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RESEARCH ARTICLE

Seasonal changes in microbial community structure and activity imply winter production is linked to summer hypoxia in a large lake

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Abstract

Carbon and nutrient cycles in large temperate lakes such as Lake Erie are primarily driven by phototrophic and heterotrophic microorganisms, although our understanding of these is often constrained to late spring through summer due to logistical constraints. During periods of > 90% ice cover in February of 2008, 2009, and 2010, we collected samples from an icebreaker for an examination of bacterial production as well as microbial community structure. In comparison with summer months (August 2002 and 2010), we tested hypotheses concerning seasonal changes in microbial community diversity and production. Bacterial production estimates were *c.* 2 orders of magnitude higher (volume normalized) in summer relative to winter. Our observations further demonstrate that the microbial community, including single-celled phototrophs, varied in composition between August and February. Sediment traps deployed and collected over a 3 year period (2008–2011) confirmed that carbon export was ongoing and not limiting winter production. The results support the notion that active primary producers in winter months export carbon to the sediments that is not consumed until the warmer seasons. The establishment of this linkage is a critical observation in efforts to understand the extent and severity of annual summertime formations of a zone of regional hypoxia in Lake Erie.

Introduction

Microbial processes in freshwater systems have been studied for decades, and observations made in these environments serve as the foundation of many ecological tenets (Lindeman, 1942). Arguably, nowhere is the role of microorganisms more important than in the processing of dissolved organic matter (DOM), a byproduct from the metabolism of all living organisms. Organic matter generated by heterotrophic microbial activity is readily incorporated back into aquatic food webs *via* protozoan grazing, providing a vital linkage within aquatic carbon cycles (Azam *et al.*, 1983). To this end, understanding how microbial processes are constrained under natural conditions and how they may vary with alterations in temperature and light, like those caused by seasonal changes, is important for the better understanding of seasonal cycles and future climate scenarios.

Collectively, the Laurentian Great Lakes are arguably the single most valuable natural resource in North America, representing some 18% of the global potable water supply (Fuller *et al.*, 2002). Lake Erie, the 12th largest lake globally, has undergone intensive research due to its role as a fulcrum of regional socioeconomics. Data-rich surveys of this lake have been conducted for decades (Charlton & Milne, 2004) and are complemented by studies of microbial activity ranging from cell abundance and biomass estimates (Rao & Burnison, 1976) to estimates of productivity (Wilhelm & Smith, 2000; DeBruyn *et al.*, 2004), diversity (Wilhelm *et al.*, 2006; DeBruyn *et al.*, 2009; Steffen *et al.*, 2012; Bouzat *et al.*, 2013; Mou *et al.*, 2013), and losses by grazing and virus-mediated lysis (Dean *et al.*, 2008; Gobler

et al., 2008). What all of these (and many other) studies suffer from, however, is an oversampling of the late spring to late summer time seasons and a dearth of microbial information regarding late fall through winter conditions (Twiss *et al.*, 2012). In marine systems, disconnects between bacterial production and carbon input have been observed in polar regions and suggest that temperature can constrain microbial carbon processing (Pomeroy & Deibel, 1986; Kirchman *et al.*, 2009). Moreover, shifts from colder to warmer waters require day-to-week time periods for microbial communities to adapt, suggesting taxonomy and not physiology is important, although small changes in temperature over extended times can lead to significant enzymatic rate changes (Pomeroy & Wiebe, 2001).

Beginning with cruises of opportunity on the Canadian icebreaker *CCGS Griffon* in February 2007 and building on historical observations that date back over 70 years (Chandler, 1940, 1942; Chandler & Weeks, 1945), significant winter phytoplankton populations dominated by the actively growing diatom *Aulacoseira* spp. have been observed throughout ice-covered as well as ice-free locations (for a review of historical sample collections in Lake Erie see Twiss *et al.*, 2012). Estimates during this period suggest that carbon fixation and phytoplankton growth rates in the winter months may approach those measured during the summer season (Saxton *et al.*, 2012; Twiss *et al.*, 2012). Given concerns about the continued occurrence (and potential expansion) of the zone of hypolimnetic hypoxia in Lake Erie during summer months (Charlton & Milne, 2004; Wilhelm *et al.*, 2006; Conroy *et al.*, 2011; Zhou *et al.*, 2012), it is important to understand how autochthonous carbon in the lake is generated and processed throughout the year (and not just within the months that sampling is logistically simple) and whether winter primary production may influence summer microbial activity (Lashaway & Carrick, 2010).

