Distinct under-ice bacterial communities in seasonally ice-covered northern lakes

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Abstract

Many northern lakes are seasonally ice-covered and it was traditionally thought that microbial communities and processes lay dormant under the ice due to cold temperature and low light levels. In this study, we investigated the spatial patterns of bacterial communities during ice-covered and ice-free periods in three limnologically distinct Canadian lakes using 16S rRNA gene sequencing approaches. Multivariate analyses of community similarity grouped samples first by lake, then by season, demonstrating that community composition was distinct during the ice-covered and ice-free periods and suggesting differences in metabolic strategies of populations living under the ice. 16S rRNA sequences from Verrucomicrobia and Planctomycetes, as well as the methylotrophic LD28 bacteria, were often abundant during ice cover. However, only a small fraction of bacterial taxa were commonly associated with ice-covered conditions across all lakes, suggesting that local lake conditions play a more important role than regional climate conditions in structuring bacterial communities in seasonally ice-covered northern lakes. These preliminary findings will guide further metagenomics-enabled research into the metabolic diversity of these important microbial communities.

Table of contents

1. Introduction

Inland waters cover about 3% of Earth's surface (Downing et al., 2006), yet they play a disproportionally large role in the global carbon cycle. Owing to the importance of inland waters in carbon cycling, there is a growing concern about the effects of climate change on these systems (Anas et al., 2015). This is particularly true for northern ecosystems, since climate change is anticipated to be more prominent at northern latitudes relative to other regions (IPCC, 2007). These changes are predicted to affect biological processes linked to dissolved organic carbon (DOC) production and transport to lakes. Likewise, shifts in physical and chemical conditions are expected to affect primary production and DOC transformations that are central to northern lake carbon cycling. Moreover, the alteration of northern lake carbon cycling may results in increased CO_2 efflux, creating a positive feedback that may further accelerate atmospheric warming (Benoy et al., 2007). Hence, in the context of the carbon cycle and environmental change, it is important to improve our understanding of freshwater ecosystems and biogeochemical dynamics.

Concerns about the consequences of climate change have stimulated research on the temporal dynamics of microbial communities and their activities in northern lakes. Most previous studies have focused on dynamics over the open water period of the year, but there is now an increasing interest from the microbial research community to understand microbial community structure and function during the ice-covered period (Bertilsson et al., 2013). The reasoning behind this is that the vast majority of high latitude lakes in the temperate and boreal climate zones are covered by ice for more than 40% of the year. Yet, our understanding of the

ecological and biogeochemical conditions of these ice-covered lakes in winter lags far behind that of the open water period (Bertilsson et al., 2013; Salonen et al., 2009; Hampton et al., 2015).

Traditionally, it was believed that due to cold temperature and low light levels, ice-covered lakes were considered dormant and biological processes were generally not studied. However, as was pointed out in a recent synthesis by Bertilsson et al. (2013), "this traditional concept fails to recognize that the winter season affects the ecology and metabolic features of freshwater microorganisms, as well as their involvement in food webs and global biogeochemical cycles throughout the year". The prevailing physical conditions during the ice-covered period do indeed influence resource availability and other factors that may reduce microbial growth. However, although bacterial biomass and productivity is generally lower in winter than summer, it is certain that bacterial communities are growing as turnover times are estimated to be a matter of a few days (Bertilsson et al., 2013). In many ice-covered lakes, light penetrating through ice and snow can fuel substantial primary production in the winter. Both short-lived and long-lived blooms of low light and cold-adapted phytoplankton, including diatoms and cyanobacteria, have been observed under the ice. For example, blooms of lowlight-adapted photoautotrophs *Fragilaria crotonensis* and *Vryptomonas erosa* developed under ice in Lake Michigan (Vanderploeg et al. 1992). A bloom of filamentous diatoms was observed directly under and imbedded in the ice in Lake Erie. The latter bloom exhibited chlorophyll a (Chl a) concentrations exceeding 73 $\mu g\ L^{-1}$ (Twiss et al., 2012), which is similar to average chlorophyll-a in eutrophic lakes during summer (Horne and Goldman, 1994). Better

understanding of the ecosystem under the ice in seasonally frozen lakes becomes crucial to predict and model these systems.

Moreover, unique microbial niches can develop and persist in ice-covered lakes. For example, in cases where ice-cover results in lower availability of labile organic substrates from phytoplankton or terrestrial input, volatile organic compounds such as methane (CH_4) and fermentation end-products produced from anoxic bottom waters or sediments can become important fuel for the microbial community. Indeed, CH_4-oxidizing bacteria are often abundant and active in winter under the ice, where they may play a critical role in lowering release of CH_4 trapped under the ice during ice off (Kankaala et al., 2006; Sundh et al., 2005). Chemolithoautotroph metabolism such as nitrification may also be important under the ice, since energy and carbon acquisition may not be as restricted by the presence of ice cover compared to phototrophs and heterotrophs (Auguet et al., 2011).

In combination, under-ice blooms of photoautotrophic and heterotrophic organisms, as well as a shift in microbial metabolism to processing of sediment-derived organic and inorganic growth substrates are likely important, but commonly overlooked, regarding their contributions to lake metabolism and nutrient cycling. Hence, knowledge on year-round community structure and metabolic traits of freshwater microorganisms is required to understand how they will influence other organisms and biogeochemical processes. In this study, we aimed to characterize the bacterial community composition within three geographically distinct freshwater lakes during the ice-covered and ice-free periods of the year and to understand the factors shaping the composition and the dynamics of these communities.

2. Materials and Methods

2.1. Sampling

Microbial samples were collected from three freshwater lakes: Lake Croche (45° 59′ N, 74° 01′W), Lake Montjoie (45° 24′ N, 72° 14′ W) and Lake Simoncouche (48° 14′ N, 71° 15′ W) in conjunction with the GRIL (Groupe de researche Interuniversitaire en Limnologie et en Environnement Aquatique) Monitoring Program. Over 3 years (2013-2015), samples were collected from the epilimnion and the metalimnion layers biweekly during ice-free periods and monthly during ice-covered periods of the year. While still on the sampling boat, lake water was pre-filtered through 53 μm mesh then collected in pre acid-washed bottles. In the lab, lake water passed through GF/D 3 μm filter to collect particles, and microbial cells were collected on a 0.22 μm Sterivex filter. After filtration, 1.8 ml of sucrose-based lysis buffer was added and filters were stored at -80° C. Each microbial sample was accompanied by a comprehensive set of meta-data that consisted of 12 physicochemical factors, including temperature, pH, conductivity, oxygen concentration, chlorophyll a, total nitrogen (TN), total dissolved nitrogen (TDN), nitrate & nitrite (NO_3^- NO_2^-), ammonium (NH_4^+), total phosphorus (TP), total dissolved phosphorus (TDP), and dissolved organic carbon (DOC) **(Supp Table 1).**

