake watershed.

METHODS

Study Sites

The Muskegon River watershed drains approximately 7,000 km^2 of west-central

Michigan. Drainage basin boundaries include portions of 12 counties and around 90 tributaries

that flow into the main stem of the Muskegon River. The river ends in a 17 km^2 drowned river

mouth lake (43.2331^{0}N, 086. 2903^{0}W), which discharges into central Lake Michigan through a

single, 1.6 km-long navigational channel. Over an 11-month period, four sites located along the

lower southwest portion of the watershed (Figure 1) were sampled once during each season to

evaluate temporal variations in community metabolism and microbial abundance, within and

between sites. The four sites are distinct yet interconnected habitats along a land-to-lake

gradient: 1. Cedar Creek (43.3057^0 N, 086.1150^0 W), 2. Muskegon River (43.2631^0 N, 086.2453^0

W), 3. Muskegon Lake (43.2261^0 N, 086.2935^0 W) and 4. Lake Michigan (43.2062^0 N,

086.4497^0 W). These landward sites are traditional sampling sites chosen by Annis Water

Resources Institute for their representativeness in the watershed. Lake Michigan site was part of

the ongoing National Oceanic and Atmospheric Administration-Great Lakes Environmental

Research Laboratory (NOAA-GLERL) long-term transect study. Cedar Creek is a cold-water

tributary of the Muskegon River and the shallow forest-canopied sampling site was located

approximately 9.5 km from its mouth at the Muskegon River. Muskegon River is approximately

350 km long with a 175 m drop in elevation between its source at Houghton Lake (44.3147^0 N,

084.7647^0 W) and the river mouth. We collected from a causeway bridge near the river mouth in

an urbanized high-traffic area amid wetland. At this location, river width is about 76 meters and

depth is ~3 m. Muskegon Lake is a drowned river mouth lake with a surface area of 17 km^2, a

mean depth of 7 m and maximum depth of 23 m. Surface water was sampled at the deepest point

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of the lake. The Lake Michigan site is at the NOAA M-45 buoy about 8 km offshore located over the 45 m isobath. All of the sites except for Cedar Creek were in open sunlight

Sample Collection

During the period from May 2010 to April 2011, at a depth of approximately 0.5 m, surface water samples were collected in each season. Four discrete 10 L water samples were collected at each site, placed in acid-cleaned carboys, transported on ice in coolers to the Annis Water Resources Institute and analyzed.

Physical and Biogeochemical Inventories

In the field, basic water chemistry (temperature, pH, conductivity and dissolved oxygen) was measured using a calibrated YSI 6600 Datasonde. In the laboratory we measured dissolved organic carbon (DOC), colored dissolved organic matter (CDOM), chlorophyll a (Chl a) and bioavailable nitrogen and phosphorus in each sample. DOC samples were filtered through 0.7 µm pre-combusted GF/F filters (4 h at 450 ^{0}C) and stored frozen in pre-combusted glass vials (4 h at 550 ^{0}C) with Teflon-lined caps until a convenient time to analyze. After thawing, sample acidification with 4-5 drops of 2N HCl and inorganic C removal by purging with ultra-pure air, measurements of DOC were determined by high temperature oxidation (680 ^{0}C) using a Shimadzu TOC-5000 carbon analyzer. Total organic carbon standards were made up from potassium hydrogen phthalate (KHP) and blanks were ultrapure deionized water (Benner and Strom, 1993). Water samples for CDOM were filtered through GF/F filters and absorption at 350 nm were then measured in a 1 or 10 cm quartz cuvette using a spectrophotometer; blanks were ultrapure deionized water. The 350 nm wavelength is a specific absorbance value representative of bulk CDOM (Helbling and Zagarese, 2003). Chlorophyll a was collected by filtering samples onto a 47mm Whatman GF/F filter. Filters were frozen for at least 24 h, followed by 90%

acetone extraction for 24 h (Parsons et al., 1984). Clarified extract was added to a 1 cm cuvette and optical density (OD) at 750 and 664 nm was read. An acidification step followed in which 100 μl of 0.1 N HCl was added to the extract in the cuvette, gently agitated and after 90 sec the OD at 750 and 665 nm were read. Samples were assayed for nitrate (NO_3), ammonia (NH_3) and soluble reactive phosphorus (SRP) according to APHA (1998) methods.

Microbial Plankton Enumeration

Prokaryotes, planktonic eukaryotes and viruses were enumerated using standard epifluorescence microscopy at 1000x magnification. Samples were preserved with 2% formalin and 500 μl aliquots were filtered onto 0.02 μm pore size, 25 mm diameter Anodisc membrane filters (Whatman) stained with SYBR Green I (Molecular Probes, Inc.) for enumeration of all microbes according to (Noble and Fuhrman, 1998). 20 ml aliquots were filtered onto 0.2 μm pore size, 25 mm diameter black Nuclepore filters and were examined for enumeration of autofluorescent phytoplankton (both prokaryotic and eukaryotic) cells. Following preparation, slides were stored frozen until enumeration when at least 10 fields of view and 300 cells were counted per sample.

Microbial Community Metabolism

Oxygen uptake in untreated and unfiltered water samples was measured in clear and darkened biological oxygen demand (BOD) glass bottles (300 ml). The bottles were incubated for 24 h in Muskegon Lake to approximate the sample temperatures and light conditions at collection sites. Changes in dissolved oxygen were measured using a titrator for automated Winkler titrations with potentiometric endpoint detection (Carignan et al., 1998). Oxygen consumption in darkened bottles is a measure of community respiration (R), and oxygen production in clear bottles measures community net primary production (NPP). Oxygen uptake

was converted to estimate R using a respiratory quotient of 1, and to estimate NPP using a photosynthetic quotient of 1 (Biddanda et al., 1994; Robinson, 2008). Bacterial secondary production was measured by means of radiolabeled [^{3}H]leucine incorporation into protein as described by Simon & Azam (1989).

<div align="center">Statistical Methods</div>

Statistical analyses were performed using R open source programming language (R Core Team, 2013) and the R Commander graphical user interface (Fox, 2005). Pearson's correlation analysis was used to test for associations between component members of the microbial community and all measured variables. One-way analysis of variance (ANOVA) was used to determine differences of: a) site or season on environmental and/or biological measures, and b) site or season on dominant microbial community. When significant ($\alpha = 0.05$) differences were found, post-hoc analysis with Tukey HSD resolved which groups were different. The data were not normally distributed; therefore, they were $\log_{10}$ transformed before analysis.

RESULTS

In all seasons, the concentrations of NO_3, NH_3 and SRP (Table 1) were significantly

higher in Cedar Creek than in other site surface waters ($F_{3,76} = 16.99$, $p < 0.001$; $F_{3,76} = 283.3$, $p <$

0.001; $F_{3,76} = 15.64$, $p < 0.001$, respectively; see Supplementary Figure 1). High nutrient values

in Cedar Creek seen in April, included concurrent spikes in CDOM, DOC and Chl a levels

(Table 1). Muskegon Lake had significantly lower NO_3 levels ($F_{4,15} = 29.2$, $p < 0.001$; see

Supplementary Figure 2A) during the months of May, July and September, when gross primary

production (GPP) peaked in the lake (Table 2), than in December and April. There was an

inverse correlation between NO_3 levels and gross primary production in Muskegon Lake (see

Supplementary Figure 2B). Across all sites and seasons, there was a significant inverse

correlation between number of autotrophs and both NO_3 and NH_3 concentrations (Table 3).

Organic carbon concentrations did not vary significantly among sites except for Lake

Michigan site, which had significantly lower concentrations of both CDOM and DOC ($F_{3,76} =$

47.7, $p < 0.001$; $F_{3,75} = 37.2$, $p < 0.001$, respectively; see Supplementary Figure 3A). April

CDOM and DOC values spiked in Cedar Creek due to stormwater runoff. When we removed

April values from the dataset to compare sites under typical baseflow conditions, DOC levels in

Cedar Creek were significantly lower than Muskegon Lake and River ($F_{3,59} = 70.51$, $p < 0.001$;

see Supplementary Figure 3B), although never as low as Lake Michigan. The ratio of

CDOM:DOC was < 1 for Lake Michigan in all seasons except December when it rose to 1.9. All

other sites had CDOM:DOC ratios > 1 for all seasons (see Supplementary Figure 3C). Both

CDOM and DOC had significant positive correlations with numbers of heterotrophs, prokaryotes

and eukaryotes (but not autotrophs) over all sites (Table 3).

Temperature was positively correlated with numbers of autotrophs (Table 3) as well as

with the metabolic processes of bacterial production, gross primary production and respiration. There was a significant negative temperature correlation with NH_3 and NO_3 concentrations (Table 4).

The number of autotrophs in Muskegon Lake was substantially higher than all other sites in July and September due to localized *Microcystis* blooms. In July Muskegon Lake had 6.5 x 10^4 autotrophs/ml, 30 times greater than Cedar Creek's 2.2 x 10^3 autotrophs/ml. In September *Microcystis* colonies were large, abundant and unevenly sized, resulting in counts that do not reflect the actual number of autotrophs present in Muskegon Lake, which should be higher than the July count (as per Chl a levels, see Table 1) but do not appear so in the data (Figure 2 and Table 5). Prokaryotes accounted for most of the micro-heterotrophs present at all sites and thus the counts were similar (Table 5). In Lake Michigan, Muskegon Lake and Muskegon River heterotrophs were approximately 90% prokaryote and for Cedar Creek 85%. In April Cedar Creek saw a sharp increase in heterotrophs, prokaryotes and eukaryotes, but not an increase in autotrophs or viruses (Figure 2). The mean virus counts for Lake Michigan, Muskegon River and Cedar Creek were 1.8 x 10^7/ml, 5.2 x 10^7/ml and 1.8 x 10^7/ml respectively (see Table 5 for seasonal breakdowns and standard errors). Virus counts in Muskegon Lake were highest in July (8.6 x 10^7/ml), September (9.5 x 10^7/ml) and December (8.3 x 10^7/ml).

Of all sites and in every season, bacterial secondary production was highest in Cedar Creek samples – ranging from a high in September of 93.0 μg carbon/L/d to a low in December of 2.6 μg carbon/L/d (see Supplementary Figure 4A). Dividing bacterial production values by prokaryotes per liter, we estimated femtograms (10^{-15} grams) of carbon utilized per cell per day. Cedar Creek's high September bacterial production value translated to 47 fg carbon/cell/d followed by Muskegon River (13 fg carbon/cell/d), Muskegon Lake (3.7 fg carbon/cell/d) and

Lake Michigan (2.2 fg carbon/cell/d) (see Supplementary Figure 4B). Although gross primary production (GPP) does not show a systematic downward trend from land to lake (Figure 3), when bacterial production as percent of concurrent gross primary production is calculated, a distinct land-to-lake gradient is evident (Table 2). Bacterial carbon demand for concurrent primary production often exceeds availability in Cedar Creek (range 63%-1515% of concurrent GPP), is lowest in Lake Michigan (range 0.3%-9.8%) and intermediate in Muskegon River (range 1.7%-68%) and Muskegon Lake (range 1.6%-110%).

Primary production varied widely between sites and seasons. In late spring, summer and fall, Muskegon Lake dominated gross primary production (GPP) among the sites (Figure 4). During the lake's large fall *Microcystis* bloom, GPP reached 1122 μg carbon/L/d – about 10-fold, 20-fold and 187-fold higher than Muskegon River (115 μg carbon/L/d), Lake Michigan (56 μg carbon/L/d) and Cedar Creek (6 μg carbon/L/d), respectively. Over the course of the study, Muskegon Lake accounted for 64% of total measured Chl a (μg/L) and 59% of all autotrophs. Proportionately, Cedar Creek added more Chl a (16%) to the total than Lake Michigan (7%), but Lake Michigan had a larger proportion of the total autotrophs (18% versus 4%) and the two sites had about the same overall rates of primary production. Lake Michigan had the most stable levels of GPP showing no significant changes from season to season ($F_{4,13} = 1.18$, $p = 0.36$).

Cedar Creek was not a major contributor to watershed primary production. Instead, the creek was a major contributor to respiration (Figure 5). The proportion of total measured respiration the creek accounted for was prominent in late spring (39%), fall (43%), winter (35%) and early spring (66%). In early spring Cedar Creek had higher counts of heterotrophs than other sites, but in all other seasons, the number of heterotrophs found at Cedar Creek were comparable in number to Lake Michigan and 2-4 times fewer than Muskegon River and Muskegon Lake.

Lake Michigan showed the least fluctuation in respiration with no significant changes from season to season ($F_{4,13} = 0.805$, $p = 0.543$) and no significant changes in number of heterotrophs between seasons ($F_{4,15} = 0.696$, $p = 0.606$). Muskegon Lake was the most variable, going from 65% of study area respiration in summer to undetectable in winter. Over the course of the study, with an April storm spike included in the dataset, the proportion of DOC was evenly distributed between Muskegon Lake, Muskegon River and Cedar Creek at 30% each and dropped off to 10% for Lake Michigan. The overall proportion of heterotrophs in the study area, most of which were prokaryotes, broke down to: Muskegon Lake, 38%; Muskegon River, 30%; Cedar Creek, 20%; and Lake Michigan, 12%.

The gross primary production to respiration ratio (GPP:R) was used to indicate net autotrophy (carbon sink) or net heterotrophy (carbon source) of a site. If GPP outweighed R, the ratio was > 1 and the site was considered net autotrophic, where more CO_2 was fixed than released into the atmosphere. Alternatively, when R exceeded GPP the site was considered net heterotrophic, where more CO_2 was released into the atmosphere than was fixed. Cedar Creek site was net heterotrophic with a ratio < 1 in every season (Figure 6). Lake Michigan's GPP:R ratio was at or close to 1 in summer, fall and winter. In spring the Lake Michigan site was net autotrophic. Both Muskegon Lake and Muskegon River were net autotrophic (carbon sinks) in all seasons during this study period. In December, due to a mean respiration level near zero ($\pm$ 0.4 SE), Muskegon River showed a large spike in net autotrophy, even though river GPP was its lowest (for values of GPP and R see Table 2). Muskegon Lake had two net autotrophy spikes, one in early spring and one in fall.

