MICROBIOLOGY OF AQUATIC SYSTEMS

Variations in Bacterial Community in a Temperate Lake Associated with an Agricultural Watershed

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Abstract Terrestrially derived carbon and nutrients are washed into lakes, providing nutritional drivers for both microbial heterotrophy and phototrophy. Changes in the quantity and diversity of carbon and nutrients exported from watersheds in response to alterations in long-term land use have led to a need for evaluation of the linkage between watershed-exported carbon and nutrients and bacterial community structure in watershed associated lakes. To learn more about these interactions, we investigated Muskrat Lake in Michigan, which has a well-defined moderately sized watershed dominated by agriculture. We measured the water chemistry, characterized the dissolved organic carbon, and determined the structure of the bacterial communities at the inlet and center of this lake (five depths per site) over the summer and fall of 2008. The lake had temporal and rain event-based fluctuations in water chemistry, as well as temporal and rain event-dependent shifts in bacterial communities as measured by terminal restriction fragment length polymorphism. Agricultural watershed inputs were observed in the lake during and after rain events. Terminal restriction fragment length polymorphism and 454 pyrosequencing of the bacterial communities indicated that there were differences over time and that the dominant phylotypes shifted between summer and late fall. Some populations (e.g., *Polynucleobacter* and *Mycobacterium*) increased during fall, while others (e.g., *Gemmatimonas*) diminished. Redundancy and partitioning analyses showed that water chemistry is highly correlated with variations in the bacterial community of the lake, which explained 34 % of the variations in the bacterial community. Dissolved organic carbon had the greatest effects on variations in the Muskrat Lake bacterial community (2 %). The results of this study provide information that will enable a better understanding of the interaction between the bacterial community of lakes and changes in chemical properties as a result of nutrient importation from the surrounding watershed.

Keywords Muskrat Lake · Agricultural watershed · Bacterial community · Water chemistry · Dissolved organic carbon

Introduction

A fundamental concern regarding freshwater aquatic microbial ecology is the bacterial community structure of lakes and the relationships between the bacterial community and allochthonous and autochthonous nutrient inputs. Proposed [1–6] potential drivers of variations in the bacteria community include pH [7], temperate [8–10], water flow [11], system photosynthetic productivity [2, 6], and nutrients including dissolved organic carbon (DOC) [12–16]. Among these, DOC is believed to be a crucial factor that controls the bacterial community structure and temporal shifts. Some investigations have shown that autochthonous and allochthonous organic carbons are utilized by phylogenetically different bacterioplankton and thus impact the structure and function

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of lake bacterial communities in different ways [17, 18]. The linkage between aquatic bacterial communities and its cognate watershed providing allochthonous nutrients and carbon is considerably more complex because of the presence of additional variables including land cover, parent soil chemistry and soil activities, and hydrologic flow.

Human activity in watersheds, such as agriculture, can impact wetlands and freshwater systems by altering the flow of allochthonous materials [19, 20]. Such differences in the allochthonous fraction (e.g., carbon, nitrogen, and phosphorus) accompanied by a variety of other factors can lead to spatially variable water chemistry in the lake that receives the inputs. For example, the N/P ratio was highest (>50 as atoms) in lakes with >90 % of their watersheds in areas impacted by row-crop agriculture [19]. A growing body of literature has identified the allochthonous fraction as comprising a considerable proportion of total nutrients and carbon in many small- to medium-sized inland lakes [21–24]. Indeed, depending on the structure of the lake and the surrounding watershed, up to 90 % of the carbon budget for pelagic bacteria may be derived from external sources [22, 25].

Terrestrially derived carbon and nutrients in aquatic ecosystems can drive the heterotrophy of the system [17]. Kritzberg et al. examined carbon sources and bacterial carbon utilization strategy in two watershed (wetland and forest) associated lakes and found that lake bacteria utilized 35–70 % of the allochthonous carbon [17]. Lennon and Pfaff used laboratory batch culture experiments to explore the relationship between watershed inputs of dissolved organic material (DOM) and freshwater bacterioplankton and found that exported DOM influences the productivity of freshwater bacterioplankton [26].

As the quantity and diversity of exported carbon and nutrients from watersheds change as a long-term consequence of land use change, lakes that receive these nutrients respond accordingly. Some studies have investigated the impact of watershed inputs on the water chemistry [19, 20] and bacterial carbon utilization strategy [17, 26] in lakes that receive the inputs. However, the linkages between water chemistry and bacterial community of watershed-associated lakes have not been well characterized. In this study, we sampled a temperate lake with a moderate watershed size that was dominated by agricultural runoff and analyzed the physiochemical parameters of the lake through summer into late fall, including during a substantial rain event, to investigate how the water chemistry impacts the bacterial community. Water chemistry and the bacterial community structure of the study lake showed temporal variations. Watershed inputs on and after rain events were observed as increasing concentration environmental factors such as DOC. Multivariate analysis indicated that water chemistry is the main driver shaping bacterial community structure and that DOC had the greatest impact on bacterial community structure variations.