2.2. DNA extraction

DNA was extracted from 0.22 μm Sterivex filters using a phenol/chloroform-method modified from Zhou, 1996. Sterivex filters were thawed on ice and the storage buffer was removed. We then concentrated the storage buffer into Amicon 30 kD filter (500 μl at a time) followed by centrifugation for 20 minutes at 10,000 g. We repeated adding 500 μl of storage

buffer each time until it is concentrated down to a final volume of 100 µl. Buffer exchange was conducted twice by washing storage buffer with 500 µl of TENP buffer (600 mg Tris, 740 mg EDTA, 580 mg NaCl, 2 g Polyvinylpyrrolidon and 100 ml milliQ, pH 8). We then broke open the Sterivex filter and removed the filter. We split the filter into halves to be placed inside a 2 ml Eppindorf tube. In order to conduct the cell lysis and digestion, we added 0.37 grams of 0.7 mm pre-sterilized Zirconium beads, 60 µl of 20 % SDS, 100 µl concentrated buffer exchanged filterate, 500 µl TENP buffer and 500 µl phenol-chloroform-isoamylalcohol (PCI) 25:24:1 to the 2 ml eppindorf tube containing the shredded filter, then we vortexed the sample for 10 min. Samples were incubated for 10 min in a 60 °C water bath followed by incubation on ice for 1 min. After that we centrifuged samples for 6 min at 10,000 rpm and 4°C. Supernatant was transferred to a clean 1.5 ml Eppindorf tube, 500 µl phenol-chloroform-isoamylalcohol (PCI) 25:24:1 was added and samples were vortexed briefly. We then centrifuged samples for 6 min at 10,000 rpm and 4 °C and transferred the supernatant to a new 1.5 ml Eppindorf tube. We repeated this PCI step until there was no longer any white precipitate at the interphase (usually 2 times). DNA was precipitated by adding 120 µl of 3 M sodium acetate followed by 1 ml of 96% ethanol. DNA was precipitated at -20 °C for at least 1.5 hours, followed by centrifugation for 60 minutes at 13,000 rpm and 4 °C. We decanted the supernatant and washed the pellet with 850 µl of 80 % ethanol. We incubated samples for 10 minutes on ice followed by short vortexing and then centrifuging samples for 15 minutes at 13,000 rpm and 4 °C. The supernatant was removed and the DNA was resuspended in 50 µl TE or Tris-HCl (pH 7.5-8).

2.3 Polymerase chain reaction (PCR) amplification and sequencing of 16S rRNA genes

The V3 region of the 16S rRNA gene was amplified using the universal primers (341F: 5'-CCTACGGGRSGCAGCAG-3' and 515R: 5'-TTACCGCGGCKGCTGVCAC-3') (Klindworth et al. 2012). Two-step PCR reactions (modified from Berry et al., 2011) were conducted in 25 µl volume contained 0.5 µM $MgCl_2$, 0.2 mM deoxynucleotide, 0.2 µM each primer and 1U of Phire Hot Start II DNA Polymerase (Finnzymes Thermofischer Scientific). The template was amplified using non-barcoded PCR primers for 20 cycles, followed by 1:50 dilution of the PCR product and 10 additional cycles of amplifications with barcoded PCR primers. The thermal program consisted of an initial 95 °C denaturation step for 4 min, a cycling program of 95 °C for 30 s, 52 °C for 30 s, and 72 °C for 60 s, and a final elongation step at 72 °C for 7 min. Reverse primers were barcoded with specific IonXpress sequence to identify samples. PCR products were purified using QIAquick Gel Extraction Kit (Qiagen), quantified using Quantifluor dsDNA System (Promega), pooled at equimolar concentration and sequenced using an Ion Torrent PGM system on a 316 chip with the ION Sequencing 200 kit as described in Sanschagrin and Yergeau (2014).

2.4. Bioinformatics analysis of 16S rRNA gene sequences

V3 16S rRNA sequences were analyzed using the open-source MOTHUR pipeline (Schloss et al., 2009). Sequences with an average quality of < 17, length < 100 bp or that did not match the IonXpress barcode and both the PCR forward and reverse primer sequences were discarded. Potential chimeric sequences were identified using UCHIME (Edger et al., 2011) and also discarded. Sequences were clustered into OTUs at 97% identity using the furthest neighbor

algorithm. Estimates of OTU richness (Chao-1 index) and evenness (Shannon) were generated from 6,974 sequences/sample using MOTHUR.

To assess taxonomic composition of bacterial communities, 16S rRNA sequences were assigned to taxonomic groups using the structure proposed in Newton et al. (2011). In this structure, a single phylogenetic framework was generated using a hierarchical taxonomic structure (phylum/lineage/clade/tribe). In this structure, each group is clustered as a monophyletic branch, which is comprised of sequences sharing different degree of similarity. The tribe is the most refined group and it consists of a group of sequences that have ≥ 97 % sequence identity. A clade represents a group of sequences have ≥ 95 % sequence identity. A lineage represents group of sequences that share maximum sequence similarity between 85-90 % A phylum is synonymous with the long-established phyla defined by bacterial systematics. The TaxAss workflow ("MacMahonLab", 2016) was used to assign the 16S rRNA sequences to the taxonomic structure. In this workflow, both the comprehensive Greengenes database (DeSantis et al. 2006) and a customized freshwater specific database were used (Newton et al. 2011), which improved the taxonomic resolution.

To explore and visualize the bacterial community structure in the three studied lakes, we performed an unconstrained non-metric multidimensional (NMS) scaling ordination of bacterial taxa distribution across lakes. The NMS was performed using Bray-Curtis distances implemented in PC-ORD (McCune and Mefford, 2011). To assess whether the bacterial community differed significantly between lakes and between ice-covered season and ice-free season in each lake, we ran multiple response permutation process (MRPP) test. The MRPP test

was performed using Euclidean distance implemented in PC-ORD (McCune and Mefford, 2011). To visualize the taxa that showed specific seasonal association in each lake, we plotted the correlation strengths of each taxon along ordination axis 1 of the NMS plots against the relative abundances in summer versus winter (ln [average relative abundances during summer/average relative abundances during winter]) for each taxon in each lake. Redundancy Analysis (RDA) was used to determine the percent of community variation explained by environmental variables (Legendre and Anderson, 1999). Prior to RDA, environmental data were tested for normality (Shapiro-Wilks test, $P < 0.05$), and variables that were not normally distributed were transformed to a near normal distribution.. RDA analyses were run in R (V.3.1.0) using the VEGAN package.

3. Results

3.1. Site description and environmental setting

In this study, we investigated the biogeographical patterns of bacterial communities in three seasonally ice-covered lakes located in temperate (Lake Croche and Lake Montjoie) and boreal regions (Lake Simoncouche) of eastern Canada (**Figure 1A**). Based on total phosphorus, all lakes were oligotrophic, but existed along a gradient in nutrient status (average TP in Croche 5.13 μg L^{-1}, Montjoie 7.9 μg L^{-1} and Simoncouche 10.29 μg L^{-1}) (**Supp**. **Table 1**). In general, all lakes exhibited the typical seasonal stratification cycle of northern lakes. The winter months were characterized by ice and snow cover. During winter, Croche and Simoncouche exhibited vertical layering with a thin cold surface layer (~1°C) atop warmer bottom water (~4°C) whereas the winter water column at Montjoie did not exhibit this thermal stratification (**Figure 1B**) Soon after the spring melt, all lakes began to thermally stratify and peak stratification was observed during August (**Figure 1B**).