DISCUSSION

Variable Inventories of nutrients and organic carbon from land to lake

Across the land-to-lake gradient studied here, the canopied surface waters of Cedar Creek site contained significantly higher levels of nutrients during all seasons than any of the other sites. A major contributing factor to this enhanced nutrient loading is the extensive 148 km^2 watershed the creek drains and its large ratio of land-margin to water area, illustrating the "river continuum concept" and the influence of land-water interface on local allochthonous inputs (Polis et al., 1997; Vannote et al., 1980; Wetzel, 1990). Terrigenous runoff undoubtedly plays a major role in Cedar Creek nutrient levels by draining a watershed of forested (~68%), agricultural (~20%), residential (~7%) and wetland (~5%) landscape (Fongers, 2004). Creek samples had fewer autotrophs than other sites, and although there is good negative correlation between number of autotrophs and NO_3 and NH_3 concentrations, low autotrophic utilization of creek nutrients is probably only a minor contributor to its high nutrient levels. Muskegon Lake, on the other hand, showed a pronounced reduction in both NO_3 and NH_3 levels that was likely due to coincident high autotrophic abundance and activity during the months of May, July and September. The lake has an average hydraulic retention time of ~23 days (Freedman et al., 1979; Steinman et al., 2008), ranging from 14 to 70 days depending on Muskegon River discharge (Marko et al., 2013). Thus compared to adjacent water bodies, lower values for NO_3 and NH_3 in Muskegon Lake are not surprising – a substantial retention time in combination with relatively large and frequent blooms (Steinman et al., 2008) provide the right conditions for phytoplankton to deplete inventories of these nutrients. Heterotrophic utilization of NO_3 and NH_3 may also be a contributing factor to nutrient reduction (Middelburg and Nieuwenhuize, 2000). However, heterotroph abundance was about the same in Muskegon Lake and Muskegon River and

although bacterial production was generally higher in Muskegon River, nutrient reduction did not occur there. These results led us to conclude that heterotrophic utilization would be a minor contributing factor to nutrient reduction in Muskegon Lake.

As expected of water bodies with frequent runoff from extensive land margins, Cedar Creek, Muskegon River and Muskegon Lake sites all had consistently higher levels of CDOM and DOC than the Lake Michigan site. However in Cedar Creek the ratio of CDOM:DOC varied widely (range ~ 2:1 to 7:1) compared to Muskegon River and Muskegon Lake where CDOM and DOC maintained fairly stable relationships to each other in all seasons (range ~ 1.5:1 to 2:1). The wide range in Cedar Creek was due to an April storm spike that increased CDOM and DOC concentrations, by 12-fold and 4-fold respectively, above their means for the previous 4 collections. Keeping in mind that storm-induced spikes in terrestrial runoff have highly influential but temporary effects in Cedar Creek inventory concentrations, the April event was removed from the dataset to compare sites under more typical baseflow conditions. Analyzed in this way, the creek had significantly less DOC than Muskegon River and Muskegon Lake. Although possible, it would be surprising if input levels of DOC from landscape surrounding the creek were significantly lower than the river, since CDOM levels are the same for both. An alternative explanation for the significantly lower DOC levels in Cedar Creek may be found in the significantly higher metabolic activity of the creek's heterotrophic bacterial populations, in combination with significantly higher primary production in the river and Muskegon Lake.

On the other end of the gradient, Lake Michigan had the lowest DOC and CDOM concentrations, which were easily explained by its location 8 km offshore and generally well out of the reach of the watershed's plume. Lake Michigan site is expected to be lower in organic carbon in general, and in some seasons rely on allochthonous subsidies to provide enough carbon

and nutrients for its planktonic populations. Biddanda and Cotner (2002) estimated that in the southern Lake Michigan basin about 10% of bacterial metabolism is dependent on terrigenous inputs of organic carbon on an annual basis. Wind driven, episodic sediment resuspension events in late winter/early spring provide Lake Michigan heterotrophic bacteria with a window of opportunity for rapid increase in biomass while decoupled from concurrent primary production which becomes temporarily light-limited (Chen et al., 2004; Johengen et al., 2008). In this study we did not have a March sampling date and thus may have missed the resuspension driven late winter/early spring heterotrophic biomass increase, or it is quite possibly a consequence of the disappearing spring bloom recorded in recent years (Evans et al., 2011; Pothoven and Fahnenstiel, 2013; Yousef et al., 2014). In April and May, we found Lake Michigan site to have low levels of heterotrophic respiration. A shift to increased primary production and net autotrophy had occurred at the site since the December collection. Similarly, early spring increases in primary production were reported by Depew et al. (2006) at several sites in cross-season measurements of PP and R in the deep east basin of Lake Erie. This comparably oligotrophic basin of Lake Erie was net autotrophic beginning with a bloom in April and May. In the present study, we found that the combination of conditions necessary for excess DOC accumulation, high primary production and comparatively low community respiration, occurred in fall as well as in spring. In fact highest DOC concentrations were recorded in fall, but CDOM was lowest which would indicate active, autochthonous DOC inputs as opposed to resuspended sediments or terrestrial sources of carbon.

Highly active heterotrophic community at the land end

Microbial processes and community structure may be variably influenced by the changing pools of limiting resources along this land-to-lake continuum (Attermeyer et al., 2014;

Dillon and Molot, 1997; Eiler et al., 2003; Fouilland et al., 2014). Although not the dominant site for sheer number of heterotrophs, Cedar Creek's heterotrophic community was the most metabolically active. It appears that influx of allochthonous nutrients, DOC, and perhaps soil-associated microorganisms, make creek surface water a hotspot for heterotrophic metabolism. Even if incoming DOC is less labile than that produced by phytoplankton, it could be associated with soil microbes capable of "priming" (Guenet et al., 2010) it for degradation and utilization by native creek bacteria and incoming soil bacteria. On the other hand, Guillemette and del Giorgio (2011) observed that river DOC has relatively high bioavailability. It has also been reported that aquatic bacteria can efficiently metabolize low molecular weight DOM derived from terrestrial sources (Berggren et al., 2010). Generally, Cedar Creek micro-heterotrophs were comparable in number to Lake Michigan's and 2-4 times fewer than those in Muskegon River and Muskegon Lake. However, in all seasons but summer, the creek's microbial community was responsible for a large proportion of respiration that occurred along the gradient. This comparatively low heterotroph abundance to respiration rate ratio indicates that Cedar Creek has a heterotrophic community that is more metabolically active than those at the other watershed sites in this survey. Net heterotrophy was reflected in a GPP:R ratio consistently < 1 and for this study categorizes creek site surface water as a year round carbon dioxide source, where respiration always outweighed primary production.

Like Cedar Creek, Muskegon River has a large land-margin that regularly delivers terrestrial subsidies in runoff. However, even with higher concentrations of heterotrophs than the creek, the river had consistently lower respiration and bacterial production rates than the creek. This leads us to conclude that overall the heterotrophs in the river were less metabolically active than heterotrophs in Cedar Creek. Situated in a large wetland area with local urbanization and

crossed by a high-traffic 4-lane road, site location may be partially responsible for lower metabolic activity in river samples. Low bioavailability of wetland DOM, particularly the DON fraction, has been shown to slow bacterial growth (Wiegner and Seitzinger, 2004). Thus, the wetland contribution to river DOM may be less labile than the forest soil runoff that supplements creek DOM (Wiegner et al. 2006). However, Hosen et al. (2014) found that with increasing impervious surface cover, there was a shift from complex, recalcitrant DOM to smaller, more bioavailable compounds. The combination of wetland and impervious land use surrounding the river site has contrasting impacts on DOM composition, and overall bioavailability to micro-heterotrophs is difficult to estimate without further study.

In addition to higher respiration rates per organism, Cedar Creek bacteria generally had the highest bacterial production rates (μg carbon/L/d). When calculated on a per cell basis (fg carbon/cell/d) secondary production was highest in Cedar Creek in all seasons. It is estimated that marine bacterioplankton in nutrient rich coastal environments contain approximately 30-50 fg carbon/cell (Fukuda et al., 1998). Using this weight per cell approximation for nutrient rich Cedar Creek, prokaryotes were increasing biomass fast enough to divide once or twice a day at their September peak. At the oligotrophic end of the spectrum, Lake Michigan prokaryotes increased biomass at a much lower rate with a doubling time of approximately eight days at their September peak, based on a smaller average cell size of ~20 fg carbon/cell (Cho and Azam, 1990; Ducklow and Carlson, 1992). In Gulf of Mexico surface waters Biddanda et al (1994) found that values for carbon assimilation ranged from ~ 4-34 fg C/cell/d off the Louisiana coast. In most seasons the estimated values for carbon assimilation per bacterium in Cedar Creek were comparable to high-end Louisiana coastal values. Except for Lake Michigan, other sites in our study had bacterial carbon utilization values that were in a range similar to low-end Louisiana

values. We found Lake Michigan values were less than low-end Louisiana coast values in most collections and attribute this to the lake's increasingly oligotrophic conditions.

<u>Peak productivity in the drowned river mouth lake estuary – a "Goldilocks Zone"</u>

Muskegon Lake was a "Goldilocks Zone" – a hotspot where conditions such as water residence time, sunlight penetration and terrigenous carbon and nutrient availability were just right to achieve maximal rates of metabolism (hot moments) and attain peak net primary production along this land-to-lake gradient (McClain et al. 2003). In September, its primary production peak for the year, Muskegon Lake contributed approximately 86% of total GPP measured across all sites. This peak came in concert with a lake-wide *Microcystis* bloom. Over the course of this study, surface water collected from the Muskegon Lake site harbored 59% of all autotrophs counted and this estimate may be conservative due to difficulty counting large *Microcystis* colonies encountered in September's bloom. The lake also produced ~ 64% of the combined Chl a extracted from all water samples over the year. Additionally, Muskegon Lake contained ~ 38% of the heterotrophs counted in this study. Though they did not have the same level of activity as Cedar Creek heterotrophs, sheer abundance of heterotrophs increased the lake's overall contribution to study-wide respiration measurements. The GPP:R ratio was always >1 (range ~ 2:1 to 8:1) in this study, making the Muskegon Lake site a highly productive net carbon sink year-round.

Muskegon River, the source of the drowned river mouth lake, dominated seasonal contributions to primary production in April (Figure 4) when Muskegon Lake autotrophs appeared less active than the river's autotrophs. Comparing the two sites in April – temperature in the lake was warmer by two degrees, nutrient levels were about the same and the number of autotrophs were the same, but there was 2-fold more Chl a in the river samples. The difference in

Chl a concentration suggests that these river autotrophs had a light harvesting advantage over Muskegon Lake autotrophs. April autotrophs in the river not only had more Chl a, but also Chl a that was producing more per unit – the GPP per Chl a ratio for Muskegon River was 61 µg C/µg Chl a/day compared to 45.5 µg C/µg Chl a/day for Muskegon Lake. Overall, primary production in the river was greater than respiration with GPP:R ratios > 1 in all seasons and thus this site, like Muskegon Lake, was a carbon sink throughout the study.

Close coupling of autotrophs and heterotrophs offshore

Of all sites, Lake Michigan had comparatively steady state primary production and respiration throughout the seasons. This site showed the least fluctuation in levels of gross primary production, with a September high that was not significantly different from December's low, and the same can be said of respiration with a July high and May low that did not vary significantly. Given the minimal fluctuation in metabolism from season to season, it is not surprising that numbers of autotrophs and heterotrophs did not vary widely between seasons. Additionally, with low concentrations of DOC and a GPP:R ratio at or near 1 in all seasons except spring, we found an expected close coupling of autotrophic and heterotrophic metabolisms in Lake Michigan's oligotrophic waters. In most seasons bacterial heterotrophs, which outnumbered autotrophs by 2-3 orders of magnitude, respired about as much carbon as autotrophs fixed, a common relationship when productivity is low (Ducklow and Carlson, 1992). Such a situation where autotrophy and heterotrophy are in near-balance, leads to little or no excess organic matter for export to benthos (Cotner and Biddanda, 2002) from the surface waters of the Lake Michigan site.

Predictable trends in the grand watershed gradient

Along the watershed gradient we found trends in inventories, processes and general

planktonic composition that were directly impacted by the amount of interface that the sites shared with surrounding landscape. At the land end, where Cedar Creek shares much of its area with the terrain it passes through, nutrients and CDOM were higher in all seasons and distinctly higher after a storm event that also brought a pronounced spike in bacterial heterotrophs and DOC. Conversely, the low concentrations of DOC and CDOM in Lake Michigan can be attributed to the sampling site's distance (~ 8 km) from land's end and the river's plume, missing rich sources of allochthonous organic nutrients. This site is therefore heavily dependent on autochthonous organic carbon, supplied by local autotrophs year round and by sediment resuspension in very early spring.