Materials and Methods

Lake Description

Muskrat Lake (latitude +42.914101, longitude −84.590701) is in the Lower Peninsula of the State of Michigan and described elsewhere [27]. Briefly, the lake area is 0.18 km^2 and a maximum depth of 4.9 m. The watershed area is 18 km^2, resulting in a lake area to watershed area ratio of 0.01. The watershed is characterized as mainly agricultural 74 % and forest 15 % (Fig. 1). Muskrat Lake is a flow-through lake with two parallel stream inputs and one stream output. Stream input is 10 m away from the inlet sampling point and 50 m away from the center sampling point. Stream output is 40 m away from the center sampling point. Because the averaged total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) during sampling were over 0.52 mg/L and 54.7 µg/L, the lake is considered eutrophic.

Sampling

Vertical profiles of the lake were taken on July 31, August 30, September 17, September 30, and November 3, 2008. Samples were taken from the inlet and center of the lake. The September 17 sampling lake was taken after a relatively large rain event that occurred on September 13 and 14. Total rain fall amounts ranged from 134 to 452 mm over the area with maximum wind speeds of 24 km/h [28]. All rain events during the sampling period (from June 1 to November 31) are presented in supplemental Fig. S1.
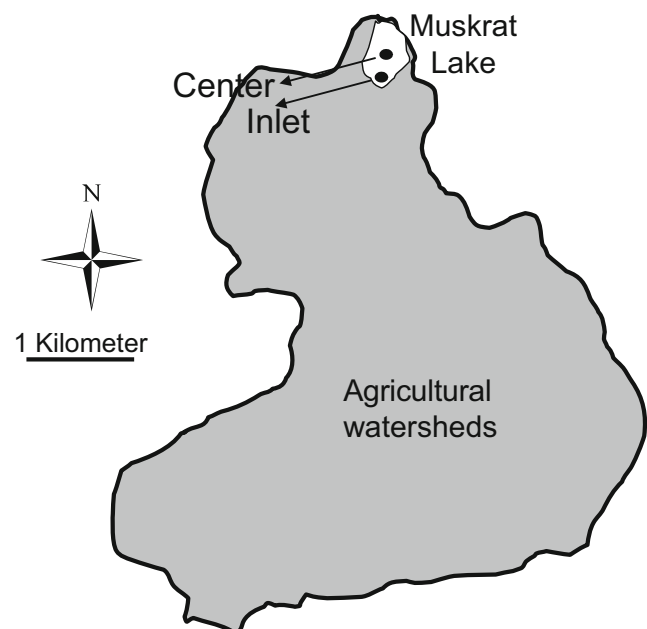

Fig. 1 Geographic information describing Muskrat Lake and its related watersheds during 2008

Water sampling protocol and water field filtration were documented in another study [27]. Briefly, water samples were collected using a 4.2-L Vertical Beta Water Sampler™ (Wildlife Supply Company, USA) at depths of 0.0, 0.5, 1.0, 1.5, and 2.0 m. Filtration was done on the field for each sample, and all sampling containers were ultra-cleaned. One liter of lake water was filtered through 0.45-μm Thermopor membrane with 50 mm diameter (AquaPrep, Pall Corporation, USA) into 1-L Nalgene HDPE sample bottles (Fisher Scientific, USA) for DOC analysis and % aromaticity determination. Of water samples for chlorophyll a (Chl-a), 0.6 L was filtered through 0.6-μm glass/fiber filters with 47 mm diameter (Sterili Tech, USA), and the filter and retentate were stored in a 250-mL dark HDPE bottle (Fisher Scientific). Of water, 250 mL was filtered through 0.45-μm ultra-clean driven filter units (Millipore Corporation) using a connected ultra-clean syringe into two 60-mL Nalgene HDPE sample bottles for anion analyses. Ultra-pure nitric acid (Fisher Scientific, USA) was added to lower the pH to <2. The remaining filtrate was kept for TDN and TDP analyses. Sixty-milliliter water was filtered through a sterilized 0.22-μm hydrophilic filter with 50 mm diameter (Millipore, USA), and the filters were stored in a 50-mL sterilized tube for bacterial community analysis.