Lakes exhibited markedly different seasonal dynamics in phytoplankton biomass as measured by Chl-a concentrations. Phytoplankton biomass was consistently highest in Croche compared to other lakes and was elevated in the metalimnion compared to the epilimnion **(Figure 2A**). Similar vertical structure in phytoplankton biomass was observed in Simoncouche, yet phytoplankton abundance was higher overall during summer 2013 compared to summer 2014 **(Figure 2C).** In contrast, phytoplankton biomass was lower in Montjoie and was at a similar level at the epilimnion and metalimnion **(Figure 2B**). Phytoplankton biomass was always higher during the open water period compared to under the ice, but significant Chl-a concentrations were observed periodically under the ice, particularly in Simoncouche and Montjoie. Most striking was a Chl-a concentration of ~3 ug/L just under the ice surface of Simoncouche in January 2013, a time of ice-cover but no snow, potentially allowing growth of phytoplankton. Similar Chl-a concentrations were observed under the ice in Montjoie during January 2014 and January 2015 **(Figure 2C).**

3.2. Bacterial 16S rRNA gene time series

To investigate the variability in bacterial diversity and community structure between ice-covered and ice-free periods of the year, we generated a 3 year time-series of bacterial 16S rRNA gene diversity. In total, we generated 16S rRNA data from 73 samples collected from the epilimnion and metalimnion, and corresponding to 6 winter time-points (January and February 2013, 2014, and 2015) and 8 summer time-points (June, July, August 2013 and 2014). The complete 16S rRNA gene dataset was comprised of 509,102 16S rRNA sequences (6,974 sequences/sample). To assess bacterial community diversity, sequences were clustered at 97%

sequence similarity, resulting in a total of 50,360 operational taxonomic units (OTUs). OTU richness estimated using the Chao-1 index varied between 2,378 and 6,239 (**Figure 3A**). On average, OTU richness was greater in Simoncouche compared to Croche and Montjoie. Moreover, average richness was consistently greater during ice-covered periods compared to ice-free periods (**Supp. Table 2**). Indeed, maximum richness was observed during winter at Simoncouche (6,141 OTUs) while minimum richness was observed during the summer in Montjoie (2,378 OTUs). In addition, we assessed the evenness of the bacterial community during the ice-covered and ice-free period at the same selected time points. As a general pattern, the bacterial community exhibited higher evenness during the ice-covered period compared to the open-water period. The maximum evenness was recorded in Simoncouche (0.88) during the ice-covered period while the minimum evenness value was recorded in Croche (0.72) during the ice-free period **(Figure 3B)**.

3.3. Community structure variability between lakes

To assess taxonomic composition of bacterial communities, 16S rRNA gene sequences were assigned to taxonomic groups using the structure proposed in Newton *et al.* (2011). 84 tribes (comprising 6 phyla) in total were selected for further analysis**.** An additional 88 taxa not represented in the Newton *et al.* 2011 taxonomy were additionally selected for further analysis. The 6 phyla were Actinobacteria, Verrucomicrobia, Planctomycetes, Bacteroidetes, Cyanobacteria/Chloroplasts and Proteobacteria (Alpha-proteobacteria, Beta-proteobacteria and Gamma-proteobacteria classes). Although all phyla were abundant in all lakes, they exhibited different patterns across lake and season **(Figure 4)**. For example, Planctomycetes and Cyanobacteria were abundant under the ice in Lake Montjoie specifically. In contrast,

Verrucomicrobia exhibited high abundances in Croche and Simoncouche during the ice-covered period.

Given the contrasting geographic settings and environmental conditions between the lakes, we hypothesized that lakes would support distinct bacterial communities. This hypothesis was supported by a MRPP test, which indicated a significant difference (A=0.1, P=0.0001) between the bacterial communities within the three lakes. To visualize the differences in community structure, we performed a NMS ordination of bacterial taxa distribution across lakes. In the ordination, samples from Croche were separated along axis 1 from a cluster of overlapping samples originating from Montjoie and Simoncouche (**Figure 5**), indicating the Croche bacterial community is relatively more distinct from the bacterial communities residing in Simoncouche and Montjoie.

Bacterial taxa that strongly distinguished Croche from Simoncouche and Montjoie were common freshwater taxa within the Actinobacteria and Beta-proteobacteria. Actinobacteria was an abundant group in all three lakes but specific actinobacterial taxa differed in their distribution between the lakes. Eight actinobacterial tribes exhibited a strong association with Croche, while 11 exhibited a strong association with Simoncouche and Montjoie. Strong partitioning was also observed for closely related tribes. For example, acI was the most abundant lineage, however variability was observed within the acI tribes; acI-A1 was most abundant in Croche, while acI-A3 was most abundant in the other two lakes. Moreover, many Actinobacteria tribes were restricted to Croche, including acI-B1, AcI-B2, AcV-A1, and AcV-A2. Within the Beta-proteobacteria, Lhab-A2 and PnecD tribes were associated with Croche, while the LD28 tribe

was associated with Simoncouche and Montjoie. Interestingly, we observed that the phytoplankton taxa Stramenopiles and Chrococcacaea were strongly associated with Croche.

3.4. Community structure variation between seasons

Since the primary objective of this study was to assess variation between winter and summer bacterial communities and since bacterial diversity was distinct between lakes, we performed comparative analyses of community structure on each individual lake. A NMS ordination (**Figure 6A**) of Croche samples revealed bacterial communities sampled during the cold ice-covered period were separated from those collected during the warm ice-free period along ordination axis 1 ($R^2 = 0.484$), suggesting distinct bacterial communities. MRPP analysis comparing winter and summer samples provided further support for distinct summer and winter communities (A=0.19, $p=3x10^{-7}$). Similar to Croche, distinct bacterial communities were associated with ice-covered and ice-free periods of the year in Simoncouche **(Figure 6B)**. NMS ordination resulted in the separation of cold winter samples from warm summer samples along ordination axis 1 (R^2=0.446) and the significance was verified by MRPP analysis (A=0.16, $p=9.7x10^{-7}$). In contrast to Croche and Simoncouche, season had a lesser effect in shaping bacterial community structure (axis 1 R^2=0.299) in Montjoie **(Figure 6C)**. MRPP analysis showed that the winter and summer separation was not significant (A=0.06, p=0.02). Instead, samples were separated in the NMS ordination into two clusters (herein referred to as clusters 1 and 2). Cluster 1 contained winter samples and most summer metalimnion samples, while cluster 2 was comprised mostly of summer epilimnion samples and a single winter sample.

From the NMS plots, it was apparent that there are unique community structures associated with each season in Croche and Simoncouche. To visualize the seasonal association of each taxon, we plotted the correlation strengths of each taxon along ordination axis 1 of the NMS (separating winter samples from summer samples) against the relative abundance ratio of summer versus winter (ln[avererage relative abundance during summer/average relative abundance during winter]) for each taxon in each lake. In **Figures 7A, 8A and 9A**, bacterial taxa located in the lower left quadrant are those that exhibited an association with winter while those located in the upper right quadrant exhibited an association with the summer period. Except for Cyanobacterial/chloroplast taxa, which are all associated with the ice-free period, most other bacterial phyla were comprised of tribes that exhibited either a winter or a summer association.