To begin to understand the fate of carbon produced by the abundant diatoms found in Lake Erie during winter months, we collected samples to measure bacterial production and diversity during surveys in February 2008, 2009, and 2010. Our goal was to test the hypotheses that winter microbial communities were genetically similar but functionally repressed (i.e. grow more slowly) compared with summer microbial communities in the lake. Our results can be used to reject at least one of these hypotheses and provide valuable insight into how temporally disconnected processes (winter primary production and summer carbon consumption by heterotrophic microorganisms) may influence bottom water chemistry and dead zone expanse in this large freshwater system.

Methods

Station location and sample collection

Stations from Lake Erie (Fig. 1) were sampled on cruises of the *CCGS Griffon* during February 2008, February 2009, and February 2010. All stations were sampled during daylight hours. Moreover, due to weather events and the 'working' nature of the ice breaker, some stations sampled during this study had to be moved (*e.g.* in some cases, ice cover was too thick to break): only stations that are geographically proximal and of similar physical property (*e.g.* ice cover) are compared and grouped (e.g. stations 949 is in effectively the same location as 1053, Fig. 1). To access the water column, the ship was used upon arrival at each station to delicately open a small lead in the ice for sampling (Twiss *et al.*, 2012). Sampling at each station was delayed for up to 1 h to let materials released from the ice to settle. To draw comparisons to the summer microbial community, water samples for microbial diversity were also collected during a cruise of opportunity in August of 2010 aboard the *CCGS Limnos*.

Water samples were collected using a 10-L Niskin bottle from two depths at each station (*c.* 1 m below surface and

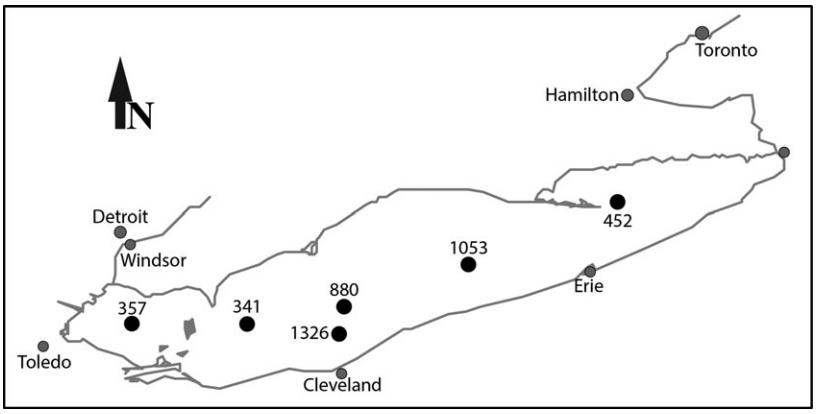

Fig. 1. Map of stations in Lake Erie.

c. 2 m above the sediment) on a metered winch. Water was processed on the ship within 1 h of sampling (see below).

Bacterial production rates

Bacterial production rates in surface waters were estimated using the ^{3}H-leucine incorporation microcentrifuge method as originally described by Kirchman (Kirchman, 2001) with adaptions for Lake Erie (DeBruyn *et al.*, 2004) for samples collected in 2008 and 2009. Water samples collected as above were held at *in situ* temperatures and processed within 1 h of sample collection. Triplicate 1.5-mL water samples plus trichloroacetic acid-killed blanks were incubated for 2 h with ^{3}H-(U)-leucine (40 nM final concentration, Perkin Elmer Life Sciences, Inc.) at *in situ* temperatures in dark containers.

For production experiments, cellular protein was isolated by microcentrifugation, and the amount of leucine incorporated was determined by liquid scintillation counting. A gross production estimate was then calculated for all samples. Where bacterial carbon production estimates are provided, the conversion factor (3.1 kg C per mol leucine) of Wetzel and Likens (2000) was employed.

Microbial community structure

Samples for molecular analysis were collected in 2010 via filtration through 0.22-μm nominal pore-size Sterivex filter units (Millipore Corp.), flash frozen in liquid N_2, and stored at −80 °C until extraction during 2010 cruises. DNA was extracted from all filters using the MoBio PowerWater DNA Isolation Kit (MoBio Laboratories Inc.) according to manufacturer's protocols. For amplification of bacterial 16S rRNA genes, bacteria-specific primers (Eurofins MWG Operon) targeting bases 338–926 (*E. coli* numbering) of the V3-V5 region of 16S rRNA gene were used to generate amplicons from DNA. We selected these primers based on a previous study (Wang & Qian, 2009). This primer set has successfully been used in other studies of 16S rRNA gene diversity coupled with 454 sequencing (Methe *et al.*, 2012). PCR reactions were performed using Invitrogen Platinum Taq (Life Technologies, Grand Island, NY) using the following protocol: 95 °C for 5 min, followed by 30 rounds of (95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s) and then a final extension step at 72 °C for 10 min. Product amplification was verified on a 1% agarose gel stained with ethidium bromide and viewed on a UV transilluminator. Individual samples were then processed to remove unincorporated primers and nucleotides using the Qiaquick PCR cleanup kit (Qiagen, Valencia, CA). Amplicon concentrations were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE).