The filtrated samples were stored on ice during transport back to the laboratory and then at −20 °C for bacterial communities and Chl-a samples and at 4 °C for water chemistry samples.

Chemical Analysis

Water chemical analysis was conducted within 1 week of sample collection. Water chemistry (DOC, TDN, TDP, chloride, nitrate, sulfate, calcium, magnesium, sodium, and potassium) was determined by standard procedure [29]. Percent aromaticity was evaluated by a Shimadzu UV-160 spectrophotometer at 280 nm and calculated as % aromaticity = 0.05 ε + 6.74 (ε is the molar absorptivity) [30].

DNA Extraction and Amplification

DNA was extracted from one half of a filter with the Ultraclean Soil DNA Kit (Mobio Laboratories Inc, USA) according to the vendor's protocol except that agitation with a Biospec Mini beadbeater for 1.5 min was used instead of vortexing. The eluate was quantified with a nanodrop ND-1000 spectrophotometer (Nanodrop, USA). DNA was amplified using 16S ribosomal ribonucleic acid (rRNA) general primers 63F (5′-CAG GCC TAA CAC ATG CAA GTG-3′) and 1389R (5′-GGG CGG WGT GTA CAA GGC-3′) [31] under the following conditions: initial denaturation at 90 °C for 1 min, followed by 25 cycles (45 s at 90 °C, 1 min 40 s at 56 °C, and 1 min at 72 °C), and a final 5-min extension at 72 °C.

T-RFLP Analysis

T-RFLP [27] analysis was used to assess the pattern of the bacterial communities over time. The 63F primer was labeled with fluorescent dye-6-FAM to enable fluorescence detection of terminal restriction fragments. Two replicate PCRs of 100 μL (each) were amplified for each sample with the same conditions described above. Combined PCR products were purified by QIAquick PCR Purification Kit and quantified by nanodrop. DNA products (300 ng) were digested with either Hha I or Msp I at 37 °C for 3 h, precipitated with EtOH, and stored at −20 °C overnight. Pelleted DNA was resuspended in 10-μL DNA/RNAase-free water and analyzed by ABI Prism 3100 genetic analyzer using size standards from 50 to 1000 bases.

Pyrosequencing Analysis

The bacterial community structure was assessed 454 pyrosequencing. Prior to emulsion PCR and sequencing, bacterial 16S V4 regions (200 bp) were amplified with high-fidelity Taq polymerase (Invitrogen, USA) under the following conditions: initial denaturization at 95 °C for 3 min, followed by 30 cycles (45 s at 95 °C, 45 s at 57 °C, and 1 min at 72 °C), and a final 4 min extension at 72 °C. Fusion forward primer (GCCTCCCTCGCGCCATCAG) combined with tags and 16S rRNA primers AYTGGGYDTAAAGNG was used as the A primer. Fusion reverse primer (GCCTTG CCAGCCCGCTCAG) combined four different 16S rRNA primers: (1) TACNVGGGTATCTAATCC, (2) TACCRGG GTHTCTAATCC, (3) TACCAGAGTATCTAATTC, and (4) CTACDSRGGTMTCTAATC, with 12:6:1:2 ratios were used as working B primer (see http://pyro.cme.msu.edu/pyro/help.jsp). PCR products were checked with electrophoresis on a 2 % agarose gel in TBE and stained with EthBr. The desired DNA fragment (200 bp) was excised by using a sterile razor blade and stored at −20 °C until extraction. The excised PCR product was extracted using QiaQuick Gel Extraction Kit, and the eluant was purified with a QiaQuick PCR Purification Kit. The final concentration of purified PCR product was quantitatively determined by using a Qubit coupled with dsDNA HS Assay Kit (Invitrogen, USA). Samples with different Fusion Tag primers were mixed together (0.5 ng/μL per sample in 10 μL total volume) and submitted to the Research Technologies Support Facility at MSU for high throughput sequencing (454-FLX platform).

The 454 raw sequences were screened for chimeras and trimmed via the infernal aligner from the Ribosomal Database Project (RDP) pyrosequencing pipeline (http://pyro.cme.msu.edu/) (release 10) [32], and sequences length shorter than 200 nucleotides and contained any ambiguous base calls (Ns) were removed. Classifier, rarefaction, Shannon and Chao I, and libcompare function of RDP projects were used for phylogenetic identification and comparison of sequence libraries.

The raw pyrosequencing data were deposited in NCBI Sequence Read Archive (SRA; http://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi) under accession no. SRX1249047.