Bacterial production was highest near land and steadily declined to lowest levels in Lake Michigan. This is not unexpected as it has been observed in both marine and freshwater systems that bacterial production is broadly correlated with bacterial growth efficiency (del Giorgio and Cole, 1998) and in marine systems it has been shown that bacterial growth efficiency is higher closer to land and decreases in less productive offshore waters (Biddanda et al., 1994; Coffin et al., 1993; Griffith et al., 1990). Bacterial production as a percent of GPP also showed a systematic decrease from land to lake. Averaged over the seasons, ~ 450% of GPP would have been necessary to satisfy bacterioplankton biomass demand for carbon at Cedar Creek site, and that number fell steadily to an average of only ~ 5% of GPP supporting bacterial biomass increase in Lake Michigan over the year. Plainly in Cedar Creek other sources of carbon and nutrients were available for the level of bacterial production occurring there. Additionally, respiration was highest at the land end, where DOC was ample and heterotrophs were particularly active and able to utilize the rich resources around them. Respiration declined in Muskegon River even though higher concentrations of DOC and more heterotrophs were present

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than in Cedar Creek. Farther down the land-to-lake gradient, respiration rose again. Muskegon Lake's high levels of respiration were undoubtedly due to dramatically high levels of primary production – with an abundance of labile organic matter being locally produced, the heterotrophic bacterial population had plenty to respire in this most productive Goldilocks Zone watershed site. Respiration predictably drops again in Lake Michigan with its low DOC levels and heavy dependence on prevailing low rates of autochthonous organic carbon production.

Although overall the creek site had higher levels of Chl a per liter than Lake Michigan site, the two had similar levels of primary production. This is not surprising considering the landscape surrounding each site, coupled with the significant differences in light-attenuating CDOM concentrations at the two sites. Since much of Cedar Creek runs through a shaded canopy of forested terrain and the creek has higher levels of light-limiting CDOM in its surface waters, creek autotrophs must produce more Chl a per cell. Increased pigment synthesis in lower light environments is well documented (Falkowski and Laroche, 1991) and, as seen here, is accompanied by a decrease in carbon fixation per unit chlorophyll. The light-rich, CDOM poor environment of Lake Michigan surface water harbored more phytoplankton, but there was less Chl a per volume in the lake site. This indicates that there was less Chl a present per cell and, due to comparatively higher GPP levels, that it was being efficiently used in these autotrophs. With warmer temperatures throughout the growing season, sufficient nutrients and an average water residence time of about one month, it's expected that Muskegon Lake would have denser phytoplankton blooms than the other study sites. In fact we found Muskegon Lake phytoplankton blooms were large enough and had a long enough residence time to elicit a significant reduction in NO_3 levels at the height of their growing season. We conclude that blooms utilized nitrogen at rapid rates in surface waters and lake stratification prohibited nitrogen replacement through

water column mixing in late spring through fall.

The carbon cycle of coastal ecosystems is a dynamic component of the global carbon cycle (Schlesinger and Berhardt, 2013). In the present study, the abundance of autotrophs was more variable than that of heterotrophs and photosynthesis was more variable than respiration, similar to findings in marine systems (Karl et al., 2003). Furthermore, it is evident that site associated photosynthesis and respiration are determined by both the type of phytoplankton and bacterioplankton present and by the inventories of nutrients and carbon available to them along this land-to-lake gradient. At the land end, as represented by Cedar Creek surface water, photosynthesis occurred at comparatively low levels while respiration and secondary production were at their highest. Creek heterotrophs were dependent on rich terrestrial subsidies. At the lake end, 8 km offshore in Lake Michigan where light is abundant but nutrients and DOC are scarce, phytoplankton appear to make more efficient use of Chl a and heterotrophic respiration was tightly coupled to primary production. How resource inventories drive community composition and how community processes transform inventories form a loop of interdependence that is difficult to untangle. In the future, next-generation sequencing of time-series environmental DNA collected at these sampling sites, may reveal site and season-specific bacterial communities that influence local biogeochemical activity in environments as different as Cedar Creek and Lake Michigan, thereby potentially providing insights into how autotrophic and heterotrophic aquatic microbes link terrigenous nutrients and carbon to aquatic food webs and contribute to regional and global biogeochemical cycles.

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FIGURE CAPTIONS

Table 1. Means (n=4; ±1 SE) for chemical parameters of surface water collected from Muskegon River watershed sampling sites in May, July, September and December 2010 and April 2011. CDOM, colored dissolved organic matter; Chl a, chlorophyll a; DOC, dissolved organic matter; SRP, soluble reactive phosphorus.

Table 2. Means (n=4; ±1 SE) of metabolic rate processes measured in surface water collected from Muskegon River watershed sampling sites in 2010 and 2011 measured in µg Carbon per liter per day (µg C/L/d).

Table 3. Pearson's correlation coefficients between microbial community biotic component and physico-chemical or metabolic variables across all sites and seasons. Correlations highlighted in bold are significant ($p < 0.05$) after Holm's adjustment of p-values for multiple comparisons. BP, bacterial production; GPP, gross primary production; NPP, net primary production; R, respiration. For chemical abbreviations see Table 1.

Table 4. Pearson's correlation coefficients between physico-chemical and metabolic process variables across all sites and seasons. Correlations highlighted in bold are significant ($p < 0.05$) after Holm's adjustment of p-values for multiple comparisons. See legends in Tables 1 and 2 for explanation of abbreviations.

Table 5. Means (n=4; ±1 SE) of microbial community abundances in surface water collected from Muskegon River watershed sampling sites in 2010 and 2011.

Figure 2. Map of the southwest portion of the Muskegon River watershed with four study sites marked. Inset map shows the location of sites, in Lake Michigan and the State of Michigan, with reference to the larger watershed.

Figure 3. Abundance of microbial plankton in each season. Site abbreviations: MI = Lake Michigan, MU = Muskegon Lake, RV = Muskegon River, and CC = Cedar Creek. Month abbreviations: MAY = May, JUL = July, SEP = September, DEC = December, and APR = April.

Figure 4. Fluctuations in net primary production (NPP), gross primary production (GPP) and respiration (Resp) levels for each site throughout the seasons. Site abbreviations: MI = Lake Michigan, MU = Muskegon Lake, RV = Muskegon River, and CC = Cedar Creek. Month abbreviations: MAY = May, JUL = July, SEP = September, DEC = December, and APR = April.

Figure 5. Seasonal proportions indicating relative importance of gross primary production at study sites (%) with mean measured rates in table below (µg C/L/d ± 1 SE). For comparison, pie charts show study site proportions of total chlorophyll a and total autotrophs measured over the eleven-month study period. For the values of measured inventories represented as percentages in pie charts see Table 1 (Chlorophyll a) and Table 5 (autotrophs).

Figure 6. Seasonal proportions indicating relative importance of respiration at study sites (%) with mean measured rates in table below (µg C/L/d ± 1 SE). For easy comparison, pie charts show study site proportions of total dissolved organic carbon (DOC) and total heterotrophs

measured over the eleven-month study period. For the values of measured inventories

represented as percentages in pie charts see Table 1 (DOC) and Table 5 (heterotrophs).

Figure 7. Ratio of gross primary production (GPP) to respiration (R) in each season for each site. Site abbreviations: MI = Lake Michigan, MU = Muskegon Lake, RV = Muskegon River, and CC = Cedar Creek. Month abbreviations: MAY = May, JUL = July, SEP = September, DEC = December, and APR = April.

TABLES

Table 1.

Watershed Site	Season	CDOM (a350 m-1)	Chla (µg/l)	DOC (mg/l)	SRP (mg/l)	NH3 (mg/l)	NO3 (mg/l)	TEMP (^{0}C)
Lake Michigan	May	0.40 ± 0.02	0.20 ± 0.07	1.00 ± 0.12	<0.005 ± 0.000	0.005 ± 0.000	0.375 ± 0.019	14 ± 0.23
	Jul	1.20 ± 0.48	0.50 ± 0.12	2.20 ± 0.04	<0.005 ± 0.000	0.011 ± 0.000	0.348 ± 0.009	23 ± 0.00
	Sep	0.60 ± 0.04	1.20 ± 0.33	2.50 ± 0.02	<0.005 ± 0.000	0.013 ± 0.001	0.374 ± 0.005	18 ± 0.01
	Dec	3.20 ± 0.22	0.70 ± 0.07	1.70 ± 0.04	<0.005 ± 0.000	0.015 ± 0.000	0.409 ± 0.019	9 ± 0.00
	Apr	1.00 ± 0.29	0.50 ± 0.20	1.90 ± 0.06	<0.005 ± 0.000	0.010 ± 0.001	0.582 ± 0.017	3 ± 0.00
Muskegon Lake	May	9.40 ± 0.09	2.90 ± 0.34	5.00 ± 0.12	<0.005 ± 0.000	0.013 ± 0.001	0.202 ± 0.066	23 ± 0.26
	Jul	8.00 ± 0.33	9.20 ± 0.43	6.10 ± 0.07	<0.005 ± 0.000	0.019 ± 0.001	0.132 ± 0.009	27 ± 0.06
	Sep	7.30 ± 0.06	16.6 ± 3.16	5.70 ± 0.06	<0.005 ± 0.000	0.014 ± 0.002	0.176 ± 0.056	19 ± 0.02
	Dec	6.00 ± 0.10	0.20 ± 0.10	4.30 ± 0.07	0.008 ± 0.000	0.077 ± 0.001	0.536 ± 0.019	4 ± 0.86
	Apr	10.1 ± 0.39	1.10 ± 0.08	6.40 ± 0.24	<0.005 ± 0.000	0.058 ± 0.004	0.731 ± 0.024	8 ± 0.01
Muskegon River	May	9.90 ± 0.04	1.30 ± 0.07	4.80 ± 0.09	<0.005 ± 0.001	0.031 ± 0.004	0.350 ± 0.010	20 ± 0.03
	Jul	10.4 ± 0.41	0.90 ± 0.13	6.10 ± 0.05	<0.005 ± 0.000	0.026 ± 0.002	0.309 ± 0.014	24 ± 0.01
	Sep	8.30 ± 0.00	1.40 ± 0.10	5.90 ± 0.05	0.013 ± 0.001	0.032 ± 0.002	0.500 ± 0.032	19 ± 0.03
	Dec	6.70 ± 0.20	0.70 ± 0.33	4.40 ± 0.02	<0.005 ± 0.000	0.069 ± 0.000	0.511 ± 0.055	1 ± 0.75
	Apr	13.2 ± 0.35	2.00 ± 0.36	6.70 ± 0.18	<0.005 ± 0.001	0.033 ± 0.006	0.654 ± 0.043	6 ± 0.01
Cedar Creek	May	7.20 ± 0.03	1.50 ± 0.10	2.40 ± 0.07	0.029 ± 0.007	0.207 ± 0.001	0.634 ± 0.046	13 ± 0.01
	Jul	7.10 ± 0.40	0.50 ± 0.08	3.40 ± 0.06	0.025 ± 0.001	0.187 ± 0.001	0.804 ± 0.002	14 ± 0.01
	Sep	10.6 ± 0.06	0.30 ± 0.20	5.20 ± 0.06	0.020 ± 0.000	0.149 ± 0.002	0.649 ± 0.002	14 ± 0.08
	Dec	6.60 ± 0.19	0.50 ± 0.03	3.40 ± 0.04	0.012 ± 0.000	0.211 ± 0.005	0.577 ± 0.007	2 ± 0.00
	Apr	91.6 ± 0.52	4.60 ± 0.53	13.3 ± 0.24	0.145 ± 0.002	0.203 ± 0.015	1.844 ± 0.018	4 ± 0.01

Table 2.

Watershed Site	Season	Community Respiration (R) (µg C/L/d)	Net Primary Production (NPP) (µg C/L/d)	Gross Primary Production (GPP) (µg C/L/d)	Bacterial Production (BP) (µg C/L/d)	BP as % GPP	Mean BP as % GPP
Lake Michigan	May	7.8 ± 1.9	21.9 ± 1.9	29.7 ± 1.9	2.10 ± 0.2	7.1	
	Jul	49.5 ± 2.8	-0.8 ± 2.9	48.7 ± 2.8	0.40 ± 0.1	0.8	
	Sep	35.6 ± 1.9	20.4 ± 1.3	56.0 ± 1.9	2.90 ± 0.4	5.2	
	Dec	16.5 ± 0.7	-0.2 ± 0.8	16.3 ± 0.4	1.60 ± 0.1	9.8	
	Apr	9.6 ± 3.7	24.0 ± 3.4	33.6 ± 3.2	0.10 ± 0.1	0.3	5
Muskegon Lake	May	139.8 ± 1.7	141.1 ± 3.4	280.8 ± 3.5	24.9 ± 1.2	8.9	
	Jul	183.1 ± 6.9	240.7 ± 4.9	423.8 ± 5.3	30.9 ± 2.2	7.3	
	Sep	132.5 ± 2.0	989.9 ± 3.1	1122.4 ± 2.8	26.5 ± 2.2	2.4	
	Dec	-8.3 ± 0.5	10.6 ± 0.5	2.3 ± 0.5	2.50 ± 0.2	110.3	
	Apr	9.0 ± 1.6	41.1 ± 1.1	50.1 ± 1.6	0.80 ± 0.2	1.6	26
Muskegon River	May	53.6 ± 2.7	153.1 ± 1.7	206.7 ± 2.9	36.7 ± 4.8	17.8	
	Jul	20.0 ± 1.1	34.3 ± 1.5	54.3 ± 1.6	36.7 ± 2.0	67.6	
	Sep	82.6 ± 1.0	32.1 ± 0.9	114.7 ± 0.9	74.5 ± 5.4	64.9	
	Dec	-16.4 ± 0.4	28.0 ± 1.0	11.6 ± 1.1	0.60 ± 0.1	5.2	
	Apr	35.2 ± 0.9	87.0 ± 0.8	122.2 ± 0.9	2.10 ± 0.3	1.7	31
Cedar Creek	May	129.1 ± 1.2	-53.3 ± 1.4	75.8 ± 1.8	81.8 ± 5.5	107.9	
	Jul	31.0 ± 5.3	-8.1 ± 5.5	22.9 ± 5.6	69.7 ± 4.2	303.9	
	Sep	192.8 ± 0.6	-186.6 ± 0.6	6.1 ± 0.5	93.0 ± 3.1	1515.3	
	Dec	9.1 ± 0.6	-8.0 ± 0.6	1.0 ± 0.7	2.60 ± 0.5	249.4	
	Apr	103.3 ± 1.3	-17.7 ± 0.7	85.6 ± 1.3	54.4 ± 0.8	63.5	448

Table 3.