Individual sample amplicons were barcoded (six additional PCR cycles: 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s) with primers that contained a unique 8-bp barcode attached to the 454 fusion primers. Our barcoding primers were designed for unidirectional sequencing on a 454 GSFLX sequencer (454 Life Sciences, Branford, CT). This strategy requires the use of the Lib-L kit (see Roche application brief 001-2009). We opted for unidirectional sequencing because our PCR product was larger than the average read length of the current 454 Titanium sequencing chemistry. This approach ensured sequences would overlap for the longest length possible. All barcoding reactions were prepared to have 0.5 ng μL^{-1} of amplicon DNA per reaction. After the barcoding reaction, we again verified our amplicons on an agarose gel and pooled all barcoded amplicons. Barcoded amplicons were processed to remove unincorporated primers and nucleotides using a single Qiagen Qiaquick column. Sequencing was completed at the *University of Tennessee/Oak Ridge National Laboratory Joint Institute of Biological Sciences* (www.ceb.utk.edu/dnasequence.html), and information deposited in the NCBI short-read archive (BioSample accession numbers SAMN02169190 – 02169204).

We used the MOTHUR software package (version 1.24.1; Schloss *et al.*, 2009) to screen sequences for sufficient length and quality. We processed our sequences similar to the Schloss SOP (http://www.mothur.org/wiki/Schloss_SOP) with some modifications of the shhh.flows command; namely, we changed the number of flows value in the shhh.flows command to 360–720 from 450 (Quince *et al.*, 2009). MOTHUR was also used to cluster sequences into operational taxonomic units (OTUs) and for phylogenetic classification (based on the RDPII database, Cole *et al.*, 2007). A 0.03 cutoff (97% identity) was chosen for OTU determination. We used MOTHUR to sort sequences into different groups based on the source of the DNA, incubation time and depth. Diversity metrics for the community, including Good's coverage (Good, 1953), species observed (sum of OTUs per sample), and inverse Simpson's (Simpson, 1949), were all determined using calculators in the MOTHUR software package.

The Primer-E software package (Version 6; Clarke & Gorley, 2006) was used to interrogate the relationships between OTUs across samples and to also look for any correlations between OTU presence/abundance and environmental parameters. The'.shared' file (a matrix file containing OTU abundances for each sample) created by MOTHUR was imported directly into the Primer-E software package. All OTUs were standardized to the total number of sequences per barcoded library (proportional abundances). The standardized abundances were square-root transformed to partially deemphasize more highly abundant OTUs. A Bray–Curtis similarity matrix was

constructed and used to perform nonmetric multidimensional scaling analysis (NMDS) for visualization of community structure relationships between the different samples. The same similarity matrix was employed for an ANOSIM analysis.

Organic carbon flux

Sediment traps were deployed at the central basin station 880 from October 2008 through July 2011 and at eastern basin station 452 from October 2008 through October 2011 (see Fig. 1). Passive sediment traps consisted of a 1-m-long piece of core tubing (6.7 cm ID) fitted with a plastic cup at the bottom. Sets of six traps were deployed for varying lengths of time (from 1 to 6 months) at 18 m depth at station 880 (Z_m 24 m) and at 50 m at station 452 (Z_m56 m). Deployment of the traps 6 m off the bottom at both sites made it unlikely for sediment that may have been resuspended by a storm event or lake turnover would be collected. Three cups from each set of the six traps were selected randomly for this study. All cups were treated independently except for one sample from station 452 (Aut-Spring, 2010–2011) which was combined into one sample due to a miscommunication: thus, while the data are an average of three independent samples, we are unable to report a range. Sediment from each cup was freeze-dried, ground, homogenized, and analyzed for total organic carbon using a high-temperature catalytic combustion method after removal of inorganic carbon by acid treatment at the National Laboratory for Environmental Testing in Burlington, ON (Environment-Canada, 1994) and results reported as means ± SD.

Results

Rates of bacterial carbon production in Lake Erie during February 2008 and February 2009 were low relative to estimates of summer rates that had previously been determined (Fig. 2). Bacterial productivity at 3 stations sampled in February 2008 was no different from production at the same stations in February 2009. Production rates also appear to be independent of phytoplankton biomass (Fig. 2, and phytoplankton biomass data from Twiss *et al.*, 2012).