In Croche, most **Actinobacteria** tribes were common in winter and summer. However, several showed a specific seasonal association. Most prominent was the acI-B2 tribe in summer and the acI-A7 tribe in winter (**Figure 7B**). Within **Alpha-proteobacteria**, the alfI-A1 tribe exhibited a preference for summer conditions, while alfI-B2 exhibited a bias towards winter conditions. The Ellin329 group was present in all winter samples and absent from all summer samples **(Figure 7C**). Within the **Beta-proteobacteria**, the LD28 and betIII-A1 tribes were more abundant during ice-covered season compared to summer. The betVII-B1 tribe, as well as tribes within the common betI (Lhab tribes) and betII (Pnec tribes) lineages, were shared across seasons, but more abundant in summer. In addition the rhodo tribe (Beta-proteobacteria) were highly associated with ice-free period but still well represented under the ice **(Figure 7D**). As is true for most freshwater lakes, **Gamma-proteobacteria** were not abundant in Croche. However,

those Gamma-proteobacterial lineages that were detected were generally more abundant during winter. The most abundant lineage was the gamI tribe, which reached highest relative abundance in February 2013. In addition, both Xanthomonadales and Alteromonadales exhibited higher relative abundance during the winter season **(Figure 7E**). Within the **Verrucomicrobia**, the two tribes (Xip-A1 and Xip-B1) of the VerI lineage exhibited contrasting patterns. The Xip-A1 tribe was strongly correlated with the winter season, whereas Xip-B1 was only detected during summer season (**Figure 7F).** In addition to VerI, additional verrucomicrobial groups were identified, including Methyloacidiphilales in the summer and Pedosphaerales in the winter **(Figure 7F**). Although Cerasicoccales was more common in the summer season, it peaked in 2015 during ice-covered period. Within the Planctomycetes, both Isospheraceae and Gemmataceae were commonly found in the winter season. Planctomycetacia were associated with the winter, particularly during 2015 **(Figure 7G**). The only tribe within the **Bacteroidetes** that showed strong correlation with winter season was the Pedo tribe within the Sphingobacteriales **(Figure 7H**). Although chloroplast taxa were highly associated with summer season, several taxa such as Stramenopiles and Cryptophyta were detected during the ice-covered season **(Figure 7I**).

In Simoncouche, the luna1-A4 and Iluma-A1 tribes (**Actinobacteria)** exhibited peak abundances during the ice-covered season while acSTL-A2, acI-A1, acI-A3 exhibited peak abundance during the summer season **(Figure 8B)**. For the **Alpha-proteobacteria**, alfVIII and LD12 tribes exhibited bias for the summer season, while the Ellin329, alfVI, and brev tribes exhibited higher relative abundances during the winter season compared to the summer **(Figure 8C)**. For the **Beta-proteobacteria,** pnecC and betVII-B1 tribes were strongly correlated with

summer season, while LD28 tribe and the two families Comamonadaceae and Rhodocyclaceae were strongly correlated with winter season. The rhodo tribe comprised relatively higher abundance during ice-covered season (especially in 2013 and 2014) compared to ice-free season **(Figure 8D)**. The **Gamma-proteobacteria** was generally associated with winter season. For example, gamI and Alteromonadales exhibited high relative abundance during the winter season **(Figure 8E)**. Cerasicoccales (**Verucomicrobia)** recorded high association with winter season as well as high abundances during winter 2015 where it constituted 10 % - 24 % of the bacterial community in the samples. In addition, Opitutales (another group of Verrucomicrobia) showed high association with winter season. In summer Xip-B1 tribe and Methyloacidiphilales exhibited high association with summer **(Figure 8F)**. Gemmataceae and Phycisphaerales **(Planctomycetes)** were associated more with the winter season rather than summer season **(Figure 8G)**. For the **Bacteroidetes,** Flavo-A2 showed high association with winter season, while the Aquir and Flavo-A1 tribes were more associated with summer season **(Figure 8H)**. Generally **Chloroplast** were strongly associated with summer season, but we identified 16S rRNA sequences originating from Stramenopiles and Cryptophyta during the winter **(Figure 8I)**.

As previously mentioned, samples from Montjoie formed two clusters in the NMS ordination. Cluster 1 was comprised of winter samples and most summer metalimnion samples whereas cluster 2 was comprised of summer epilimnion samples and a single winter sample. Many taxonomic groups were partitioned between these clusters. For example, in **Actinobacteria**, all of the acI-A1, acI-A5, act-A4, AcI-A6 were strongly associated with warm surface water samples (cluster 2), while both of acI-c1 and Iluma-A1 showed strong associations with cold winter and deep summer samples (cluster 1) **(Figure 9B)**. Within the **Alpha-**

proteobacteria, the Brev tribe was strongly correlated with cold and deep water (cluster 1) and the Sphingo tribe was only found under the ice **(Figure 9C)**. Within the **Beta-proteobacteria**, the LD28 and jnab tribes as well as families Comamonadaceae and Rhodobacteraceae were associated with cold and deep waters, while betVII-B1 and PnecC tribes were very abundant during summer and in surface samples. The rhodo tribe recoded high abundances in both clusters but one sample in cluster two which comprised most surface and summer samples had the highest value **(Figure 9D)**. For the **Gamma-proteobacteria**, we found the Pseudomonadales and Alteromonadales families were highly associated with cluster 1 **(Figure 9E).** For the **Verrucomicrobia,** Cerasicoccales and Opitutales were more abundant during summer and surface samples **(Figure 9F).** Phycisphaerales and Gemmataceae (**Planctomycetes**) exhibited high relative abundances in cluster I (cold and deep waters) and were nearly absent from cluster II (surface and warm waters) **(Figure 9G)**. For the **Bacteroidetes**, the Flavo-A2 tribe was identified under the ice cover. Both Aquir and bacI-B1 tribes were highly associated with summer **(Figure 9H).** Chloroplast sequences from Stramenopiles were generally more abundant in cluster two (mostly summer and surface samples). In addition, Haptophyceae exhibited a relative high abundance (6.6%) under the ice in 2015 and this sample was included in cluster 2 as well **(Figure 9 I).**

3.5. Seasonal bacterial core taxa

Following the lake specific seasonal analysis, we were interested in identifying the presence of taxa commonly associated with ice-free and ice-covered condition across all three lakes. To do so, we defined this "winter core" as the set of bacterial taxa negatively associated with NMS axis 1 ($R \leq -0.45$) in all three lakes. Similarly, the "summer core" was defined as the set of bacterial

taxa positively associated with NMS axis 1($R \geq 0.45$) in all the three lakes. The winter core was comprised of a larger number of taxa compared to the summer core. Of the 61 taxa that were winter associated in at least one lake, only 9 were members of the winter core. The winter core was comprised mainly of taxa within the Proteobacteria, including Alpha-proteobacteria (Ellin329 and alfI-B2), Beta-proteobacteria (LD28, Lhab-A4 and Janb) and Gamma-proteobacteria (Xanthomonadales, Pseudomonadales and Alteromonadales) as well as a single Actinobacteria tribe (luna1-A4) **(Figure 10A)**. During the summer season, of the 46 taxa that were summer-associated in at least one lake, a summer core of only three taxa was identified, and was comprised of cyanobacterial and actinobacterial taxa **(Figure 10B).** The majority of summer associated taxa were only associated with summer within a single lake.