	TEMP	Chl a	CDOM	DOC	NH$_3$	NO$_3$	SRP	GPP	R	NPP	GPP/R	BP	Viruses
Autotrophs	**0.47**	**0.47**	-0.18	0.10	**-0.69**	**-0.46**	-0.22	0.57	0.07	**0.53**	**0.40**	-0.01	0.32
Heterotrophs	0.28	**0.59**	**0.63**	**0.66**	-0.07	0.02	0.33	**0.56**	**0.42**	0.32	0.16	**0.60**	**0.52**
Prokaryotes	0.28	**0.59**	**0.59**	**0.63**	-0.11	-0.01	0.30	**0.58**	**0.42**	0.33	0.18	**0.59**	**0.54**
Eukaryotes	0.05	**0.44**	**0.71**	**0.73**	0.29	0.34	**0.46**	0.25	0.24	0.07	0.01	**0.47**	0.26
Viruses	0.08	0.34	0.20	0.33	-0.30	-0.37	-0.37	**0.50**	0.07	0.38	0.39	0.08	1.00

Table 4.

	BP	CDOM	Chl a	DOC	GPP	GPP/R	NH$_3$	NO$_3$	NPP	R	SRP	TEMP
BP	1											
CDOM	**0.53**	1										
Chl a	0.40	**0.43**	1									
DOC	**0.45**	**0.90**	**0.51**	1								
GPP	0.40	0.19	**0.86**	0.32	1							
GPP/R	-0.22	-0.09	0.15	0.01	0.24	1						
NH$_3$	0.32	**0.53**	-0.08	0.28	-0.32	**-0.43**	1					
NO$_3$	0.10	**0.59**	-0.09	**0.45**	-0.40	-0.24	**0.65**	1				
NPP	-0.13	-0.06	**0.54**	0.04	**0.58**	**0.43**	-0.40	-0.31	1			
R	**0.68**	0.35	**0.52**	0.34	**0.56**	-0.40	0.14	-0.04	-0.20	1		
SRP	0.39	**0.69**	0.25	**0.54**	-0.08	-0.30	**0.63**	**0.83**	-0.17	0.27	1	
TEMP	**0.51**	-0.15	0.25	-0.05	**0.45**	-0.05	**-0.43**	**-0.51**	0.09	**0.48**	-0.25	1

Table 5.

Watershed Site	Season	Autotrophs ($\times 10^6$/L)	Heterotrophs ($\times 10^9$/L)	Prokaryotes ($\times 10^9$/L)	Eukaryotes ($\times 10^8$/L)	Viruses ($\times 10^{10}$/L)
Lake Michigan	May	8.2 ± 0.4	2.4 ± 0.4	2.3 ± 0.4	0.7 ± 0.2	1.5 ± 0.2
	Jul	10.0 ± 0.4	2.8 ± 1.2	2.4 ± 1.1	4.2 ± 1.3	2.4 ± 1.0
	Sep	8.5 ± 1.0	1.5 ± 0.3	1.3 ± 0.2	1.3 ± 0.2	1.1 ± 0.1
	Dec	2.0 ± 0.2	2.3 ± 0.2	2.1 ± 0.2	1.8 ± 0.2	1.6 ± 0.1
	Apr	4.3 ± 0.7	2.1 ± 0.1	1.9 ± 0.1	1.9 ± 0.2	2.3 ± 0.1
Muskegon Lake	May	7.4 ± 0.6	7.4 ± 0.2	7.1 ± 0.2	1.9 ± 0.4	4.5 ± 0.4
	Jul	65.0 ± 0.5	12.0 ± 1.6	11.0 ± 0.7	13.0 ± 1.0	8.6 ± 0.2
	Sep	30.0 ± 3.4	7.6 ± 0.9	7.1 ± 0.8	5.3 ± 0.6	9.5 ± 0.1
	Dec	3.7 ± 0.9	4.7 ± 0.3	4.4 ± 0.2	2.8 ± 0.7	8.3 ± 0.3
	Apr	3.3 ± 0.1	2.8 ± 0.1	2.5 ± 0.1	3.4 ± 0.3	5.2 ± 0.2
Muskegon River	May	3.7 ± 0.9	6.3 ± 0.3	5.8 ± 0.5	5.3 ± 0.3	4.5 ± 0.9
	Jul	19.1 ± 2.3	8.0 ± 0.6	6.7 ± 0.3	9.7 ± 1.0	4.9 ± 0.2
	Sep	6.0 ± 0.2	6.3 ± 0.4	5.9 ± 0.3	3.7 ± 0.4	5.0 ± 0.8
	Dec	3.9 ± 0.3	3.9 ± 0.2	3.6 ± 0.2	3.4 ± 0.3	6.0 ± 0.2
	Apr	3.3 ± 0.3	2.7 ± 0.3	2.3 ± 0.3	3.9 ± 0.4	5.4 ± 0.8
Cedar Creek	May	0.6 ± 0.1	3.0 ± 0.2	2.7 ± 0.2	2.8 ± 0.2	2.8 ± 0.2
	Jul	2.2 ± 0.3	2.7 ± 0.1	1.9 ± 0.1	5.9 ± 0.6	1.7 ± 0.0
	Sep	1.0 ± 0.1	2.3 ± 0.3	2.0 ± 0.3	3.4 ± 0.1	2.0 ± 0.3
	Dec	0.8 ± 0.0	1.2 ± 0.1	1.0 ± 0.1	2.1 ± 0.2	1.6 ± 0.1
	Apr	3.4 ± 0.9	8.7 ± 0.3	7.6 ± 0.3	11.0 ± 1.0	1.1 ± 0.2

Figure 2.
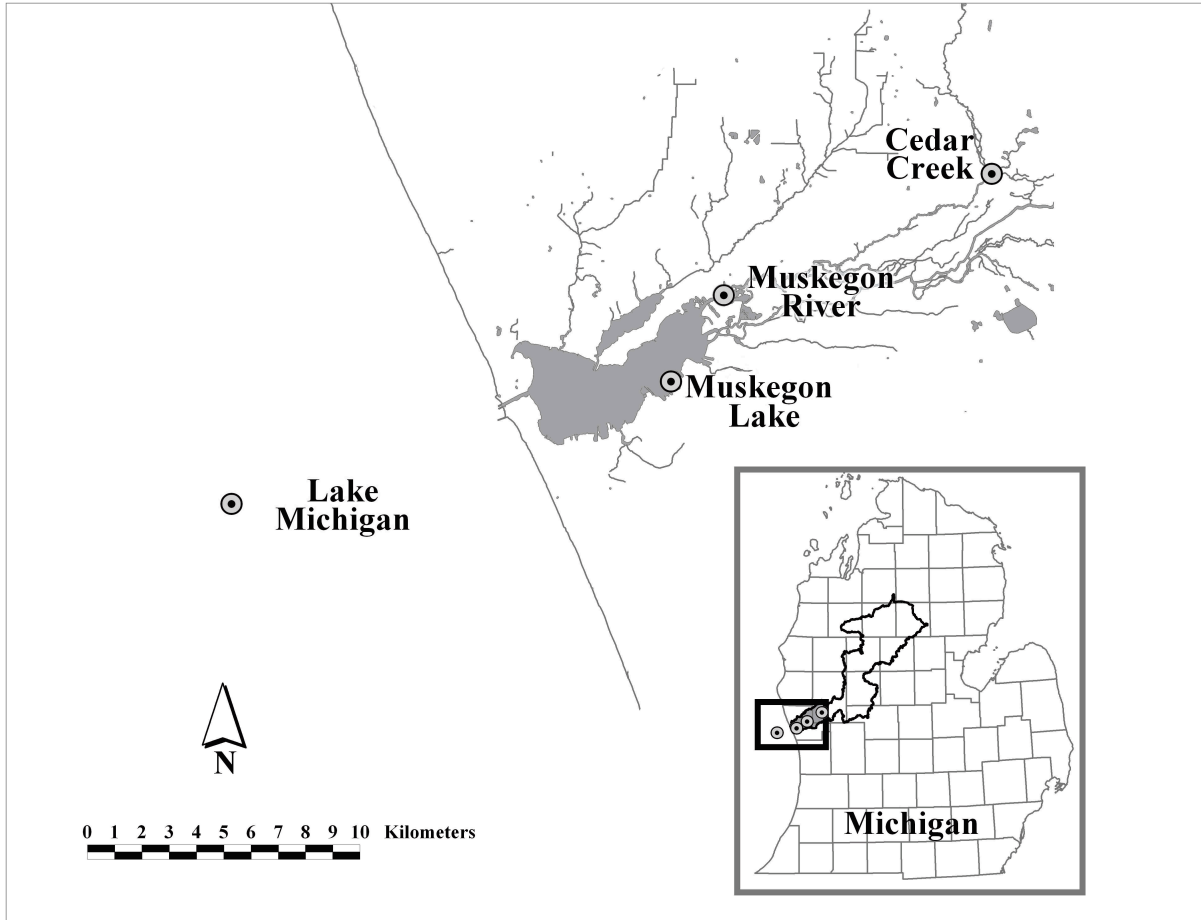
Figure 3.

Figure 4.

Figure 5.

	MAY		JUL		SEP		DEC		APR	
✎ Lake Michigan	29.7	(1.9)	48.7	(2.8)	56.0	(1.9)	16.3	(0.4)	33.6	(3.2)
▦ Muskegon Lake	280.8	(3.5)	423.8	(5.3)	1122.4	(2.8)	2.3	(0.5)	50.1	(1.6)
▨ Muskegon River	206.7	(2.9)	54.3	(1.6)	114.7	(0.9)	11.6	(1.1)	122.2	(0.9)
■ Cedar Creek	75.8	(1.8)	22.9	(5.6)	6.1	(0.5)	1.0	(0.7)	85.6	(1.3)

Proportion of Chlorophyll a

Cedar Creek 16%
Lake Michigan 7%
Muskegon River 13%
Muskegon Lake 64%

Proportion of Autotrophs

Cedar Creek 4%
Lake Michigan 18%
Muskegon River 19%
Muskegon Lake 59%

Figure 6.

	MAY		JUL		SEP		DEC		APR	
Lake Michigan	7.8	(1.9)	49.5	(2.8)	35.6	(1.9)	16.5	(0.7)	9.6	(3.7)
Muskegon Lake	139.8	(1.7)	183.1	(6.9)	132.5	(2.0)	0.0	(0.5)	9.0	(1.6)
Muskegon River	53.6	(2.7)	20.0	(1.1)	82.6	(1.0)	0.0	(0.4)	35.2	(0.9)
Cedar Creek	129.1	(1.2)	31.0	(5.3)	192.8	(0.6)	9.1	(0.6)	103.3	(1.3)

Proportion of DOC

Cedar Creek 30%
Lake Michigan 10%
Muskegon River 30%
Muskegon Lake 30%

Proportion of Heterotrophs

Cedar Creek 20%
Lake Michigan 12%
Muskegon River 30%
Muskegon Lake 38%

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Figure 7.