From these observations, we generated the hypothesis that the microbial community composition in Lake Erie is constant but that activity is seasonally repressed by temperature. To address the composition component of this hypothesis, we completed a survey of the microbial community using pyrosequencing of 16S rRNA gene amplicons (Table 1). A total of 33 787 sequences remained after QA/QC of our data. These sequences averaged 195 bp and provided a range of observations (623–3474) across our stations. Estimates from the

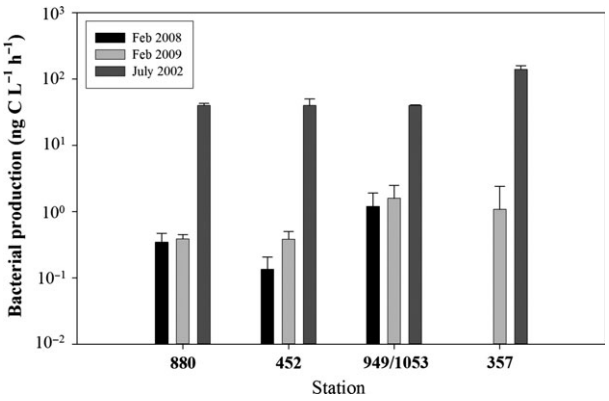

Fig. 2. Bacterial production rates from surface samples at stations in Lake Erie collected in 2008 and 2009. Data from summer samples at similar stations from DeBruyn *et al.* (2004) are provided for reference. During 2008, we were unable to sample station 357.

MOTHUR program suggested a return of *c.* 93–98% of the total community for this level of effort.

To make analyses more clear, we separated the putative heterotrophic (746 total) from putative phototrophic (82 total) OTUs and focused on the most dominant OTUs (Fig. 3a and b). Surveys of heterotrophic bacterial community members during winter and summer months demonstrated that different populations were abundant in different seasons. The most striking contrast between the two seasons was a predominance of OTUs phylogenetically aligned with Actinobacteria in summer samples (particularly a *Modestobacter* spp.) in contrast to groups aligned with the Flavobacteria in winter months (Fig. 3a). In examination of the entire population, only a few OTUs (phylogenetically classified as *Nitriliruptor* spp., the *Modestobacter* sp., and an *Ottowia* sp.) were consistently found in all libraries in all seasons. Other OTUs, including an *Illumatobacter* sp., a *Nubsella* sp., two *Flavobacterium* spp., and a *Roseomonas,* were almost consistently confined to a single season.

With regard to OTUs identified as putative phototrophs, sequences returned from winter months were dominated by a single OTU most closely identifying with the diatom species, *Aulacoseira spp.* (Fig. 3b; OTU1 –Bacillariophyta). In contrast, summer samples were consistently dominated by sequences from a cyanobacterial GP IIa sequence (i.e. either *Synechococcus/Cyanobium*) member of the community. Overall, there were a larger number of OTUs appearing only in summer relative to the winter stations (with only one OTU unique to the winter samples vs. 15 OTUs unique to the summer phototrophic community).

To specifically test our hypotheses concerning the similarity of summer and winter communities, nonmetric multidimensional scaling plots were generated for the independent heterotrophic and phototrophic community

Table 1. Sequencing results from Lake Erie winter and summer samples from 2010. A total of 22 011 sequences (746 OTUs) were assigned to heterotrophic bacteria and 11 696 sequences (82 OTUs) were assigned to phototrophs. Good's coverage, Species Observation Estimate and Inverse Simpson's were all estimated in MOTHUR (Schloss *et al.*, 2009)

Sample	Depth (m)	Total sequences	Identities		Good's Coverage Estimate (%)	Species Observed Estimate	Inverse Simpsons
			Heterotrophs	Phototrophs			
452							
Winter	1	1969	1446	523	97.6	117	19.198
Summer	1	3135	2725	410	96.8	217	8.769
Winter	52	2163	1537	626	96.9	160	19.885
Summer	52	623	603	20	93.4	96	23.249
1053							
Winter	1	2370	297	2073	98.1	86	1.636
Summer	1	3474	2986	488	97.9	172	7.108
Winter	18	1443	280	1163	95.7	98	2.088
Summer	20	2201	1883	318	94.6	236	23.974
880							
Winter	1	1513	873	640	96.6	121	6.577
Summer	1	2683	2306	377	97.0	168	4.677
Winter	18	2782	1166	1616	97.1	169	3.704
Summer	19	1750	1614	136	96.2	151	9.800
1326							
Winter	1	2523	2041	482	96.2	205	29.189
Summer	1	2005	1492	513	96.7	159	6.368
341							
Winter	1	3073	762	2311	97.7	151	4.038
Summer	1	Sequences not available					

members (Fig. 4a). An examination of the results for the heterotrophic OTU groupings clearly delineates between the summer and winter populations despite the depth of sampling. In winter months, depth-resolved samples were most similar to other samples from the same station (i.e. for stations 1953, 452, 84). In summer months, the effect of depth was enhanced, with the surface (1 m) samples collectively clustering progressively away from the deeper communities (but still more similar to these deep lake communities than the winter communities). ANOSIM analysis, used to consider the effects of three different variables (season, station, and depth), found that only season had a significant ($P > 0.001$) effect. A similar story with the OTU designations associated with phototrophs appears in Fig. 4b. Samples from winter and summer months cluster separately. In these samples, depth once again appears to play a more important role in the winter months relative to the summer phototrophic communities, but ANOSIM showed only season to be significant.