3.6. The influence of environmental factors in structuring the ice-covered and ice-free communities between lakes

Owing to the differences in the ice-covered communities between lakes, we performed a redundancy analysis (RDA) to explore the role of environmental factors in explaining the 46variation of under-ice bacterial communities between the three studied lakes. In addition, the same analysis was run on the ice-free bacterial communities. During the ice-covered period, the RDA model statistically explained 59 % of the variation between the under ice samples. Croche samples were separated from Simoncouche and Montjoie samples along axis 1 (rda1) which explained 30.28 % of the explained variation (**Figure 11**). Conductivity, dissolved phosphorus (TDP), ammonium (NH_4^+) and dissolved organic carbon (DOC) were found to be the environmental factors that statistically best explained the variations in the under-ice bacterial

community composition between the three lakes. Simoncouche and Montjoie were associated with higher DOC and TDP. On the other hand, Croche was associated with higher NH_4^+.

During summer, environmental factors had a lesser role in shaping the bacterial community in the three lakes compared to the ice-covered period **(Figure 12).** The RDA model only explained 36 % of variation between the summer samples. Similar to the winter season, Croche samples were separated from Simoncouche and Montjoie samples along the axis (rda1), which explained 19.92 % of the explained variation. Nitrogen and phosphorus were the main explanatory factors followed by pH and conductivity.

4. Discussion

Generally the physical environment under the ice is different than during the ice-free period. Ice cover acts as a shield over the lake surface, which reduces light intensity and hence the production of phytoplankton-derived organic substrates, as well as the input of terrestrial terrestrial nutrients and organic matter. Ice cover also hinders gas exchange with the atmosphere (Bertilsson et al. 2013) The main focus of this study was to characterize bacterial communities during the ice-free and ice-covered period within different temperate and boreal lakes and to recognize dynamics of different bacterial groups between the two focal seasons. Such observation should provide insights about the potential activities and role of the bacterial community under the ice cover and during the ice-free period. In addition we investigated the role of environmental factors in shaping these bacterial communities.

In this study, bacterial richness and evenness was generally higher during the ice-covered season compared to the ice-free season. We were unable to identify any studies that previously

assessed bacterial drichness during winter and summer seasons in freshwater lakes. However, in marine waters, Gilbert and colleagues (2012) reported maximum bacterial diversity during winter and minimum diversity during summer in 6-years study in the English Channel. In addition, El Swais *et al.* (2015) reported high bacterial richness and evenness in late autumn and winter and low richness and evenness in spring and summer in the Northern Atlantic Ocean. One possible explanation is the different structure of the water column between the two seasons. During summer the water column is vertically stratified. During autumn, stratification becomes less stable and wind can mix the lake. In addition, under-ice mixing can be introduced by heat flow from the sediments or convection currents (Malm et al. 1997, Welch and Bergmann 1985, Petrov et al. 2007, Bertilsson et al. 2013). Previous studies showed that convective mixing was an important factor in maintain non-motile phytoplankton in the photic zone of the upper water column (Kelley 1997, Vehmaa and Salonen 2009), thus we propose that water column mixing regimes during autumn and during ice-covered period led to the introduction of bacteria from the deep water to the surface layer (and vice versa) which lead to increase the overall under-ice diversity.

We hypothesized that bacterial community structure under the ice would differ significantly compared to that found during the ice-free period due to the differences in the lake physical environment. Our results supported this hypothesis in both Croche and Simoncouche. However in Montjoie, samples were separated into two clusters with most winter samples appearing more similar to summer metalimnion samples (cluster 1), which were distinct from summer epilimnion samples (cluster 2). Our data reported a mixed water column under the ice in each of the three studied years in Montjoie. In addition, relatively high chlorophyll a values were

recorded within these mixed layers, which indicated possible autochthonous carbon source availability (normally very scarce during ice-covered period). We propose that these factors may have contributed to a different environment under the ice in Montjoie compared to Croche and Simoncouche and hence the seasonal bacterial community structure differed in this lake. Our observations are in agreement with previous work that showed lake-mixing regime as a significant indicator of bacterial community composition (Shade et al. 2007).

Although there was significant variation between lakes with respect to winter-associated taxa, a cores set of winter taxa common to all lakes was identfied. One interesting taxon within the winter core was the LD28 tribe (Beta-proteobacteria). The LD28 tribe was previously implicated in the metabolism of one-carbon (C1) compounds, in particular methanol (Chistoserdova 2011, Ramachandran et al. 2015). LD28 and other C1-oxidizing bacteria have been observed in winter previously (Salcher et al. 2008). In our study, we also identified the gamI tribe as a winter taxa in two of the three lakes. The gamI tribe is a member of known methane-oxidizers (Wartiainen et al. 2006) and were previously identified during the winter (Garcia et al. 2013). Methane-oxidizing bacteria are often abundant and active in winter under the ice, where they may play a critical role in lowering release of CH_4 trapped under the ice during ice off (Kankaala et al., 2006; Sundh et al., 2005). Gamma-proteobacteria were also considered as dominant the methane oxidizer responsible for methane removal in freshwater lake (Oswald et al., 2016). This can give us some insights about the potential activities of the bacterial community during the ice-covered season in the studied three lakes. Interestingly, Luna1-A4 (Actinobacteria) was also considered as a winter core taxa. Although Luna1-A4 were rare, this tribe showed a persistent association with winter conditions in all studied lakes.

Although the detection of a winter core community is insightful regarding the potential activity and metabolism under the ice, this core still constituted a small portion of the bacterial community in each lake. Other bacterial groups showed interesting seasonal patterns only in one or two lakes. For example, Verrucomicrobia were abundant in Croche and Simoncouche, especially under the ice. This phylum is widespread in terrestrial, aquatic, and intestinal tract environments (Arnds et al. 2010). Many previous studies have implicated Verrucomicrobia in polysaccharides degradation, which constitutes one of the key bottlenecks in the carbon cycle (Arnosty 2011**)**. More interestingly, population of Verrucomicrobia differ significantly at finer phylogenetic level. In Croche, they were comprised mainly of families Pedospherales and Opitutales. In contrast, Verrucomicrobia were comprised mainly of the Xip-A1 tribe in Simoncouche. Diversity and abundance of Verrucomicrobia was much reduced in Montjoie especially during the ice-covered period. Further targeted studies of these Verrucomicrobia taxa should provide information of their ecological roles in freshwater environment.

The Planctomycetes is another under-investigated group of aquatic bacteria that was associated with winter conditions. In Simoncouche, members of the Planctomycetes exhibited a strong winter association and were abundant in many samples. Planctomycetes were highly abundant in deep cold water (cluster 1) in Montjoie. Planctomycetes are still among the less understood bacterial phyla in terms of diversity and dynamics. More focus is given these days to study the role of Planctomycetes in the functioning of aquatic ecosystems given their involvement in nitrogen and carbon cycles (Tadonleke RD 2006, Schbert et al., 2006). In addition, some studies have shown significant changes in Planctomycetes composition during

and after cyanobacteria blooms (Morris et al., 2006.) This is in line with other genomic studies demonstrating that Planctomycetes poses genes involved in the degradation of plant detritus and algal polymers (Woebken et al. 2007).