CHAPTER III

Once upon a time, there was a little girl named Goldilocks. She went for a walk in the forest. Pretty soon, she came upon a house. She knocked, and when no one answered, she walked right in. At the table in the kitchen, there were three bowls of porridge. Goldilocks was hungry. She tasted 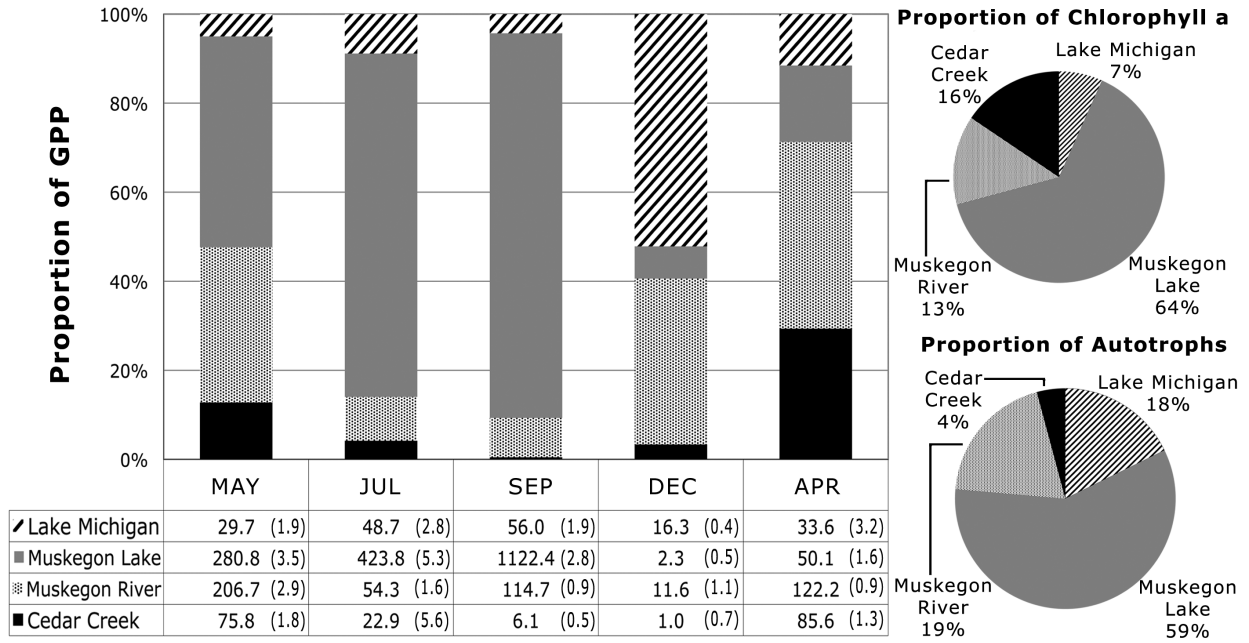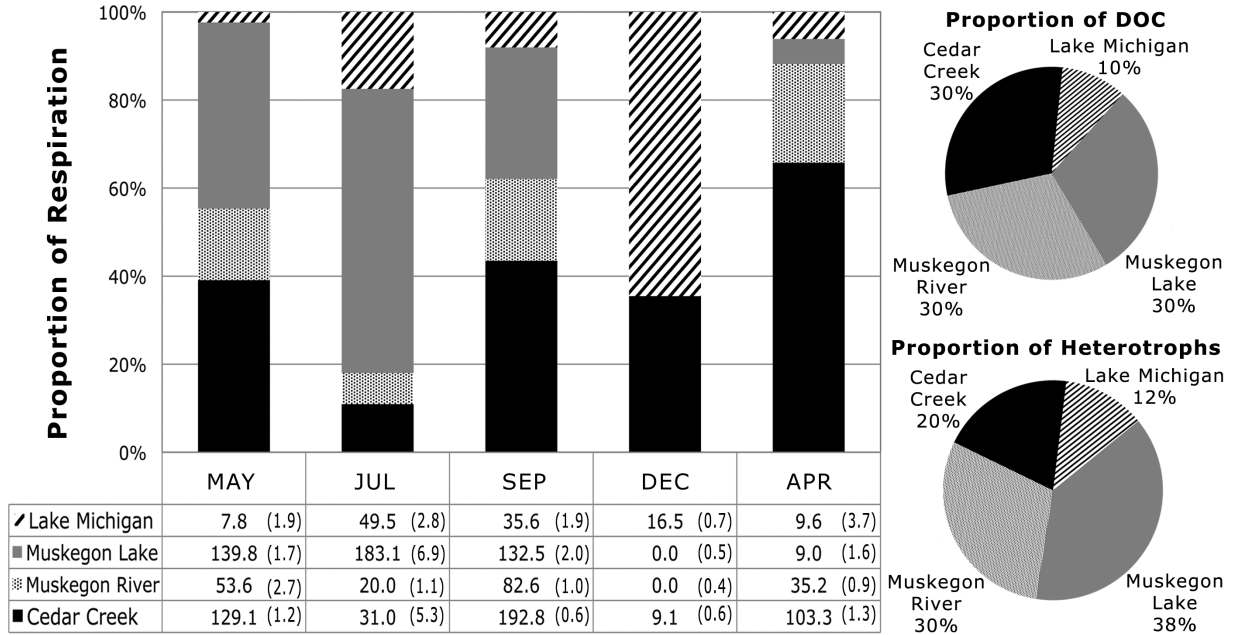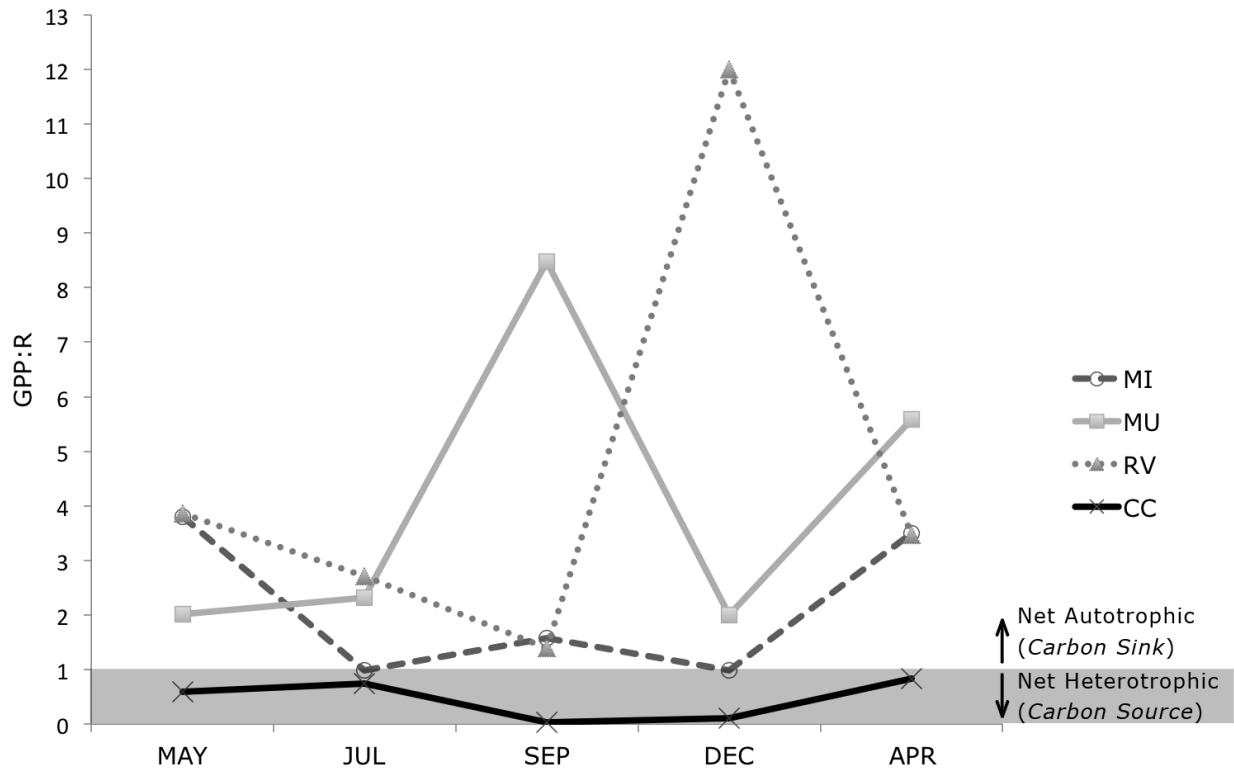the porridge from the first bowl. "This porridge is too hot!" she exclaimed. So, she tasted the porridge from the second bowl. "This porridge is too cold," she cried. So, she tasted the last bowl of porridge. "Ahhh, this porridge is just right," she said happily and she ate it all up.......

– *Goldilocks and the Three Bears (A Fairy Tale, circa 1813).*

EXTENDED REVIEW OF LITERATURE

Carbon and nutrient runoff from terrestrial sources, load streams, rivers, wetlands and small lakes with intermittent but consistent subsidies to the metabolic processes that take place in these varied aquatic environments (Beman et al., 2005; Biddanda and Cotner, 2002; Weinke et al., 2014). Our results show planktonic autotrophs and heterotrophs perform biogeochemical processes at increased levels in these tributary waterways before nutrient loads have a chance to enter larger receiving basins, such as the great lakes. In particular, we found the river-mouth estuary was a biogeochemical hotspot or "Goldilocks zone" where terrestrial subsidies combined with increased residence time to allow plankton more time to assimilate nutrients and carbon (Essington and Carpenter, 2000; Slattery and Phillips, 2011; McClain et al., 2003; Marko et al., 2013). Figure 8 is a conceptual diagram that shows the Muskegon Lake estuary's domination of metabolic activity and planktonic biomass in the land-to-lake transect observed in this study, where approximately 80% of measured NPP took place in Muskegon Lake compared to merely 5% in Lake Michigan. Additionally, the robust metabolic activity in Muskegon Lake

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that peaked during the summer-fall season was apparent in every season compared to oligotrophic Lake Michigan (Figure 9), which never contributed more than ~10% of total measured NPP in any given season.

Net autotrophy and a buildup of phytoplankton biomass in this estuary Goldilocks zone sustains a level of productivity that supports a dynamic estuarine food web (Figure 10), leading to a viable nursery for riverine and lake species and sustainable fisheries (Biddanda and Dila, 2015; Bhagat and Ruetz, 2014; Carter et al., 2006). It is likely that Goldilocks Zones, where environmental conditions are "just right" for maximum productivity, are common biogeochemical hot-spots of ecological hot-moments in the landscape (McClain et al., 2003) just prior to the large diluting effects of the world's great lakes, as they are in the estuaries of oceans. In Weinke et al. (2014) the authors summarized data from 2003 through 2013 monitoring the rates of carbon metabolism in summer surface waters collected at multiple sites in the estuary and out into Lake Michigan. They tracked changes in GPP, R and NPP from year to year and found a systematic decrease in metabolism in surface waters extending from Muskegon Lake to offshore Lake Michigan (Figure 1). Our current study finds the same decrease from the estuary to lake Michigan, but provides additional insights by moving farther up the watershed to feeder tributaries where, although land-margin is comparatively higher, metabolism and plankton abundance are generally lower than Muskegon Lake. This demonstrates the important impact of residence time on taking full advantage of terrestrial subsidies – hydrologic forces at play in streams and rivers limit full utilization of concentrated sources of runoff and wetland flush input. Inserting the upstream element to the watershed study area adds a missing piece to the gradient puzzle and expands on the Weinke et al (2014) Muskegon Lake conceptual diagram (Figure 8), further describing the spatio-temporal environmental dynamics of surface water that make

Muskegon Lake stand out as a hot spot of productivity (Figure 9).

The Cedar Creek site is partially open canopy surrounded by a riparian zone classified as Palustrine Forest/Shrub wetland (FWS, National Wetlands Inventory, 2016) and is thus intermittently subsidized by wetland flush of highly utilizable carbon and nutrient resources (Fishbeck et al., 2011) in addition to overland runoff after rainfall events. In the present study, the post-rain collection in April 2011 (Table 6) showed a spike in Chl a, which could be from combined terrestrial vegetation sources and sediment periphyton since autotrophic phytoplankton counts did not rise after the event. Ogdahl et al (2010) found that periphyton was a more important contributor than phytoplankton to Chl a in Cedar Creek and that periphytic biomass was highest in Cedar Creek sediment (as well as in sediment of all sites in their study area). Based on surface water turbidity visible during the April collection, creek sediment as well as resuspended periphytic biomass could easily have been an important component of the water sample collected and a major factor in the observed high Chl a measurements. The study area in Ogdahl et al (2010) was similar to the Muskegon River watershed transect studied here, and the author's found that periphytic metabolism was a much larger portion of overall site metabolism than the surface water planktonic metabolism we examine here. We emphasize that our study compares rates of surface water contributions from selected sites, and for larger modeling studies it is critical to consider the influence of microhabitats within sites as well as the areal scale of each watershed component.

Wetland flush at the Cedar Creek site could stimulate rapid heterotrophic bacterioplankton metabolism by intermittent introduction of rich carbon sources and account for the lower concentration of DOC found there. Bacterial growth efficiency (BGE) in aquatic systems varies widely but is estimated to range between 10% and 50% (Biddanda et al., 1994;

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del Giorgio and Cole, 1998; Kirchman, 2012). Subtracting the small values for total autotrophic abundance (separate counts were not made for prokaryotic autotrophs) from the much larger values of total prokaryotes, we get a good estimate of total heterotrophic prokaryotes that can be used to compute how much BP is heterotrophic and how much measured respiration is due to heterotrophic bacterial metabolism. Heterotrophic bacterial respiration was calculated using the following equation:

BGE = BP/(BR+BP)

Where BGE was estimated at .50 and .30, BP is the average of the heterotrophic fraction of seasonally measured values (60.3 µg C/L/day) and BR is the calculated bacterial respiration. Assuming a conservative bacterial growth efficiency of 50% with average Cedar Creek BP of ~60 µg C/L/day the calculated heterotrophic BR is 60 µg C/L/day. Thus at 50% BGE, heterotrophic bacteria could account for ~65% of the average respiration measured in BOD analysis (~93 µg C/L/day). Using the less conservative 30% BGE that del Giorgio and Cole (1998) found was common for river samples, calculated BR would be 140 µg C/L/day and heterotrophs could be responsible for 100% of measured BOD respiration in Cedar Creek surface water. In either case, heterotrophic bacterioplankton appear to be the major contributors to Cedar Creek respiration values.

With reference to Cedar Creek, the Muskegon River site likely receives comparatively recalcitrant carbon sources from terrestrial surroundings as it is located in a large diverse emergent/shrub wetland complex (FWS, National Wetlands Inventory, 2016) that is set within an urbanized location. In 2003 Marko et al (2013) found that ~53% of a 1800 metric ton (MT) load of particulate organic carbon (POC) and 33% of a 24 MT load of total phosphorus (TP) entering Muskegon Lake from Muskegon River was never discharged to Lake Michigan. However, only

~3% of a 3400 MT DOC load was intercepted by Muskegon Lake, while most of the DOC load was discharged to Lake Michigan. The larger fractional reduction of POC and TP could be due to consumption by heterotrophic invertebrates, larger eukaryotes and autotrophs in this highly productive mesotrophic lake (Bhagat and Ruetz, 2011; Carter et al., 2006; Larson et al., 2013), while the DOC source may be a more recalcitrant than the sources that subsidize Cedar Creek heterotrophic bacterioplankton. Even under the increased residence time of Muskegon Lake, Marko et al (2013) found that the subsidy of DOC was underutilized. It is also possible that in many seasons the availability of easily consumable autochthonously produced DOC makes the additional riverine source unnecessary, especially if it is a harder substrate to metabolize to begin with.