To rule out substrate limitation of bacterial production, organic carbon (C) fluxes were determined using passive sediment traps. Carbon export was highest during the late autumn through winter sampling periods and lowest during the spring and summer at both stations 880 (Fig. 5a) and 452 (Fig. 5b). At central basin station 880, the late autumn–winter C flux for 2009–2010 was 8.3 times higher than it was for the following 2010 spring–summer period and 2.7 times higher for equivalent periods in 2010–2011 (Fig. 5a). The C flux for station 452 in the eastern basin was overall 6–7 times lower than for the central basin station but the seasonal differences were comparable. The late autumn–winter C flux for 2009–2010 was 3.8 times higher than it was for the following 2010 spring–summer period and 6.0 times higher for the equivalent 2010–2011 periods (Fig. 5b).

The C : N atomic ratio can indicate the sources of the organic matter that make up the suspended particulates in lakewater. C : N ratio of freshwater planktonic sources ranges from 6 to 10, and terrestrial organic matter sources are > 20 (Meyers *et al.*, 1984; Meyers, 1994). The C : N of the suspended sediment from station 880 averaged 7.88 ± 1.02 (Range: 6.94–10.3). For the particulates collected from station 452, the mean C : N is 9.11 ± 1.65 (Range: 7.39–12.4). The values for both sites are consistent with those reported by Lean *et al.* (1983), Hecky *et al.* (1993), and Guildford *et al.* (2005) for Lake Erie particulate matter.

Discussion

Large lakes are dominant features of the landscape they occupy, profoundly shaping carbon and nutrient cycles