Other interesting groups common in Quebec lakes were taxa that are putatively aerobic anoxygenic phototrophs (AAPs). AAPs are facultative phototrophic that can grow in the dark on organic carbon substrates. On the other hand, they can also derive a significant portion of their energy requirements from light (Hauruseu and Koblizik 2012; Kirchman and Hanson 2013). AAPs were recovered from freshwater lakes (Salka et al. 2011, Cepakove et al. 2016) mainly during summer season. The Rhodo tribe (Beta-proteobacteria) is an APP bacterium, which was reported repetitively in our database within the three studied lakes. In Croche, it was recorded in higher abundance during the ice-free season compared with ice-covered season. In contrast, in Simoncouche, Rhodo tribe exhibited high abundances under the ice, especially in 2013 and 2014. In Montjoie, the Rhodo tribe was more associated with surface summer season (cluster 2). These results suggested that AAP may contribute to energy/ carbon cycling year around in Quebec lakes.

In this study, environmental filtering was an important mechanism in shaping bacterial community structure. Under the ice, the variations in the bacterial community were mainly explained (59%) by conductivity, TDP, NH_4^+ and DOC. On the other hand, during the ice-free season, nitrogen and phosphorus, pH and conductivity were the factors that explained (36%) the variations in the bacterial community. Previous work confirmed the importance of the different environmental factors in shaping the bacterial community. For example, lake trophic status

(Lindstrom 2004, Yannarell et al. 2003, Yannarell and Triplett 2004), pH, retention time (Lindstrom et al., 2005), hydrology (Yannarell and Triplett 2005), lake mixing regime (Shade et al. 2007), and nutrient concentration (Stepanauskas et al. 2003, Van der Gucht et al. 2001) impact the bacterial community structure across lake and time.

In conclusion, we confirmed that the bacterial community composition under the ice differed significantly compared to the ice-free period. The under-ice bacterial community structure shared some similarities within the lakes, however we propose that the under-ice bacterial community is more lake-specific and it depends to a large extent on the local conditions of each lake. In addition, we found that trying to generalize the under-ice bacterial community will lead one to ignore the complexity and specificity of the under-ice community between lakes. However, this pilot study opens the door for future studies focusing on specific tribes and groups to investigate their actual role in the water column either under the ice or during the ice-free period.

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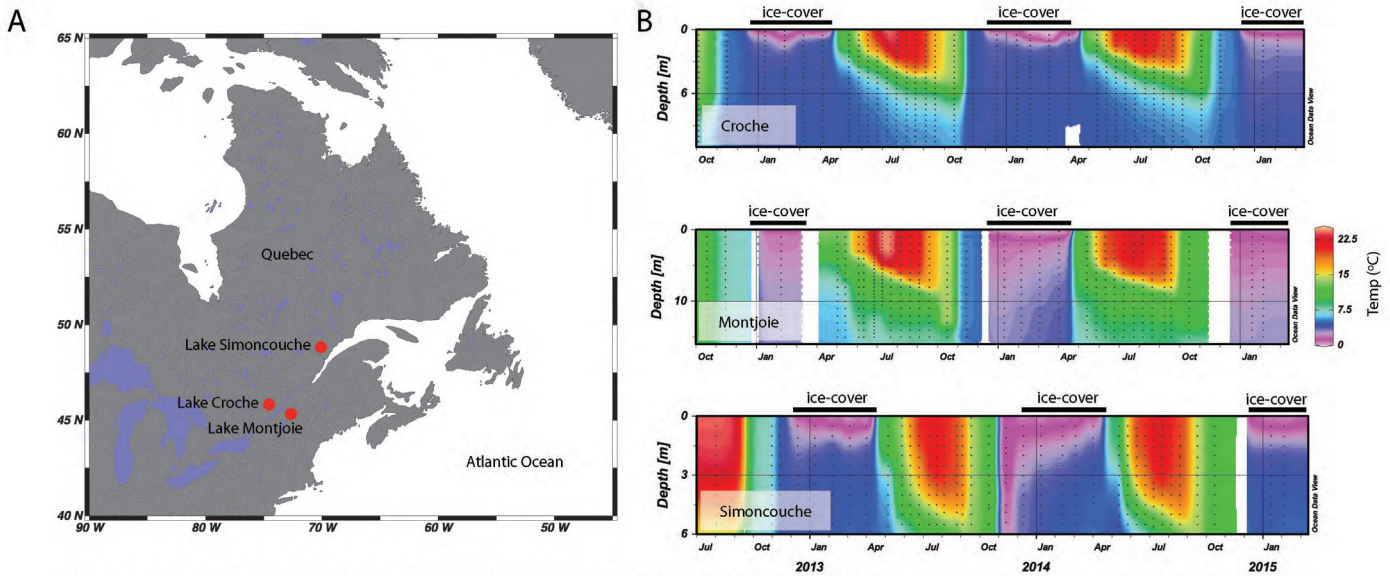


Figure 1. Location and seasonal conditions of the lakes in this study. (A) Lake Croche and Montjoie are temperate lakes located in southern Quebec, while Simoncouche is a boreal lake located in cetral Quebec. (B) The existing time-series and sample collection in the focal lakes.