Data Analysis

Terminal restriction fragment sizes between 50 and 900 bp were determined using GeneScan analytical software 3.0 (Applied Biosystems, USA), and the peak area profiles were binned with T-Align with 0.5 cutoff [33]. To further eliminate the bias, a peak accounting for >0.2 % of the total peak area of fragment was retained. The T-Align exported average fluorescence intensity comparison files were used for downstream analysis.

The exported T-RFLP data of Hha I was ordered by principal component analysis (PCA) based on Euclidean distance matrix with Hellinger transformation [34]. The bacterial community structure difference between location within lake (inlet versus center) and between sampling time was also tested by analysis of similarity (ANOSIM) at PAST [35]. To determine the relationship between environmental variables and bacterial community, detrended correspondence analysis (DCA) [36] was employed first to determine which model (linear or unimodal) best fit the bacterial community dataset [37]. This was followed by redundancy analysis (RDA) to test which environmental variables best explained the variation in bacterial community structure. Significant variables for the analysis were preselected by forward model selection, and the significance of RDA model and of the selected variables was tested by 999 Monte Carlo permutations. The respective effects of variables (groups or single) on the variation in bacterial community structure were further examined by partitioning analysis [38].

The environmental variables (Table S1) were log10-tranformed (except pH, depth, temperate, turbidity, sampling time) to normalize their distribution before analysis. Geographic data was firstly transformed from decimal degree (WGS84) to UTM-WGS84 (WGS84) at PAST (functional category, geome; subcategory, coordination transformation) and then log10-tranformed. Sampling time was treated as descriptive variable "time" and recorded the sampling times as "0.0, 1.0, 1.5, 2.0, and 3.0" to represent the time line with equal intervals. All the analysis was conducted at program R with the Vegan package except the significant variables selection by forward model selection at CANOCO [39] and ANOSIM at PAST.

Results

Temporal Variation in Water Profiles

Muskrat Lake, which is located in a watershed with moderate agricultural activity, was investigated during summer and fall. During this period, there was a rain event on September 13–14 that resulted in over 134 mm of precipitation. The lake was sampled 3 days after this rain event. Sampling was conducted at the indicated sites at the inlet and center of the lake. The trends and structure of detected environmental parameters were similar at both sampling sites as illustrated in the upper panels (center) and lower panels (inlet) of Fig. 2.

DOC and % aromaticity were relatively constant over depth, whereas chlorophyll a, TDN, TDP, and nitrate varied. Monthly variations in Chl-a, DOC, aromaticity, TDN, TDP, and nitrate were observed (Fig. 2). Chl-a readings were lowest 24 h after the rain event, then peaked 2 weeks later (Fig. 2a, b). DOC increased immediately after the rain event (September 17), then further increased to the maximum value on September 30 (Fig. 2c, d). The % aromaticity was lowest on July 31 and August 30 and highest 72 h after the rain event (Fig. 2e, f). TDN was highest 2 weeks after the rain event (Fig. 2g, h). In contrast, TDP peaked in late August and 72 h after the rain event but was relatively constant at other times (Fig. 2i, j). Nitrate also peaked 72 h after the rain event (Fig. 2k, l). Only minor variations were seen over the sampling depths.

Agricultural watershed inputs were observed in the lake during and after rain events, as evidenced by immediate increases in alkalinity, % aromaticity, TDN, TDP, nitrate, DOC, and Chl-a. Alkalinity increased from 119.9 mg/L (average) to 213.0 mg/L (average). The % aromaticity increased from 17.3 to 28.0, while NO^{-3} increased from 1.0 to 15.3 mg/L. TDN increased from 1.0 to 2.1 mg/L, and TDP increased from 127.8 to 154.2 μg/L. Specifically, the DOC concentration of the lake increased from 9.8 to 11.8 mg/L and the Chl-a increased from 9.8 to 55.1 μg/L.