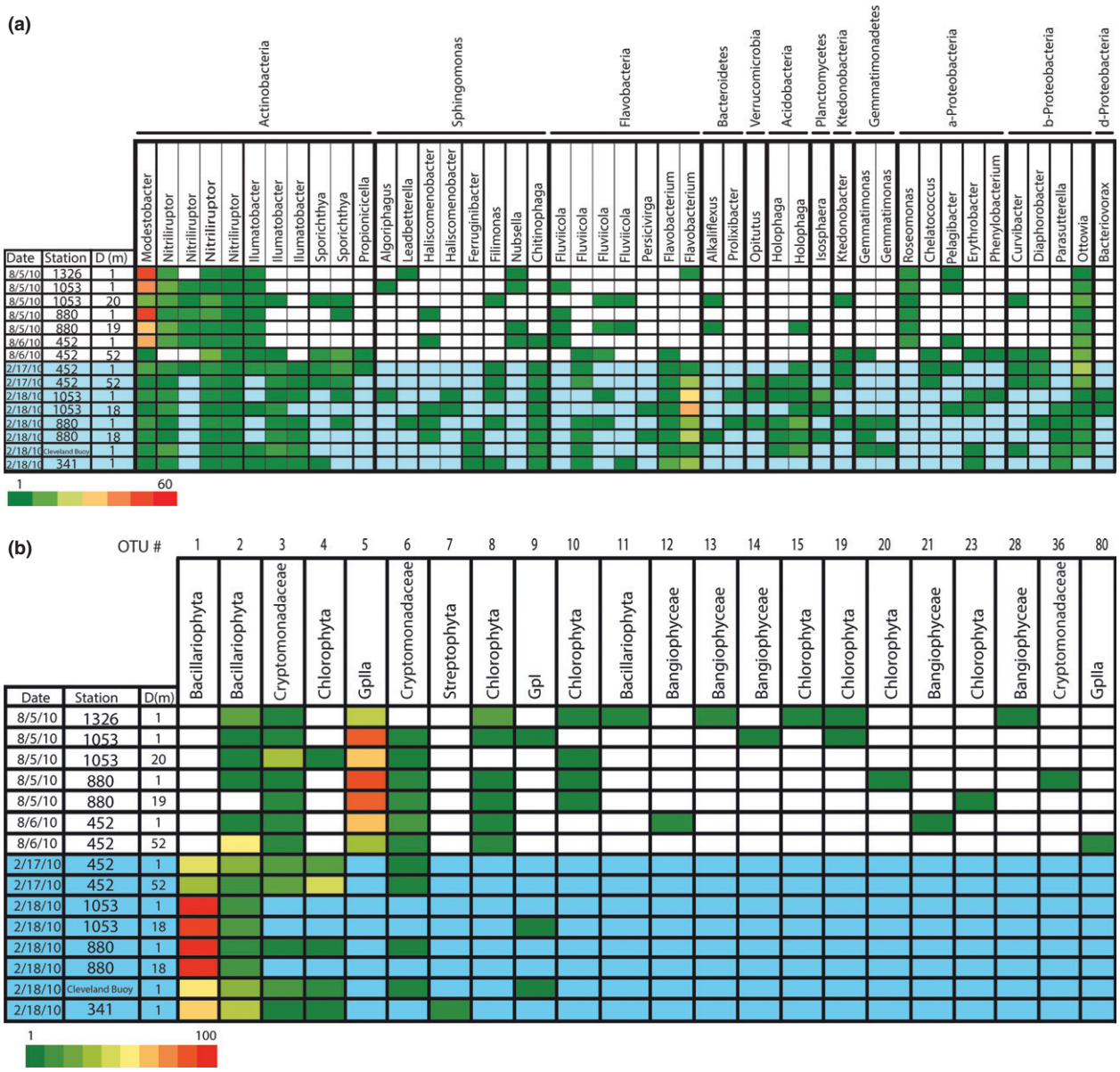

Fig. 3. (a) Phylogenetic affiliations of the top heterotrophic bacterial OTU groups from summer and winter libraries based on BLAST analyses. Due to space constraints, an OTU must have been present in at least two libraries at relative abundances > 1%. White = summer < 1% OTU abundance, Blue = winter < 1% OTU abundance. (b) Identities of top phototrophic (cyanobacteria and chloroplast) OTU groupings from summer and winter libraries. White = summer < 1% OTU abundance, Blue = winter < 1% OTU abundance.

within regional ecology. Lake Erie, the most anthropogenically affected of the Laurentian Great Lakes, provides both an important and unique opportunity to study the influences of seasonal cycles on such systems. Given that the biogeochemical cycles in this system are driven by microbial communities, we felt it prudent to determine how the communities changed between summer and winter seasons. Our results demonstrate that heterotrophic microbial carbon production in the system was decreased during winter months and that the dominant community is made up of significantly different members than seen in summer months. We couch these data within the context of the carbon export from abundant and prolific diatom blooms seen in winter months, and the formation of a large regional zone of hypoxia (aka the Lake Erie '*dead zone*') during late summer months.

In the current study, we observed that microbial community function (i.e. bacterial carbon production as estimated by ^{3}H-leucine incorporation) is impaired in the winter relative to summer. Researchers in marine systems