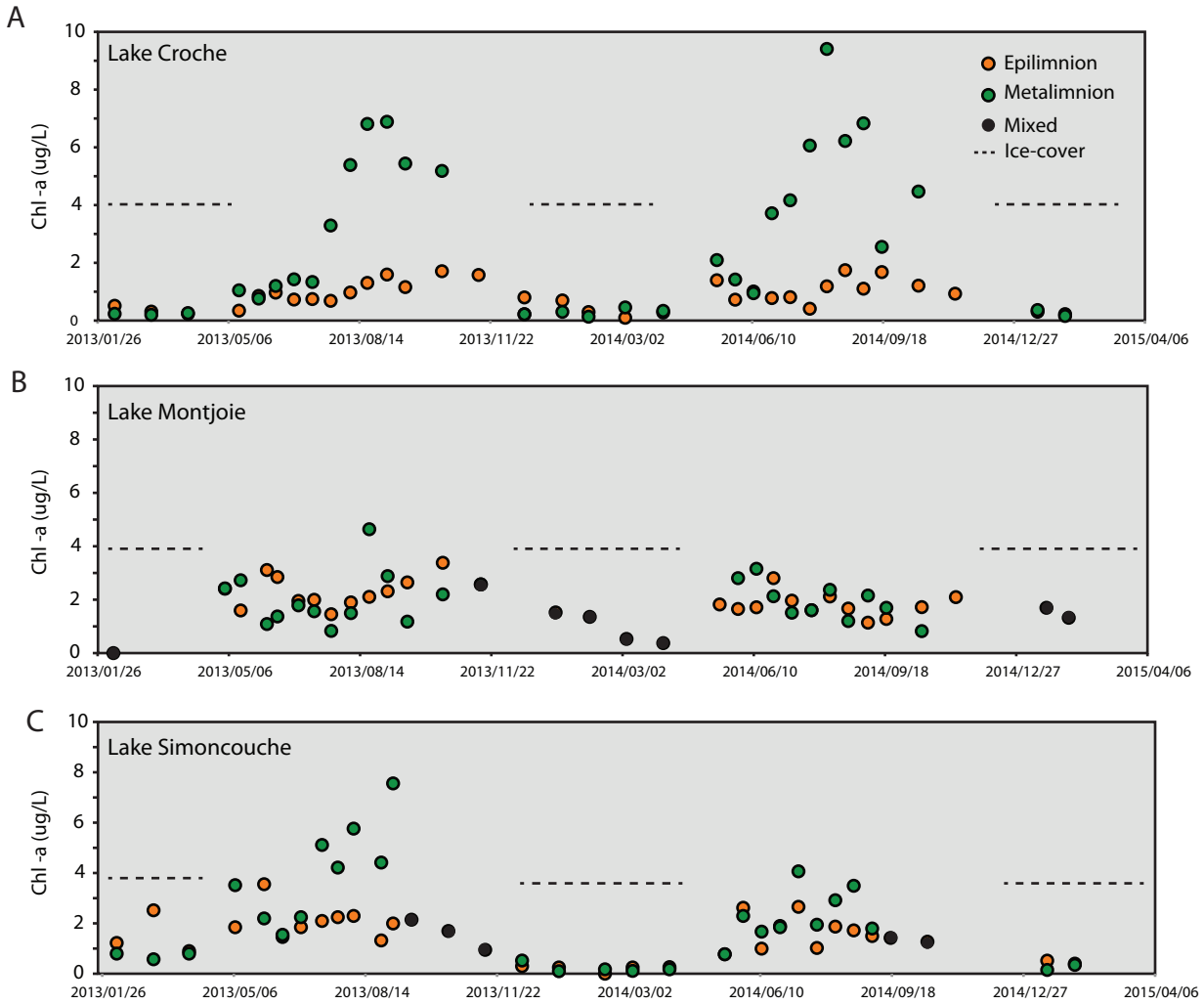


Figure 2. Chlorophyll-a concentration (µg/L) in epilimnion and metalimnion layers through years 2013-2015 within (A) Lake Croche. (B) Lake Montjoie. (C) Lake Simoncouche.

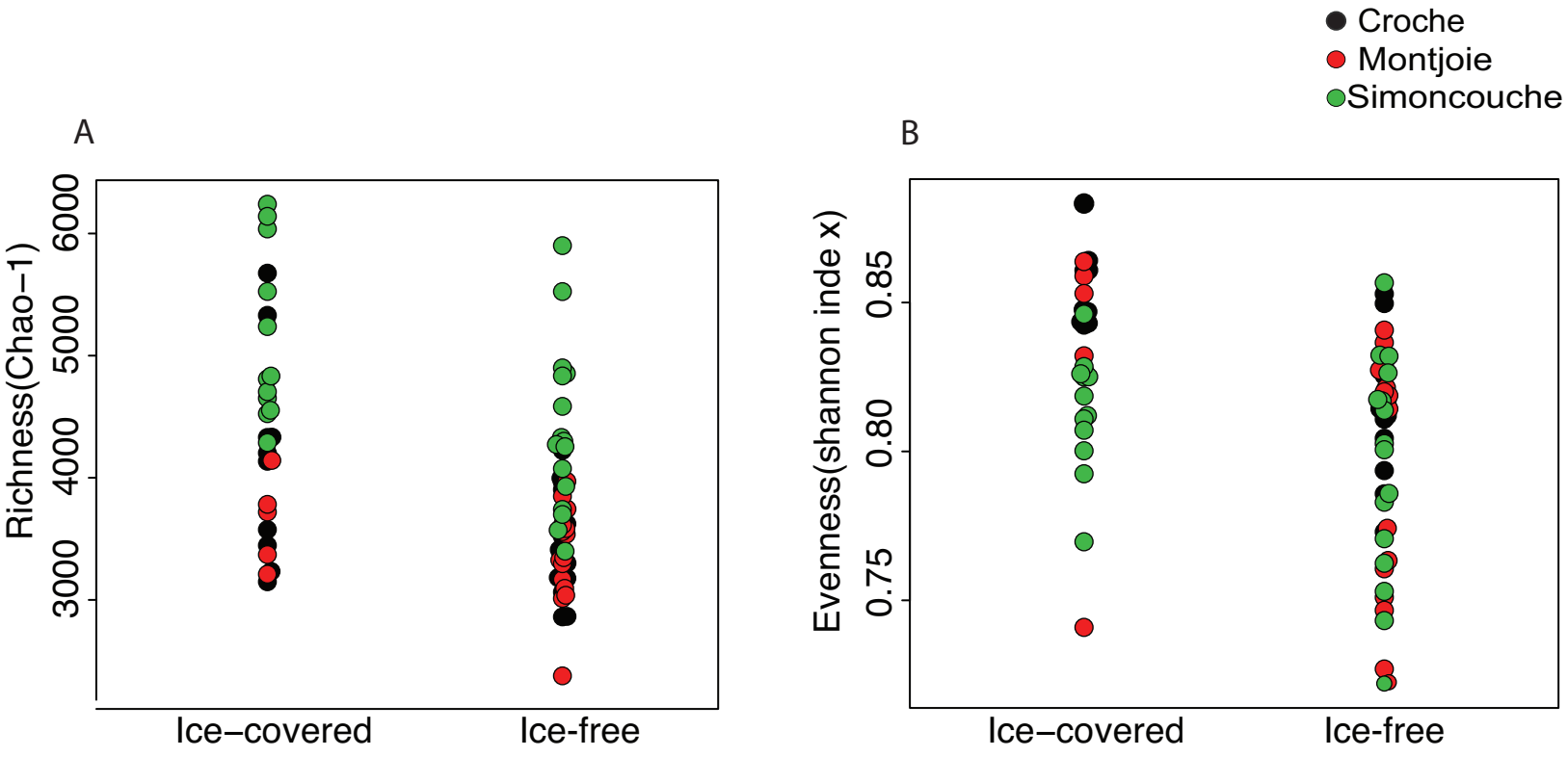


Figure 3. Seasonal community diversity indices. (A) Seasonal community richness (Chao 1 index). (B) Seasonal community evenness (Shannon-evenness index)