How resource inventories drive community level process, and how autotrophic and heterotrophic aquatic microbes link the fate of terrigenous nutrients and carbon is partially explored in this thesis, but future studies using high-throughput sequencing of environmental samples would add a deeper resolution of microbial communities, based on taxonomic identification, and associated metabolic activities along the gradient studied here. Tremendous diversity within microbial communities is being uncovered quickly by a growing number of studies that use next-generation sequencing methods on the 16S rRNA region of bacterial DNA samples collected from every type of environment (Andersson et al., 2009; Baker and Dick, 2013; Eiler et al., 2012; Eren et al., 2014; Galand et al., 2009; Newton et al., 2011; Sogin et al., 2006). Using ecological scaling laws reformatted for large-scale predictions, Locey and Lennon (2016) recently estimated that Earth is inhabited by close to a trillion (10^{12}) bacterial species, 99.999% of which are still undiscovered. Many environmental factors influence this diversity, but dispersal and environmental condition are two primary drivers. Dispersal based 'mass

effects' and environmental condition based 'species sorting', vary widely in importance to local microbial diversity (Besemer et al., 2012; Crump et al., 2007; Crump et al., 2012; Leibold et al., 2004; Székely et al., 2013). In freshwater ecosystems these spatial and environmental processes can be especially important to diversity along the highly variable land-to-lake environmental gradient. In fact the bacterial meta-community of tributary streams should be strongly linked to the River Continuum Concept (Vannote et al., 1980), in which hydrological flow conditions, the riparian zone, physical substrate and food are all important factors in determining community structure along the entire river system. The influence of landscape runoff, as an intermittent but regular source of terrestrial microbes to stream communities, can be an intense introduction of both microbes and fresh nutrient to the constituent microbial communities, and to a lesser extent, the communities of receiving waters that that tributaries feed.

Differences in microbial community structure between tributaries and adjacent water bodies have been found to be dependent on various parameters including; upslope or upstream inoculation, type of available organic matter, hydrological residence time, and season (Crump et al., 2012; Fortunato and Crump, 2011; Fortunato et al., 2013; Judd et al., 2006). Crump et al (2012) found a 10x decrease in alpha diversity of microbial communities across a land to lake gradient, from a headwater tributary to oligotrophic Toolik Lake during spring thaw in an Alaska watershed. Peak diversity in the headwater stream suggested that small streams are mixing zones where terrestrial and aquatic communities combine and as they move toward lake environments there is a loss of the upslope soil water community and an increase in community members that are best adapted to lake conditions. Which of the upslope bacteria are actually viable in small streams with short residence times is questionable, but regular introduction from the landscape keeps diversity high. Mass effects of species dispersal are only important in environments with

short residence times (Lindström et al., 2006; Nelson, 2009; Shade et al., 2007), but this terrestrial reservoir could prevent the extinction of rare taxa in lakes, allowing emergence of these species when appropriate conditions are present (Jones and Lennon, 2010). In fact Crump et al (2012) found that approximately half of abundant lake taxa were rare in upslope environments and suggested that taxa that were mixed and transported across hydrologically linked ecosystems could be quite adaptable to different niches along the continuum and ultimately dominate a downstream environment. In the western Michigan watershed studied for this thesis project it would not be surprising to see very similar ranges of diversity from Cedar Creek to Lake Michigan given their extremely different ecosystems. In the Toolik Lake study the headwater inlet stream was small like Cedar Creek, and studied during a time of year when runoff would have a strong impact on microbial community structure, and although Toolik Lake is much smaller than Lake Michigan, it is also a very deep and oligotrophic system. The large range of diversity would be especially true in the early spring collection when prokaryotes in Cedar Creek were particularly abundant after a runoff event and Lake Michigan was still cold, but spring mixing might emphasize the lakes dominant microbial community.

My thesis provided a spatio-temporal survey of the changes in planktonic microbial abundance and community metabolism along a land-to-lake gradient, and the changes seen in community structure of these autotrophs and heterotrophs at the individual study sites during different seasons. How spatial and temporal variations in the distribution and make-up microorganisms impacted the biological activity measured in a sample, was not always easy to assess based on the broad categories used to define the plankton communities. Within sites, comparisons could be made between production and respiration levels and changes in numbers of autotrophs and heterotrophs from season to season. However it was less straightforward to

compare changes in community structure with reference to community metabolism when evaluating different sites with fundamentally different aquatic ecosystems. Environmental parameters were so different from Cedar Creek (with its short hydraulic residence time of a few days and large proportional land margin with associated terrestrial runoff) to offshore Lake Michigan (where residence time is approximately 62 years (Quinn, 1992), terrestrial input is comparatively limited and autotrophic production is the primary carbon source for heterotrophs in a nutrient-sparse environment) that simple slide counts did not resolve variations between the sites. A closer look at the microbial taxa that inhabit these diverse niches in different conditions would tell a fuller story of their influence on carbon and nutrient metabolism in freshwater systems that vary in essential ways. Sequencing variable regions of 16S rRNA genes for taxa identification would not indicate which genes are active among microbes in different environments, but it should be possible to identify differentiating taxa and search for fully sequenced and annotated genomes in the public database. This approach could outline broad similarities and differences in genes necessary for proliferation or survival in these highly varied freshwater environments. From land to lake, freshwater communities of abundant and rare microbes are essential to biogeochemical processes that produce and recycle carbon and nutrients on a globally relevant scale, and better understanding this complex microbial ecology is a fascinating future study for this dynamic Lake Michigan watershed.

EXTENDED METHODOLOGY

Study Sites

The Muskegon River watershed drains approximately 7,000 km^2 of west-central Michigan. Drainage basin boundaries include portions of 12 counties and around 90 tributaries that flow into the main stem of the Muskegon River. The river ends in a 17 km^2 drowned river mouth lake (43.2331^{0}N, 086. 2903^{0}W), which discharges into central Lake Michigan through a single, 1.6 km-long navigational channel. Over an 11-month period, four sites located along the lower southwest portion of the watershed were sampled to evaluate temporal variations in community metabolism and microbial abundance, within and between sites. The four sites are distinct yet interconnected habitats along a land-to-lake gradient: 1. Cedar Creek (43.3057^0 N, 086.1150^0 W), 2. Muskegon River (43.2631^0 N, 086.2453^0 W), 3. Muskegon Lake (43.2261^0 N, 086.2935^0 W) and 4. Lake Michigan (43.2062^0 N, 086.4497^0 W). These landward sites are traditional sampling sites chosen by Annis Water Resources Institute for their representativeness in the watershed. Lake Michigan site was part of the ongoing National Oceanic and Atmospheric Administration-Great Lakes Environmental Research Laboratory (NOAA-GLERL) long-term transect study. Cedar Creek is a cold-water tributary of the Muskegon River and the sampling site was a partially open canopy surrounded by wetland located approximately 9.5 km from its mouth at the Muskegon River. The riparian zone is classified as Palustrine Forest/Shrub wetland that is seasonally flooded (FWS, National Wetlands Inventory, 2016). Muskegon River is approximately 350 km long with a 175 m drop in elevation between its source at Houghton Lake (44.3147^0 N, 084.7647^0 W) and the river mouth. We collected from a causeway bridge near the river mouth surrounded by an emergent/shrub wetland complex (FWS, National Wetlands Inventory, 2016) amid urbanized land use. At this location, river width is about 76

meters and depth is ~3 m. Muskegon Lake is a drowned river mouth lake with a surface area of 17 km^2, a mean depth of 7 m and maximum depth of 23 m. Surface water was sampled at the deepest point of the lake. The Lake Michigan site is at the NOAA M-45 buoy about 8 km offshore located over the 45 m isobath. All of the sites except for Cedar Creek were in open sunlight

Sample Collection

On May, July, September, and December of 2010, and April of 2011, at a depth of approximately 0.5 m, surface water samples were collected in each season (see Table 6 for dates and weather conditions). Collection bucket and sample containers were rinsed 3 times with sample water before collection. Four discrete 10 L water samples were collected at each site, placed in sample rinsed acid-cleaned carboys, transported on ice in coolers to the Annis Water Resources Institute (AWRI) and analyzed.

Physical and Biogeochemical Inventories

In the field, basic water chemistry (temperature, pH, conductivity and dissolved oxygen) was measured using a calibrated YSI 6600 Datasonde. In the laboratory we measured dissolved organic carbon (DOC), colored dissolved organic matter (CDOM), chlorophyll a (Chl a) and bioavailable nitrogen and phosphorus in each sample.

DOC samples were filtered through 0.7 μm pre-combusted GF/F filters (4 h at 450 ^{0}C) and stored frozen in pre-combusted glass vials (4 h at 550 ^{0}C) with Teflon-lined caps until a convenient time to analyze. After thawing, sample acidification with 4-5 drops of 2N HCl and inorganic C removal by purging with ultra-pure air, measurements of DOC were determined by high temperature oxidation (680 ^{0}C) using a Shimadzu TOC-5000 carbon analyzer. Total organic carbon standards were made up from potassium hydrogen phthalate (KHP) and blanks were

ultrapure deionized water (Benner and Strom, 1993).

Water samples for CDOM were filtered through GF/F filters and absorption at 350 nm were then measured in a 1 or 10 cm quartz cuvette using a spectrophotometer; blanks were ultrapure deionized water. The 350 nm wavelength is a specific absorbance value representative of bulk CDOM (Helbling and Zagarese, 2003).

Chlorophyll a was collected by filtering approximately 1000 ml of sample onto a 47mm Whatman GF/F filter in a darkened room. Filters were then frozen for at least 24 h. After freezing and still in a darkened room, filters were placed in cold grinding tube with 3–5 ml of cold buffered acetone solution (90 parts acetone with 10 parts saturated $MgCO_3$ solution of 1.0 g $MgCO_3$ in 100 ml Type I deionized water), then ground for 1 minute with a grinding stone attached to a drill. Tubes of Chl a extract were covered in foil, refrigerated and steeped for 24 h before reading (Parsons et al., 1984). Clarified extract was added to a 1 cm cuvette and optical density (OD) at 750 and 664 nm was read. An acidification step followed in which 100 μl of 0.1 N HCl was added to the extract in the cuvette, gently agitated and after 90 sec the OD at 750 and 665 nm were read.

For nutrients, samples were given to the Rediske analytical chemistry lab at AWRI for assay according to standard APHA (1998) methods. For soluble reactive phosphorus (SRP) and nitrate (NO_3), samples were prepped by filtering 25 ml through a 25 mm 0.45 μm pre-rinsed nitrocellulose filter, immediately iced and then frozen until assayed. The nitrocellulose filters were pre-rinsed with 5 ml of 25% HCl followed with 25 ml of deionized water and then blown out with air before sample was added for filtration. Prep for ammonia (NH_3) assay included acidification of 250 ml of sample with 250 μl of concentrated H_2SO_4 after which samples were put on ice then refrigerated at 4^0. All prepped samples were stored for no longer than 28 days

before being assayed.

Microbial Plankton Enumeration

Prokaryotes, planktonic eukaryotes and viruses were enumerated using standard epifluorescence microscopy at 1000x magnification. Samples were preserved with 2% formalin and 500 µl aliquots were filtered onto 0.02 µm pore size, 25 mm diameter Anodisc membrane filters (Whatman) stained with SYBR Green I (Molecular Probes, Inc.) for enumeration of microbes according to Noble and Fuhrman (1998). Staining was done after filters were completely dry. When stain was added filters were kept in the dark (a lab bench drawer) for 15–20 minutes. After staining, anti-fade mounting solution was used to mount filter to slide and cover-slip was added. SYBR green stains DNA where the smallest stained particles are counted as viruses, larger fully-stained particles are counted as prokaryotes, and eukaryotes are recognized by shape and stained nuclei (not present in prokaryotes). For autotroph enumeration, 20 ml aliquots were filtered onto 0.2 µm pore size, 25 mm diameter black Nuclepore filters and no dye was added. Chlorophylls of phytoplankton fluoresce red under UV light and are visible against the black filter. Counts were made of autofluorescent phytoplankton including cyanobacteria, diatoms, flagellates, and undefined. Following preparation, all slides were stored frozen until enumeration when at least 10 fields of view and 300 cells were counted per sample.

Microbial Community Metabolism

Oxygen uptake in untreated and unfiltered water samples was measured in clear and darkened biological oxygen demand (BOD) glass bottles (300 ml). Quadruplicate clear and darkened BOD bottles were filled using tubing to allow overflow for 20 seconds to ensure no air contamination, and incubated for 24 hours in situ in Muskegon Lake at a depth of 0.5 m suspended on a wire rack. Winkler titrations for the determination of dissolved oxygen were

carried out directly in 300 ml BOD bottle using Micro Winkler titration with potentiometric endpoint detection using a combined platinum Ag/AgCl reference electrode (Biddanda et al., 2001; Carignan et al., 1998; Weinke et al. 2014). Oxygen consumption in darkened bottles measured community respiration (R), and oxygen production in clear bottles measured community net primary production (NPP) after 24 hours incubation. GPP was then calculated as NPP + R as described by Wetzel and Likens (2000). Time zero (T_0) bottles measure the initial oxygen concentration in the samples and they were immediately treated with standard BOD pickling reagents and kept in darkness until the 24 hour light ($T_{24}L$) and dark ($T_{24}D$) bottle incubations were completed and all bottles were assayed.

Variables of R, NPP and GPP were calculated as follows:

$R = T_0 - T_{24}D$

$NPP = T_{24}L - T_0$

$GPP = NPP + R$

The few negative R values found in our dataset most likely represent anomalous production of oxygen in dark bottles. Oxygen consumed was converted to carbon respired assuming a molar respiratory quotient of 1.0 (Biddanda et al. 1994; Robinson 2008), and oxygen produced was converted to carbon produced using a molar photosynthetic quotient of 1.0 (Robinson 2008). GPP:R ratios indicate the potential for positive or negative NPP in the system, with a value of 1.0 indicating perfect carbon balance.