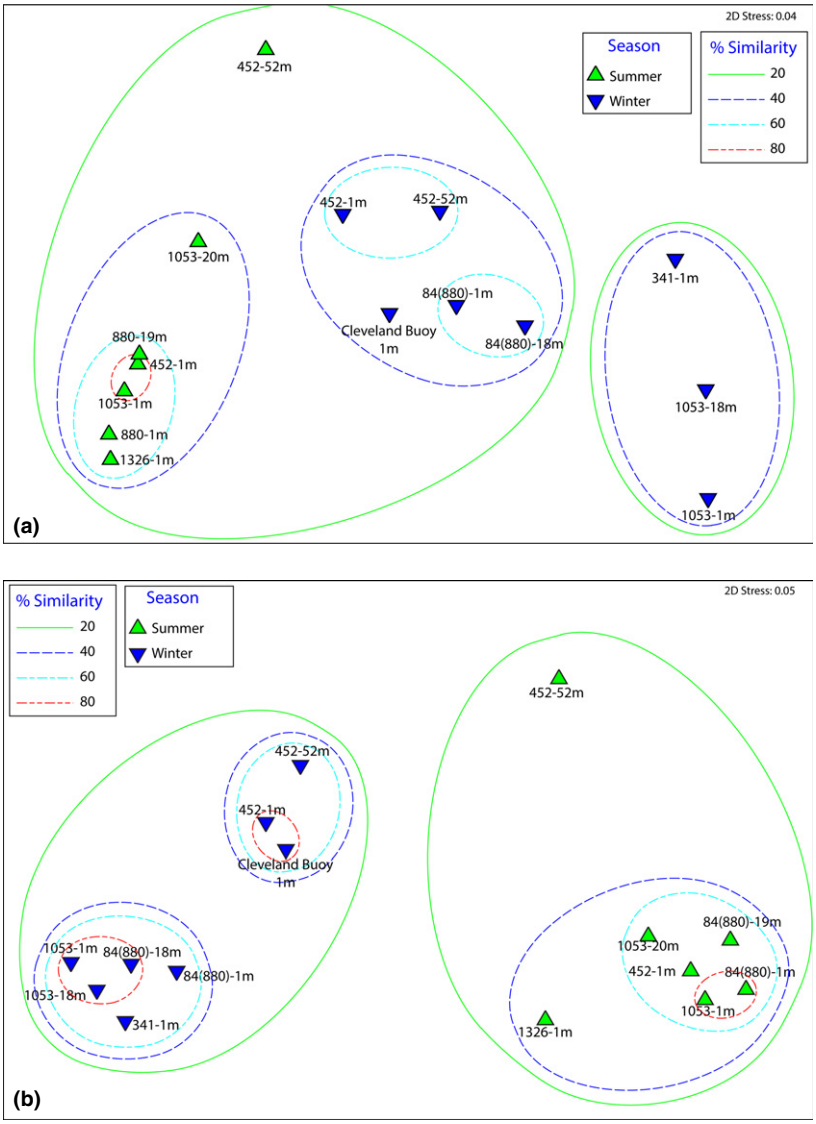

Fig. 4. Nonmetric multidimensional scaling plots of microbial community structure for summer and winter samples collected in 2010. (a) Sequences for heterotrophic bacteria. (b) Sequences for chloroplasts/cyanobacteria.

were able to demonstrate some time ago that bacterial production during colder winter months is disconnected from primary production and that temperature was the primary cause (Pomeroy & Deibel, 1986; Pomeroy & Wiebe, 2001). However, it is unclear whether such a relationship could be extrapolated to lake systems, where differences in stratification/annual mixing and greater seasonal temperature shifts might alter the community. In our observations, bacterial production rates in the winter months were *c.* 1% of rates observed in summer months. Given that rates of primary production as well as phytoplankton biomass (determined using chlorophyll *a* as a proxy) observed during this same period were similar to rates during the summer period (Twiss *et al.*, 2012) and that chlorophyll deposition to the sediments is generally within a factor of two between winter months and other seasons (Lashaway & Carrick, 2010), our observations concerning bacterial activity suggest that carbon produced during the winter may accumulate and be primed for mineralization when conditions are more favorable. This potentially contributes to the excess oxygen demand observed in summer months.

Phylogenetic reconstruction of microbial communities has been used for decades to infer potential functional capabilities of members of the microbial community (Woese & Fox, 1977; Woese, 2000), and the advent of modern molecular biological tools has led to a renaissance in efforts to link bacterial identity to community function (e.g. see Cole *et al.*, 2007; Schloss *et al.*, 2009). As part of the current study, we wanted to test the hypothesis that the phylogenetic structure of microbial communities in Lake Erie was similar between summer

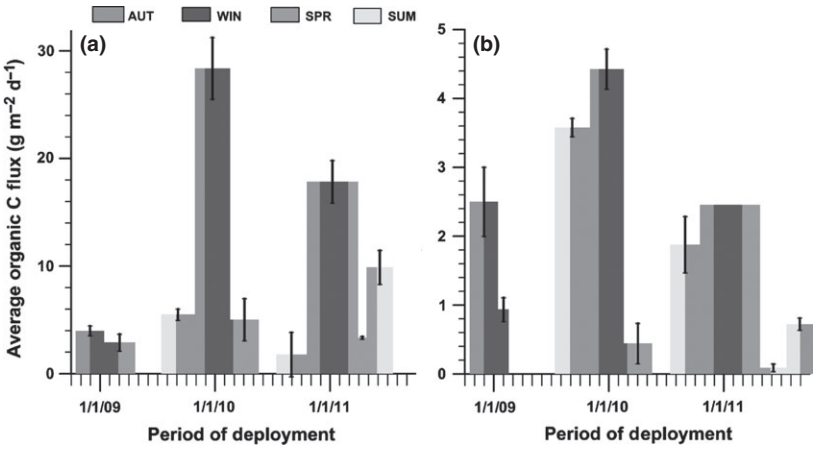

Fig. 5. Sediment trap data. (a) Carbon export at central basin station 880 estimated from passive sediment traps deployed during 2009, 2010, and 2011 at 18 m (6 m above the bottom). Seasons are delineated: Autumn (September – November), Winter (December – February), Spring (March – May), and Summer (June – August); error bars represent one standard deviation for average C flux and are within the histogram boundary when not shown. (b) Carbon export at eastern basin station 452 estimated from passive sediment traps deployed during 2009, 2010, and 2011 at 50 m (6 m above the bottom). Seasons and error bars (with exception noted in methods) same as a.