Other physicochemical parameters of the lake are presented in supplemental Figs. S2 and S3 and Tables S1 and S2. Temporal variations in temperature (Fig. S2a, b), dissolved oxygen (DO) (Fig. S2e, f), and alkalinity (Table S1) were detected in the lake. The highest temperature was observed in July and the lowest in November. DO values in the upper portion of the water column clustered around 10 mg/L and decreased with depth during July and August. Peak alkalinity was observed in November, while the lowest value was observed in July and it appeared to be diluted by the rain event. In addition, turbidity (Fig. S2g, h) was highest shortly after the rain event and lowest in July. Conductivity was variable (Fig. SS2i, j), with peak values in July and minimum values shortly after the rain event, presumably due to dilution. pH values were constant over depth but varied from 7.3 to 8.6 temporal (Fig. S2c and Fig. 2). Concentrations of Cl^{-1}, SO_4^{-2}, Ca^{+2}, Mg^{+2}, and Na^+ (Fig. S3) were lowest shortly after the rain event, then increased. Furthermore, concentrations of SO_4^{-2} and Ca^{+2} reached their greatest value in November. Conversely, concentrations of K^+ increased shortly after the rain event and remained high through the end of September, then decreased in November, although they remained higher than the values observed in July and August.
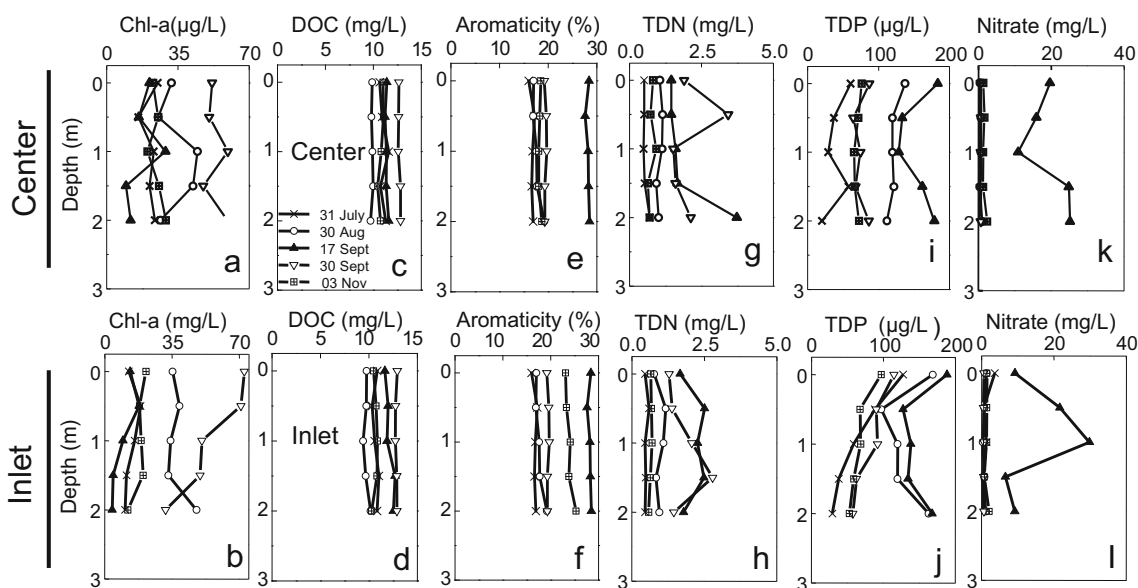
Fig. 2 Vertical profiles of chlorophyll a (Chl-a), dissolved organic carbon (DOC), aromaticity %, total dissolve nitrogen (TDN), total dissolved phosphorus (TDP), and nitrate for the center of Muskrat Lake (**a**, **c**, **e**, **g**, **i**, **k**, respectively) and inlet of Muskrat Lake (**b**, **d**, **f**, **h**, **j**, **l**, respectively)

Variations in Bacterial Community Structure

Figure 3 presents the redundancy analysis of T-RFLP profiles from two sites at five depths from all five sampling times. Profiles clustered according to sample time and the groups were significantly separated (ANOSIM $R = 0.9891$, $p < 0.0001$). The bacterial communities at the inlet and center of Muskrat Lake were similar to each other in July, immediately after the rain event, and in late September (ANOSIM $R_{July} = 0.1$, $p = 0.1522$; $R_{Rain} = 0.1385$, $p = 0.2905$; $R_{September} = 0.072$, $p = 0.2507$), but significantly different in August and November (ANOSIM $R_{August} = 0.325$, $p = 0.044$; $R_{November} = 0.692$, $p = 0.015$). The difference or similarity

Fig. 3 Redundancy analysis (RDA) of sampling locations at Muskrat Lake based on structure of microbial communities determined by T-RFLP analysis. *White circle* denotes the inlet of Muskrat Lake; *black circle* denotes the center of Muskrat Lake. The variables significantly influencing microbial community structure selected by forward selection procedure were fitted to the ordinations

between monthly samples is in agreement with the Bray-Curtis dissimilarity scores (average and range; Table S3).

Correlation Between Environmental Variables and Bacterial Community

All environmental variables were correlated and the results are provided in Table S2. Briefly, DOC was positively correlated with TDN ($r = 0.60$) and pH ($r = 0.61$), while pH was positively correlated with TDN ($r = 0.60$) and negatively correlated with Cl^- ($r = -0.59$), SO_4^{2-} ($r = -0.65$), Mg^{2+} ($r = -0.58$), and K^+ ($r = -0.61$).