Bacterial secondary production (BP) was estimated from rates of protein synthesis using radiolabeled [3H]leucine incorporation (Kirchman et al. 1985, Simon and Azam 1989). Triplicate 1 ml unfiltered whole lake water samples were incubated in the dark at in situ temperatures (± 2°C). Incubations were run for 1 to 3 h with 20 nM (final concentration) of [3H]leucine (2.52 Å~

1012 Bq/mmol1), together with a trichloroacetic acid (5% final concentration) killed control. Incorporation rates were converted to bacterial carbon production (BP) using the conversion factor of 2.3 kg C produced/mol leucine uptake that assumes a 1.5-fold internal isotope dilution (Biddanda et al. 1994).

<div align="center">Statistical Methods</div>

Statistical analyses were performed using R open source programming language (R Core Team, 2013) and the R Commander graphical user interface (Fox, 2005). Pearson's correlation analysis was used to test for associations between component members of the microbial community and all measured variables. One-way analysis of variance (ANOVA) was used to determine differences of: a) site or season on environmental and/or biological measures, and b) site or season on dominant microbial community. When significant ($\alpha = 0.05$) differences were found, post-hoc analysis with Tukey's honest significant difference (HSD) test resolved which groups were different. The data were not normally distributed; therefore, they were $\log_{10}$ transformed before analysis.

FIGURE CAPTIONS

Table 6. Rain on day of collection, 24 hour antecedent rainfall (ARF), 48 hour ARF and overall

weather conditions for sampling date. * = snowfall measurement.

Figure 8. The generalized relationship between watershed sites along the land-to-lake gradient

and their percent contribution to total autotrophic and heterotrophic biomass, and NPP.

Figure 9. A comparison of mesotrophic Muskegon Lake (Estuary) and oligotrophic Lake

Michigan (Lake) contributions to percent of total NPP during each season.

Figure 10. Schematic diagram of microbial cycling of carbon and other bioactive elements in a

model lake. Terrigenous inputs to receiving watersheds include dissolved and particulate

organic matter and inorganic nutrients (not shown here). Phytoplankton growing on terrigenous

and recycled nutrients also produce dissolved organic matter (DOM) through primary

production. The fate of most DOM is bacterial respiration, which results in regeneration of

mineral nutrients and carbon dioxide. Bacterial production (secondary production) moves DOM

to higher trophic levels through grazing by protozoa and aggregation into metabolic "hot spots"

called lake snow. Inorganic nutrients are also recycled. Microbial metabolism, sloppy protist

grazing and excretion work together to make inorganics biologically available for further

phytoplankton production. The viral shunt, microbial loop and classic grazer food web combine

to move the elements of life through the aquatic community, while lake snow delivers them to

the benthos for sediment burial.

TABLES

Table 6.

Date	Site	Rain			Condition
		Collection Day	24 ARF	48 ARF	
5/26/10	MI, MU, RV, CC	0.00	0.00	0.00	Lowflow
7/21/10	MI, MU, RV, CC	0.00	0.02	0.08	Lowflow
9/22/10	MI, MU, RV, CC	0.00	0.47	0.00	Lowflow
12/4/10	MI	0.00	0.00	2.90*	Lowflow
12/15/10	MU, RV, CC	0.00	0.10*	0.00	Lowflow
4/19/11	MI, MU	0.80	0.80*	0.00	Rain
4/20/11	RV, CC	0.05	0.80	0.80*	Rain

Figure 8.

Figure 9.

Figure 10.

Phytoplankton

CO$_2$ O$_2$

Leaky & Dead
Cells

C, H, O, N, P, S, Si

Aggregation
& Sinking

DOM

Viruses

Protozoa   Zooplankton   Fish

Bacterioplankton

Lake Snow
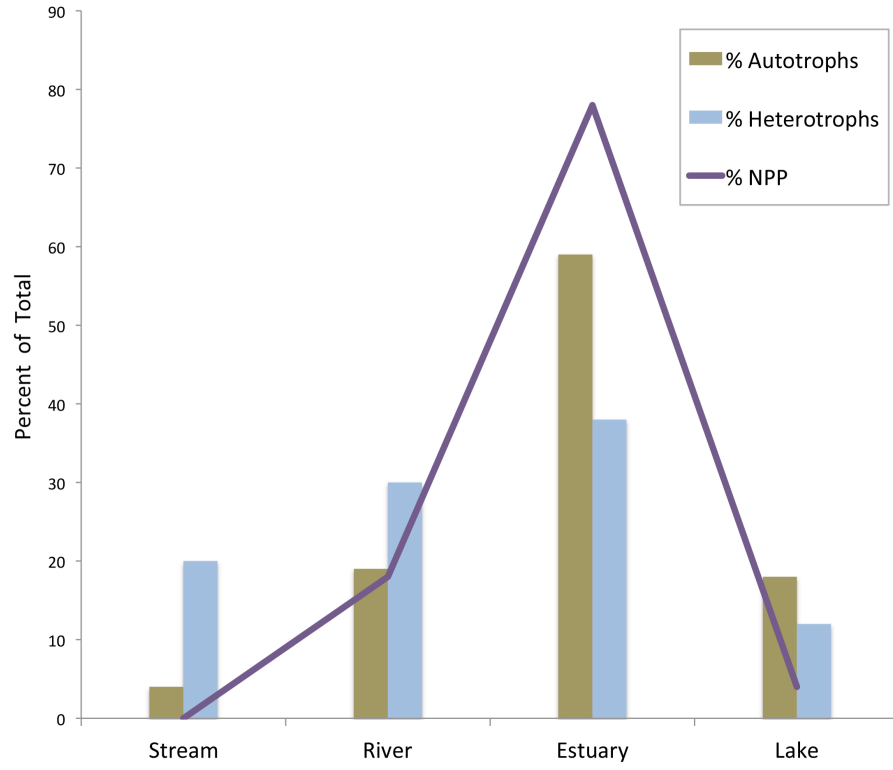
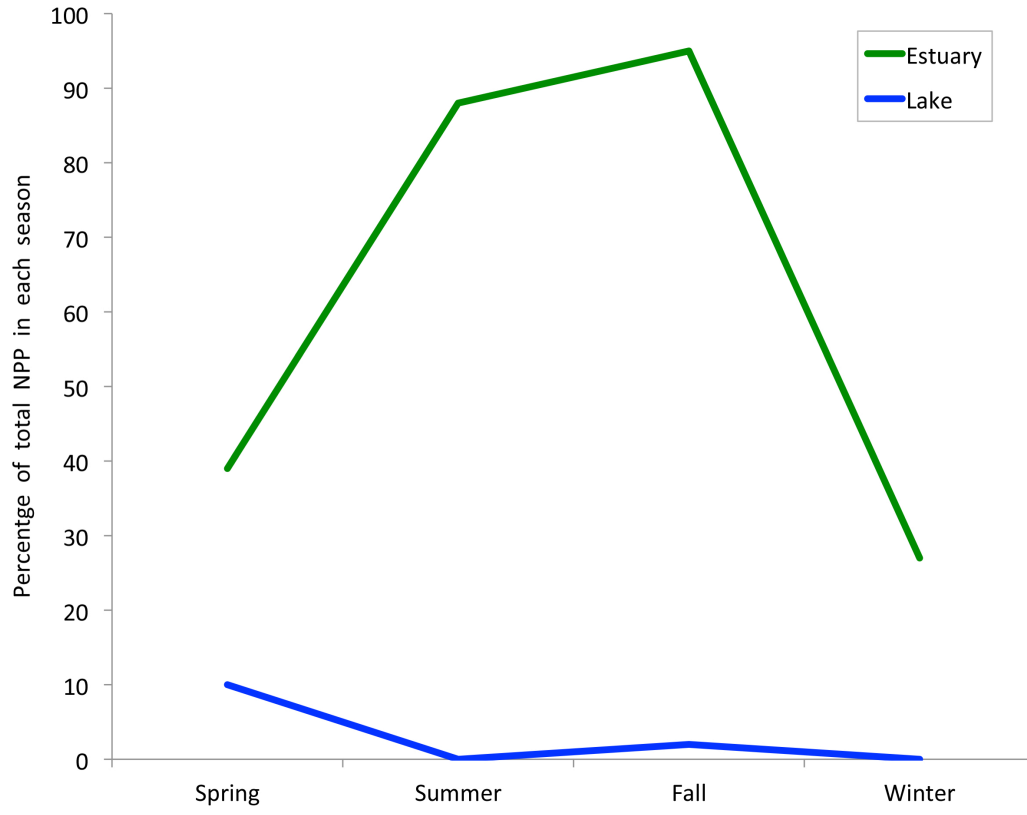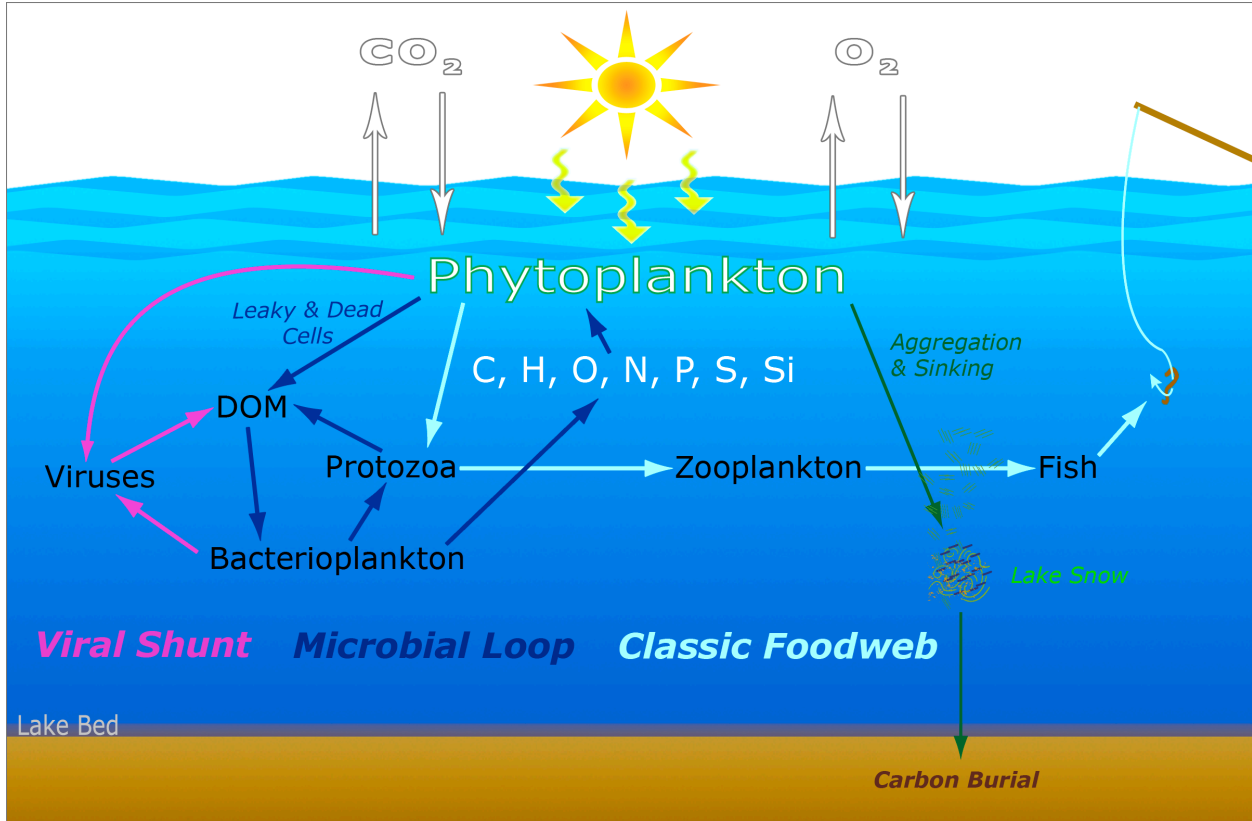**Viral Shunt   Microbial Loop   Classic Foodweb**

Lake Bed

Carbon Burial

FIGURE CAPTION FOR SUPPLEMENTARY FIGURES

Supplementary Figure 1. (A) Mean nutrient levels for all seasons and study sites along the Muskegon River watershed (see Table 1 for standard errors). (B) Boxplots of total nutrients plotted by study site. Different letters above boxplots indicate significant differences (NO_3 = $F_{3,76}$ = 16.99, p < 0.001; NH_3 = $F_{3,76}$ = 283.3, p < 0.001; SRP = $F_{3,76}$ = 15.64, p < 0.001) between sites as determined by ANOVA and Tukey HSD. (Note: $F_{3,76}$ denotes 3 degrees of freedom for number sites and 76 degrees of freedom for the number of samples.)


Supplementary Figure 2. (A) Boxplots of seasonal NO_3 (mg/L) levels in Muskegon Lake. Different letters above boxplots indicate significant differences ($F_{4,15}$ = 29.2, p < 0.001) between seasons as determined by ANOVA and Tukey HSD. (B) Linear regression of NO_3 (mg/L) relative to gross primary production (µg Carbon/L/d) values in Muskegon Lake. (Note: $F_{4,15}$ denotes 4 degrees of freedom for number seasons and 15 degrees of freedom for the number of samples.)

Supplementary Figure 3. (A) Mean colored dissolved organic matter (CDOM) and dissolved organic carbon (DOC) levels for all seasons and study sites (see Table 1 for standard errors). (B) Boxplots of total CDOM and DOC plotted by study site, with April values removed as outliers. Different letters above boxplots indicate significant differences (p < 0.001) between sites as determined by ANOVA. (C) Ratio of colored dissolved organic matter (CDOM) to dissolved organic carbon (DOC) in each season for each site.