and winter months (with the subsequent subhypothesis that the activity of these communities is similar). Our observations, for both heterotrophic bacteria as well as for phototrophs we identified, rejected these hypotheses. Statistical comparisons of community structure as well as the assignment of OTUs to phylogenetic groups clearly demonstrate distinct communities were present in winter and summer samples we collected. Previous efforts from Lake Erie (Sharma *et al.*, 2009; Mou *et al.*, 2013) have suggested that Actinobacteria are important components of the microbial community. Other studies have also documented the seasonally dependent (spring–summer) abundance of Actinobacteria in freshwater lakes (e.g. Van der Gucht *et al.*, 2001; Allgaier *et al.*, 2007). In the current study, this remained the case, especially for the summer samples. OTUs classified (99% identity) as *Modestobacter* spp. were dominant across all stations in summer months. A survey of the literature suggests these bacteria are consistently identified in biological soil crusts (Mevs *et al.*, 2000; Reddy *et al.*, 2007), highlighting the potential linkage between the biological communities in Lake Erie and allochthonous inputs – especially in summer months, where significant run-off is an important driver of system ecology (Saxton *et al.*, 2011; Michalak *et al.*, 2013). Several other over-represented OTUs all identified most closely (95–100% sequence identity) with *Nitriliruptor* spp. Previous examinations of this genus have identified the ability to hydrolyze aliphatic nitriles to carboxylic acid and ammonia (Sorokin *et al.*, 2009), resulting in a potential nitrogenous growth source. In contrast, the winter community was heavily dominated by OTUs consistent with (100% identity) *Flavobacterium* spp. This genus contains a large number of species that have not only been observed previously in Lake Erie (Wilhelm *et al.*, 2006), but in lakes around the globe, especially during periods of high productivity (Eiler & Bertilsson, 2007). Our observations, however, are somewhat in contrast to those of Eiler & Bertilsson (2007), who sampled in the spring through autumn (May – October) months. Moreover, the optimum temperature range for most *Flavobacterium* spp. is 20–30 °C (Bernardet & Bowman, 2006), although at least one isolate (the fish pathogen *F. psychrophilum*) has been shown to have a series of genes up-regulated at colder temperatures (at 8 °C, Hesami *et al.*, 2011). That said, Lake Erie may host different summer populations than the Swedish lakes studied above, as a recent study of Sandusky Bay and the Western Basin of Lake Erie provided observations similar to ours (Mou *et al.*, 2013).

Equally striking within our datasets are the differences in 16S rRNA genes associated with phototrophs (i.e. plastids) within the sequence libraries. For summer samples, the phototrophs at all stations were dominated by sequences consistent with cyanobacteria (*Synechococcus* or *Cyanobium*). Several studies have reported on the abundant distribution of these cyanobacteria across the Great Lakes using both optical and molecular techniques (Fahnenstiel & Carrick, 1992; Carrick & Schelske, 1997; Wilhelm *et al.*, 2006; Ivanikova *et al.*, 2008). Consistent with molecular observations, Matteson *et al.* (2011) reported direct epifluorescence enumerations for Lake Erie cyanobacteria that were *c.* two orders of magnitude higher for summer (10^5–10^6 mL^{-1}) relative to winter months (2.6–3.7×10^3 cells mL^{-1}). In the winter months, concurrent microscopic observations (Saxton *et al.*, 2012; Twiss *et al.*, 2012; D'souza *et al.*, 2013) have

confirmed the abundant distribution of diatoms, particularly *Aulacoseira islandica*. Currently, no molecular data on this diatom are available, although we anticipate that it is closely related to, and therefore consistent with, the OTU we identified for the dominant winter phototroph (*Aulacoseria granulata*, 100% identity to sequence).

The overarching questions that remain unanswered by this study concern the fate of carbon produced by phytoplankton during winter months: where does it go, when is it consumed and by whom? Efforts to examine carbon export during winter months have met with the difficulties of working with sediment traps in systems covered in ice (making retrieval difficult) and challenged by storms (resulting in sediment resuspension in this relatively shallow large lake). In the current study, our observations (C : N ratios from sediment traps) at station 880 demonstrate export consistent with a planktonic source of materials. And while the higher C : N ratio at station 452 could indicate that a portion of the materials in the trap has a terrestrial source, the results are consistent with materials derived from plankton grown under N-deficient conditions (Hecky *et al.*, 1993; Guildford *et al.*, 2005), supporting our previous rate observations concerning active photosynthetic carbon production during winter months (Saxton *et al.*, 2012; Twiss *et al.*, 2012).

From our first winter observations (2007), we have attempted to address questions concerning the importance of winter production and summer hypoxia. The current study moves us one step closer: we have demonstrated that bacterial carbon cycling is markedly decreased in winter months which implies that carbon production during this period must be consumed at a later point. Furthermore, the heterotrophic microbial community responsible for consumption is distinctly different than the winter assemblage. Finally, we have further demonstrated that there is significant export of organic carbon in the water column during winter months, and the chemical nature (C : N ratio) of this material is consistent with phytoplankton biomass. The potential for this seasonal cycle cannot be ignored nor can potential future effects of seasonal variations in temperature (and ice cover) that may exacerbate this carbon loading. Overall, these observations establish a foundational baseline for future research efforts to better develop this linkage. They further point to the need for seasonal sampling in systems like the Laurentian Great Lakes, as obviously important microbial and geochemical processes are occurring that have previously been missed.

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