Variations in bacterial community structure of Muskrat Lake as extracted by PCA consisted of two major axes explaining 59.4 and 21.1 % of the total variation in T-RFLP (data not shown). The gradient observed upon DCA was 1.1, which was shorter than 3.0. Therefore, RDA was performed using forward model selection to further determine the environmental variables associated with changes in the bacterial community structure. Variations in the bacterial community structure of Muskrat Lake could be explained by two main axes accounting for 67.7 and 14.7 % of the total variation (Fig. 3), with selected environmental variables explaining 88 % of the variation (Table 1). Temperature, sampling time, DO, and sulfate explained the first RDA axis and DOC, pH, conductivity, and alkalinity explained the second RDA axis (Fig. 3).

Variation partitioning analysis was used to determine the effects of each factor on variations in community patterns. The explanatory variables were divided into water chemistry (sulfate, pH, DO, conductivity, alkalinity, and DOC), sampling time, and temperature. All pure and co-varying factors explained 84 % of the variations in Muskrat Lake (Fig. 4).
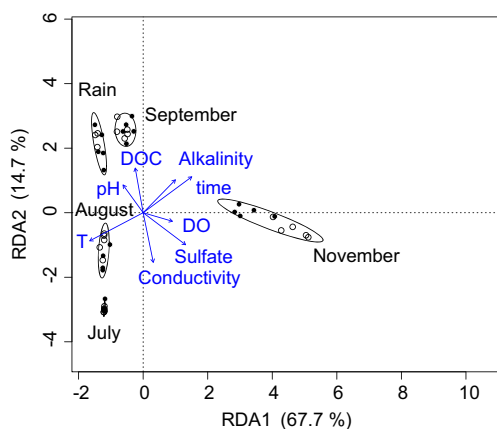
Table 1 Conditional effects of forward model selected environmental variables determined by redundancy analysis (RDA)

Environmental variable	Lambda-A	F ratio	Environmental variable	Lambda-A	F ratio
Sulfate	0.25	32.97***	Chloride	0.01	1.32[NS]
Time	0.41	31.04***	DO	0.01	1.32[NS]
DOC	0.02	3.79***	Na	0	1.2[NS]
pH	0.01	3.59***	Depth	0	1.14[NS]
Alkalinity	0.01	3.16**	TDP	0.01	0.92[NS]
Conductivity	0.11	19.68***	Mg	0	0.8[NS]
Temperate	0.05	11.4***	K	0.01	0.85[NS]
Nitrate	0	1.86*	TDN	0	0.84[NS]
Turbidity	0.01	1.42[NS]	Cu	0	0.81[NS]
Chl a	0.01	1.32[NS]	Ca	0	0.66[NS]

Lambda-A represents the variance explained by each variable in the model

Significance is indicated by ***$p \leq 0.001$, **$p \leq 0.01$, *$p < 0.05$, and NS (not significant), as determined by 999 Monte Carlo permutations under full models

Water chemistry, sampling time (all season), and temperate explained 34 % ($p < 0.001$), 1 % ($p < 0.001$), and 1 % ($p < 0.001$) of the variation in the Muskrat Lake community as pure factors. In addition, 14 % of the variation in the Muskrat Lake bacterial community was explained by the combined effects of water chemistry, sampling time, and temperature. DOC and pH had the greatest effects on variations in the Muskrat Lake bacterial community (2 % for both) as individual factors (Table 2).

Bacterial Community Composition

The bacterial diversities observed in the water samples in July and November (1 m depth) in the center of Muskrat Lake were determined by 16S rRNA-based pyrosequencing with a 3 % cutoff used to define an operational taxonomic unit (OTU). Based on the rarefaction curves (Fig. 5a insert), the coverage

Fig. 4 Partitioning of Muskrat Lake microbial community variations into the significant variables alone or in combination. Significant contribution of pure fractions to microbial community variation are indicated by ***$p \leq 0.001$ and *$p < 0.05$, as determined by 999 Monte Carlo permutations