Supplementary Figure 4. (A) Mean (n=4; ±1 SE) bacterial secondary production as measured by [^{3}H] leucine incorporation into protein. (B) Mean (n=4; ±1 SE) bacterial production per cell – estimated by dividing measured bulk bacterial production rates (in units of μg carbon/liter/day) by microscopically determined bacterial abundance (in units of cells/liter).
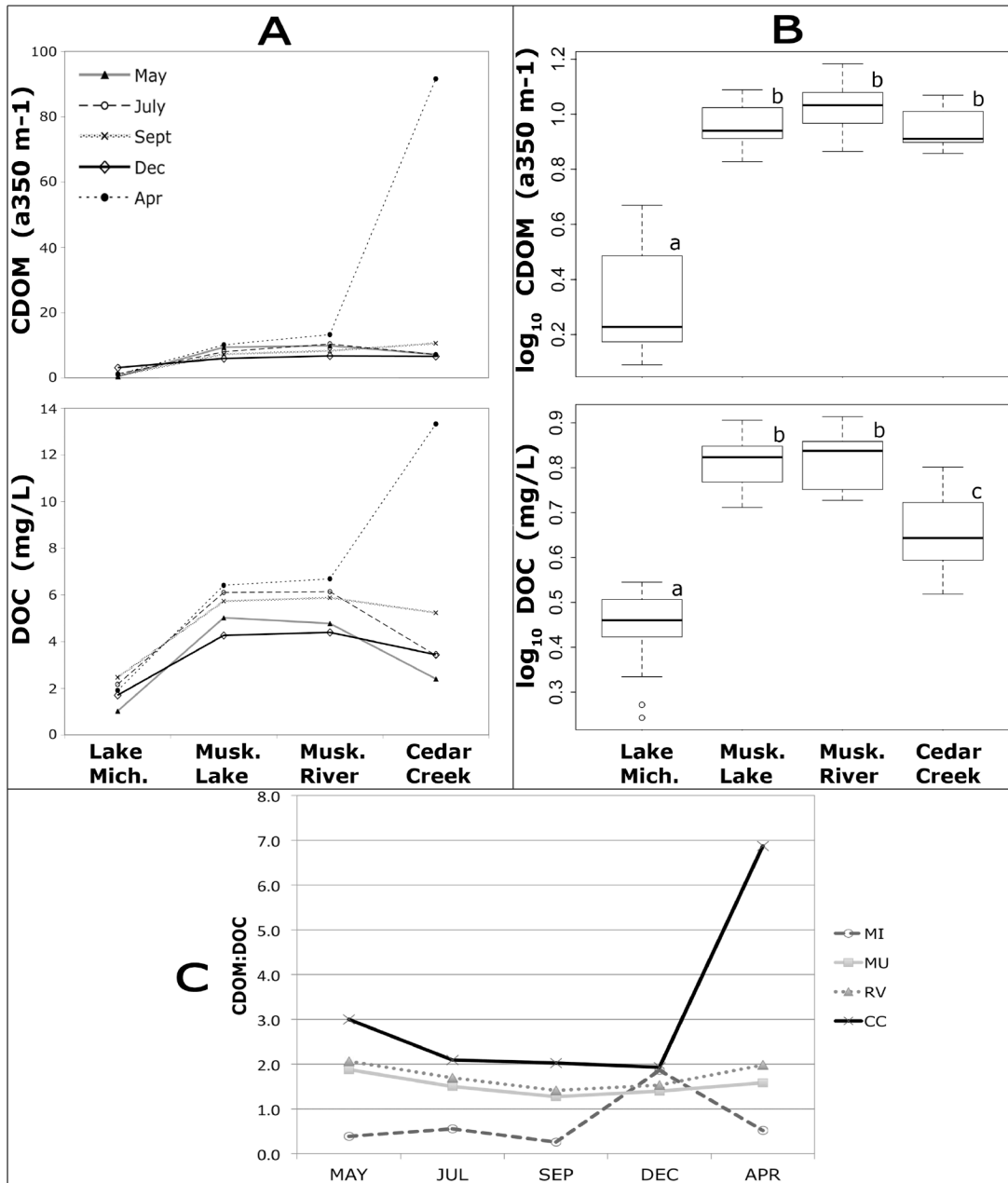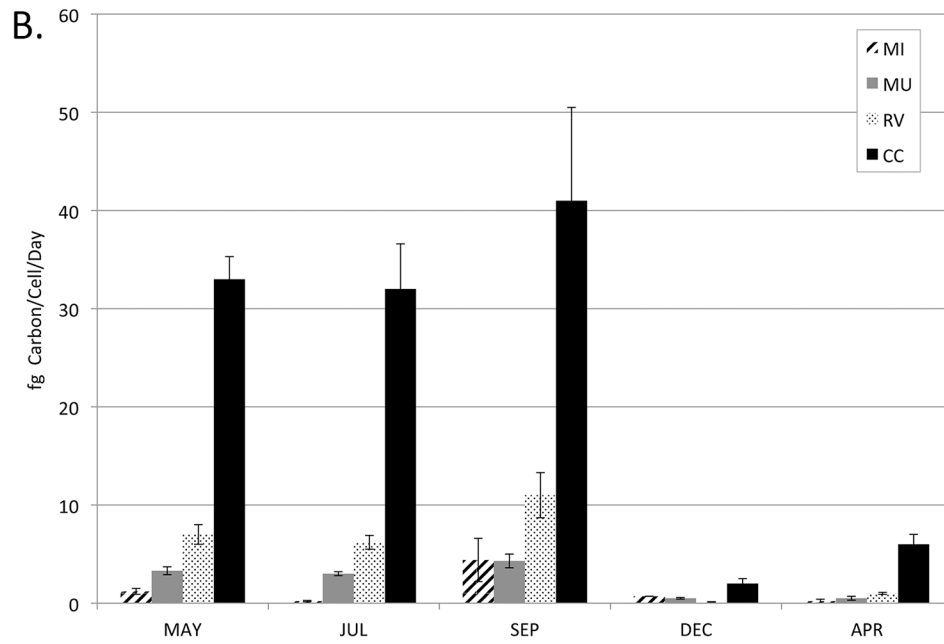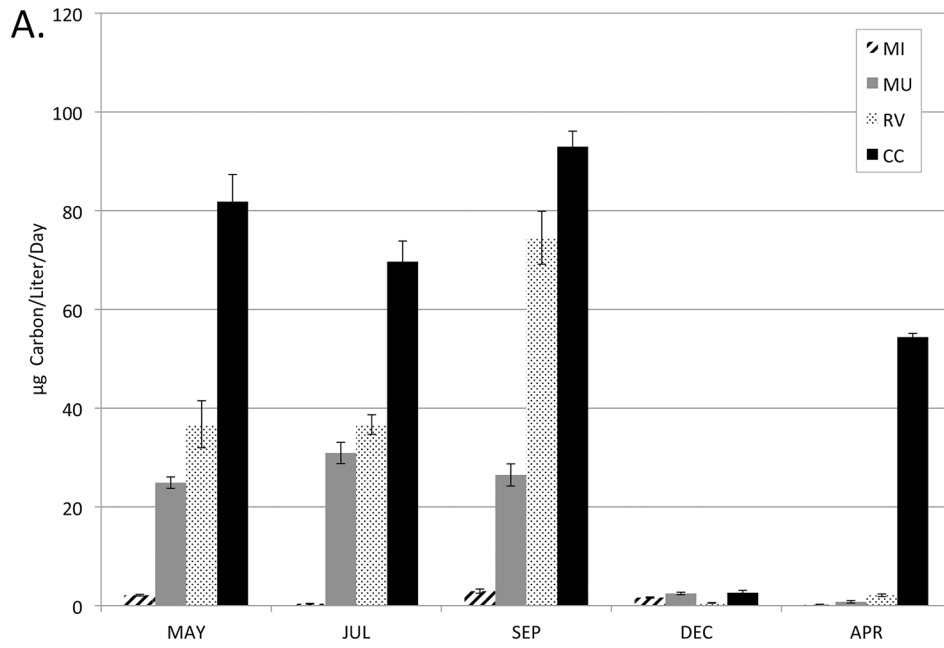
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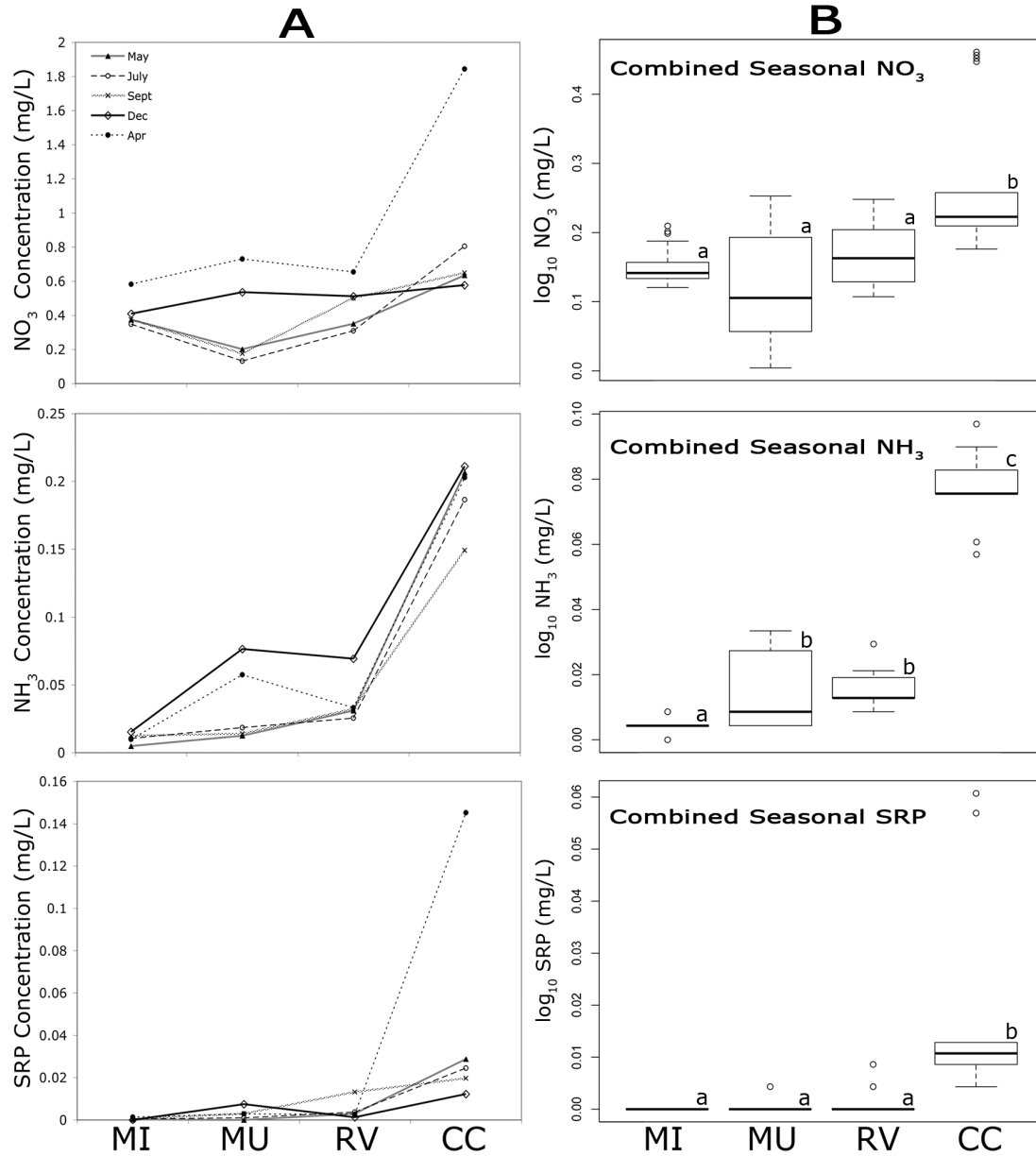
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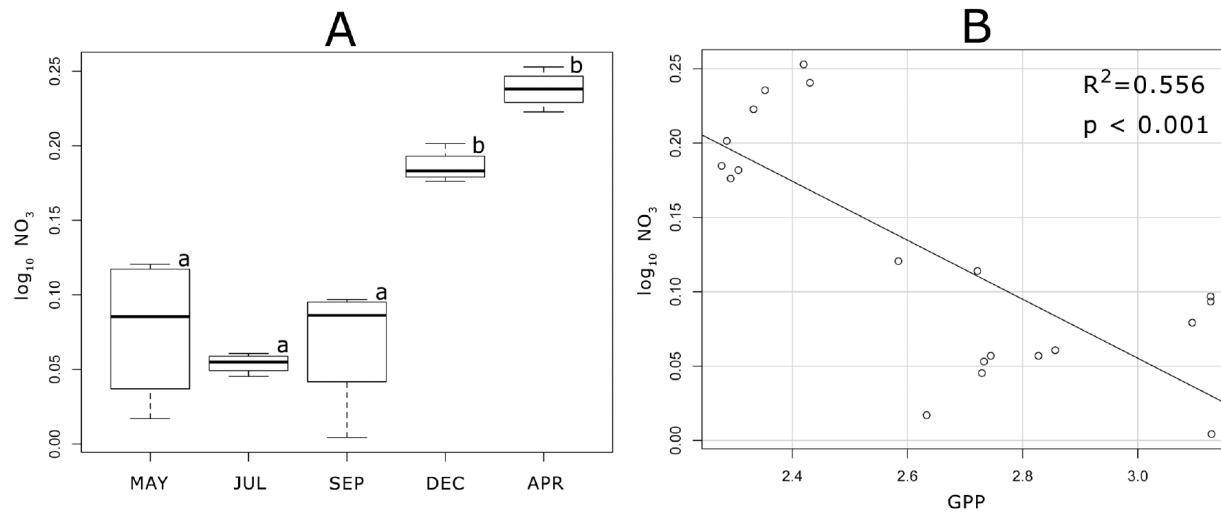
Supplementary Figure 3.

Supplementary Figure 4.

LITERATURE CITED

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