was incomplete. A total of 3180 and 3160 sequences were obtained for July and November, respectively, and 385 and 346 OTUs were assigned. The Chao I estimates and Shannon-Weiner diversity indices for July and November were 626.6 and 1061.0 and 4.54 and 4.33, respectively. Both samples contained previously detected phyla (Fig. 5a) and genera (Fig. 5b) typically found in freshwater systems [40, 41]. Muskrat Lake was dominated by *Verrucomicrobia*, *Proteobacteria*, *Actinobacteria*, and unclassified bacteria in July. While the *Verrucomicrobium* were dramatically reduced in November, the *Proteobacteria*, *Actinobacteria*, and unclassified bacteria remained abundant, with a 1.5-fold increase of *Proteobacteria* and *Actinobacetria* and a 3-fold increase of the *Cyanobacteria*. The genera-level analysis revealed a temporal shift in *Spartobacteria genera incertae sedis*, a poorly characterized genus of *Verrucomicrobia* that was dominant in July, accounting for 63 % of the sequences, but greatly reduced in November. In addition, *GPIIa*, *GPI*, *Mycobacteria*, and *Polynucleobacter* increased significantly in November.
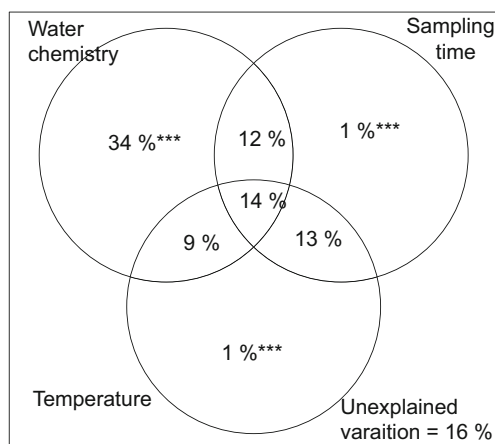
Table 2 Partitioning of variations in Muskrat Lake microbial community into significant variables alone

Environmental variable	Explanation percent
Sampling time	1[NS]
Sulfate	1***
Conductivity	1[NS]
Temperature	1***
pH	2**
DOC	2***
Alkalinity	1***
DO	1***
Nitrate	0[NS]

Significant contribution of single factors to microbial community variation are indicated by ***$p \leq 0.001$, **$p \leq 0.01$, *$p < 0.05$, and NS (not significant), as determined by 999 Monte Carlo permutations

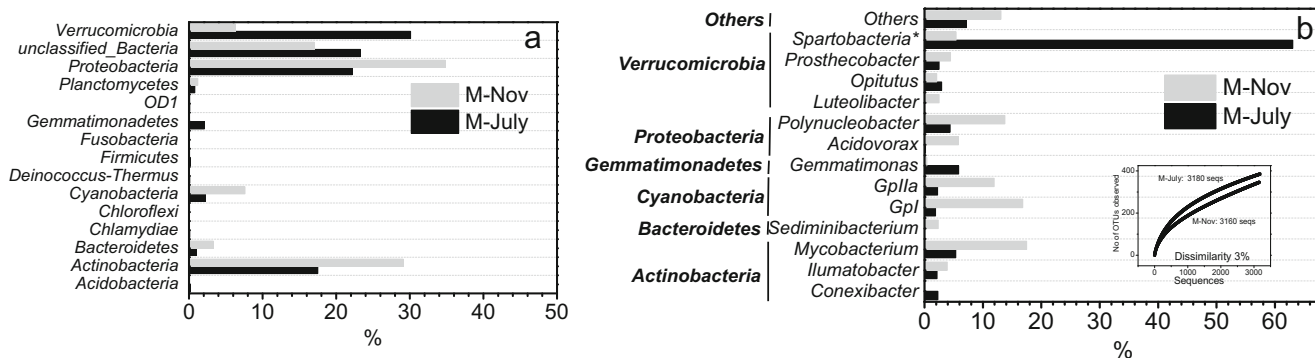**Fig. 5** Comparison of microbial community phylum (**a**) and genera (**b**) of Muskrat Lake based on 454 sequencing of 16S rRNA gene fragments. Inserted figure denotes the rarefaction data of 454. Samples were collected on July 31, 2008 and November 3, 2008. Phylogenetic groups accounting for ≥2 % of the sequences are summarized in the artificial group "others" in Fig. 5b. *Spartobacteria**: *Spartobacteria_genera_incertae_sedis*

Discussion

Muskrat Lake is a seepage temperate lake adjacent to a moderately sized agricultural watershed. Agricultural watershed inputs can lead to variable water chemistry in lakes. Temporal variations in Muskrat Lake water chemistry were observed in Chl-a, DOC, aromaticity, TDN, TDP, and nitrate. Moreover, agricultural watershed inputs were observed in the lake during and after rain events, as evidenced by immediate increases in alkalinity, % aromaticity, TDN, TDP, and nitrate. Rain events might also cause short-term increases in pH because of the ubiquitous limestone in soils in the southern part of Michigan [27]. However, only one rain event occurred during our study period, which is insufficient to establish a link between agricultural watershed inputs into the lake via runoff. Accordingly, more sampling during rain events is necessary for future investigations.

Environmental heterogeneity plays an important role in structuring bacterial communities [8, 10, 41–44]. The export of nutrients and carbon from an agricultural watershed into a lake can lead to variable water chemistry in the lake. Such changes in the physical and chemical environment can influence the bacterial community structure in the lake. Langenheder and Ragnarsson found that environmental conditions played a major role in the planktonic bacteria composition on a small spatial scale (<500 m) [43]. Logue and Lindström investigated the bacterial community structure of eight lakes during four seasons and found that local environmental factors were the main drivers of bacterial community structure [45]. In the present study, temporal variations in bacterial community structure were characterized by their T-RFLP profiles. The changes were correlated with water chemistry (sulfate, pH, DO, conductivity, alkalinity, and DOC), suggesting that water is the main determinant shaping bacterial community structure. Temperature [8, 10] and sampling time [37] were also important determinants in shaping the lake microbial community.

Allochthonous material, especially organic carbon, sustains the metabolism of the aquatic microbial community.

Allochthonous carbon accounts for 35–70 % of the carbon utilized by bacteria in the lake [17] and plays an essential role in maintenance of aquatic food webs [46]. The effects of autochthonous and allochthonous organic carbon on the structures of aquatic bacterial communities have been considered individually and in combination [47]. Moreover, there is evidence that bacterial biomass and activity is positively correlated with phytoplankton production [48] and allochthonous DOC [49, 50] in different trophic statuses. Because Muskrat Lake is associated with an agricultural watershed dominated by groundwater hydrology, it is expected that agricultural land use and hydrology will contribute substantial allochthonous inputs, including DOC, to the lake, and that this would influence the bacterial community. Although DOC explained 2 % of the variation in the bacterial community structure of Muskrat Lake, it had the greatest effect of any individual factor, suggesting that it is one of the main environmental factors influencing bacterial community structure in Muskrat Lake.

TDP and TDN are often correlated with bacterial and phytoplankton growth [51, 52], and their effects on bacterial communities have been demonstrated [53]. Although variations in TDP and TDN were detected, they were not identified as important factors in this study, possibly because their effects were subsumed by other explanatory variables used in the RDA model [10].

The 454 pyrosequencing revealed that both July and November samples contained typical freshwater system taxa, including *Verrucomicrobia*, *Proteobacteria*, and *Actinobacteria*. *Actinobacteria* [54] and *Proteobacteria* [55] are believed to play vital parts in organic matter turnover. *Mycobacteria* can use a wide range of carbon compound and nitrogen resource [56]. *Polynucleobacter* has been widely found at freshwater [57, 58] and been proposed to utilize photochemically DOM [59]. Accordingly, the abundance of phyla *Actinobacetria* and *Proteobacteria* and genera *Mycobacteria* and *Polynucleobacter* increased in November, 49 days after the rain event, most likely due to the watershed input DOC and nutrients. Conversely, *Verrucomicrobium* were dominant in

July but dramatically reduced in November. *Verrucomicrobia* is a universal phylum present in a variety of habitats such as aquatic ecosystem and soil, but its ecological function is rare known [55]. Recent study indicated that inorganic nitrogen addition decreased the relative abundances of *Verrucomicrobia* across North America [60]. Likewise, the increased inorganic nitrogen (e.g., nitrate) from the watershed at rain events might decrease the abundance of *Verrucomicrobia*. Additionally, abundant *Cyanobacteria* were detected during the November sampling in levels 3-fold higher than those detected in July. Similarly, the genera of *Cyanobacteria*, *GPIIa*, and *GPI* were also increased. *GPIIa* and *GPI* are related to nitrogen fixing [61] and have been detected at freshwater [61] and seawater [62] and coastal microbial mats [63]. The increased TDN and TDP from the watershed at rain events likely increased the abundance of *Cyanobacteria*, *GPIIa*, and *GPI*.

In conclusion, our results showed variations in the bacterial community structure of an agricultural watershed-associated lake that were correlated with changes in water chemistry. Agricultural watershed inputs were observed in the lake during and after rain events, as evidenced by an immediate increase in DOC and other nutrients. Differences in bacterial community composition with time were correlated with the local environmental, including water chemistry, sampling time, and temperature, with water chemistry explaining 34 % of the variation in the bacterial community. Of the investigated parameters, DOC accounted for the largest variations in the bacterial community (2 %).

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