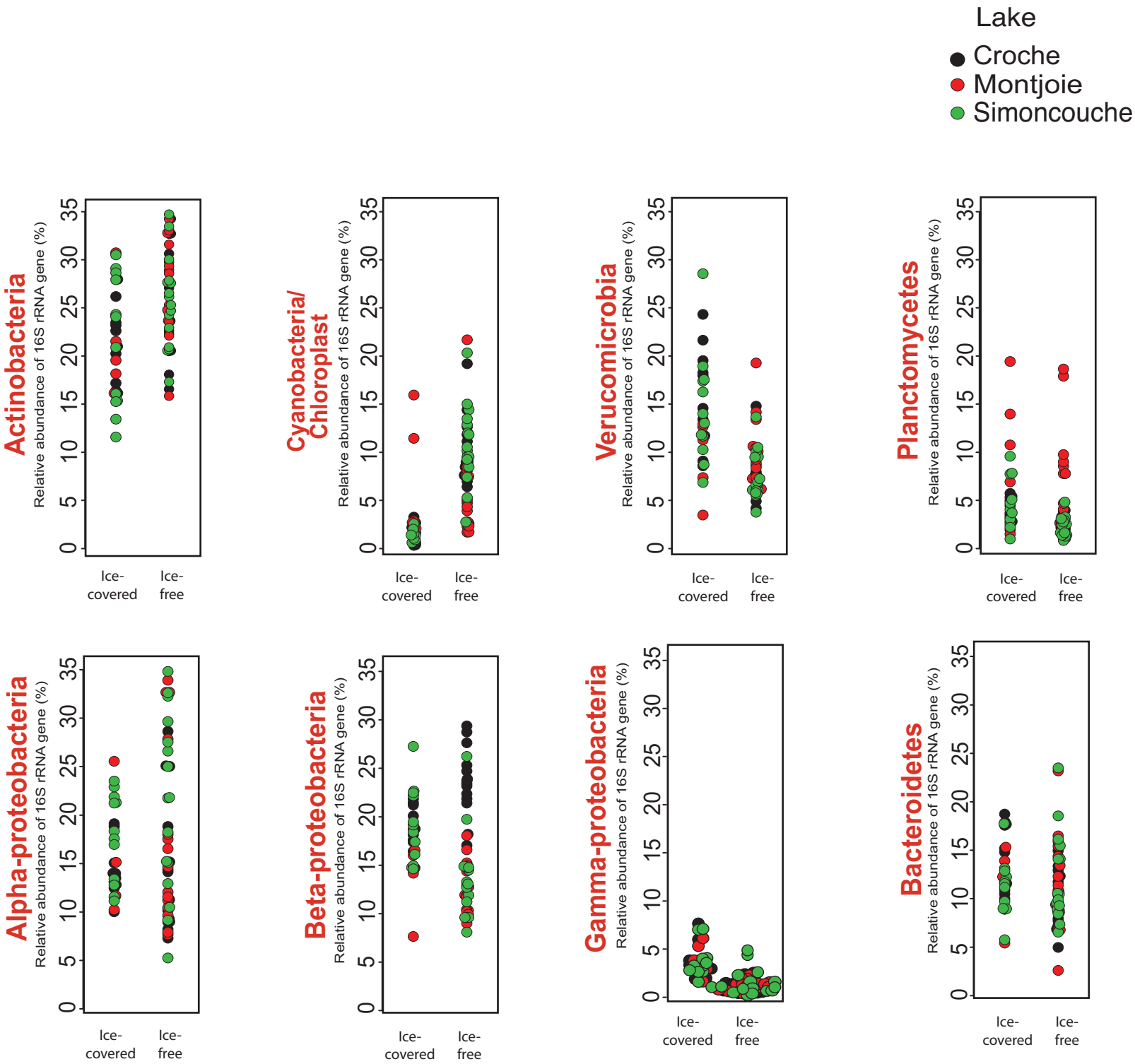


Figure 4. Relative abundances of 16S rRNA gene of select major bacterial groups during ice-covered and ice-free seasons within the three focal lakes.

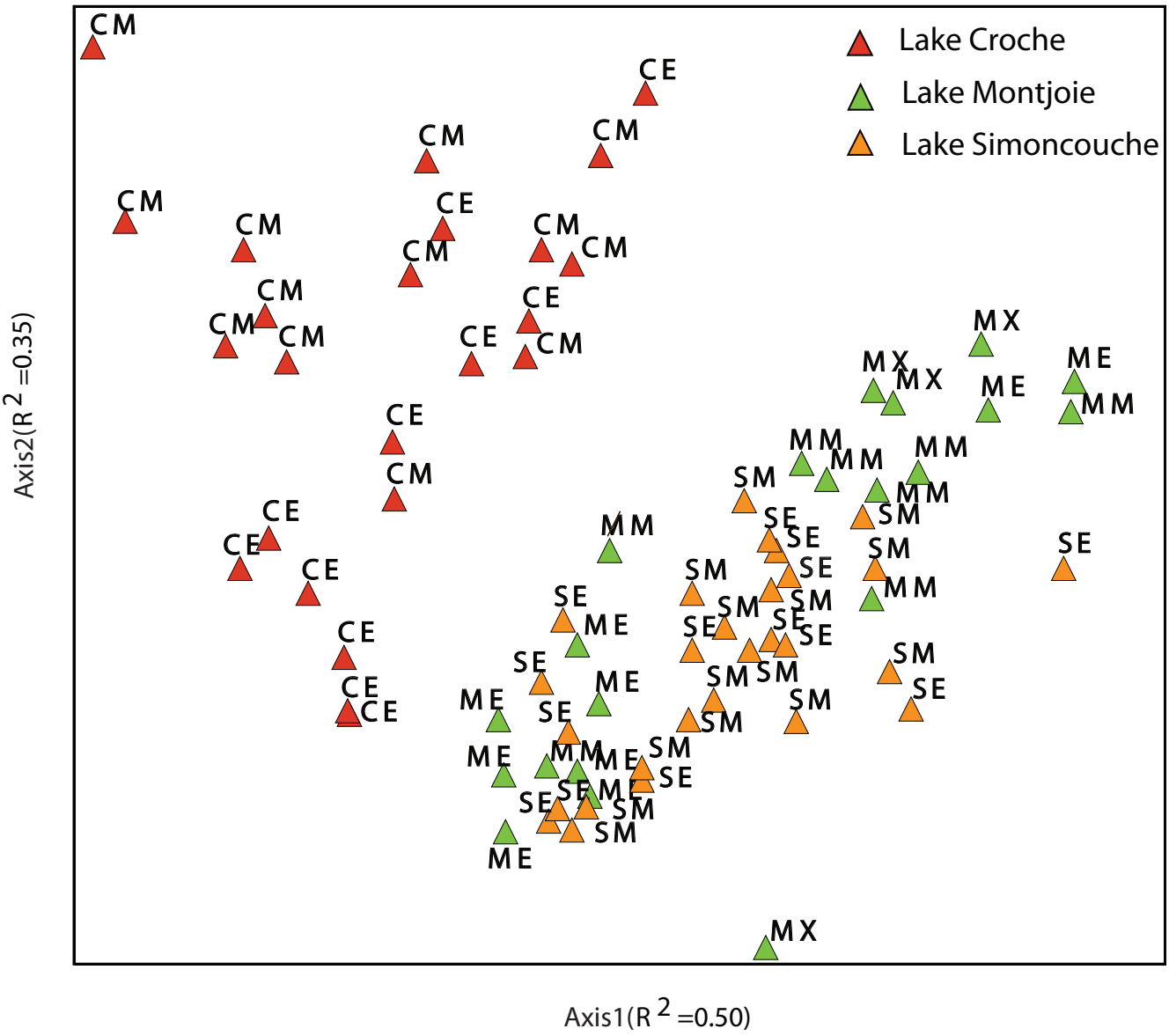


Figure 5. NMS ordination of all samples collected from Lake Croche, Lake Simoncouche and Lake Montjoie. The first letter represents the name of the lake (C=Croche, S=Simoncouche, M=Montjoie). The second letter represents the layer (E=eplimnion, M= metalimnion, X= mixed layer)

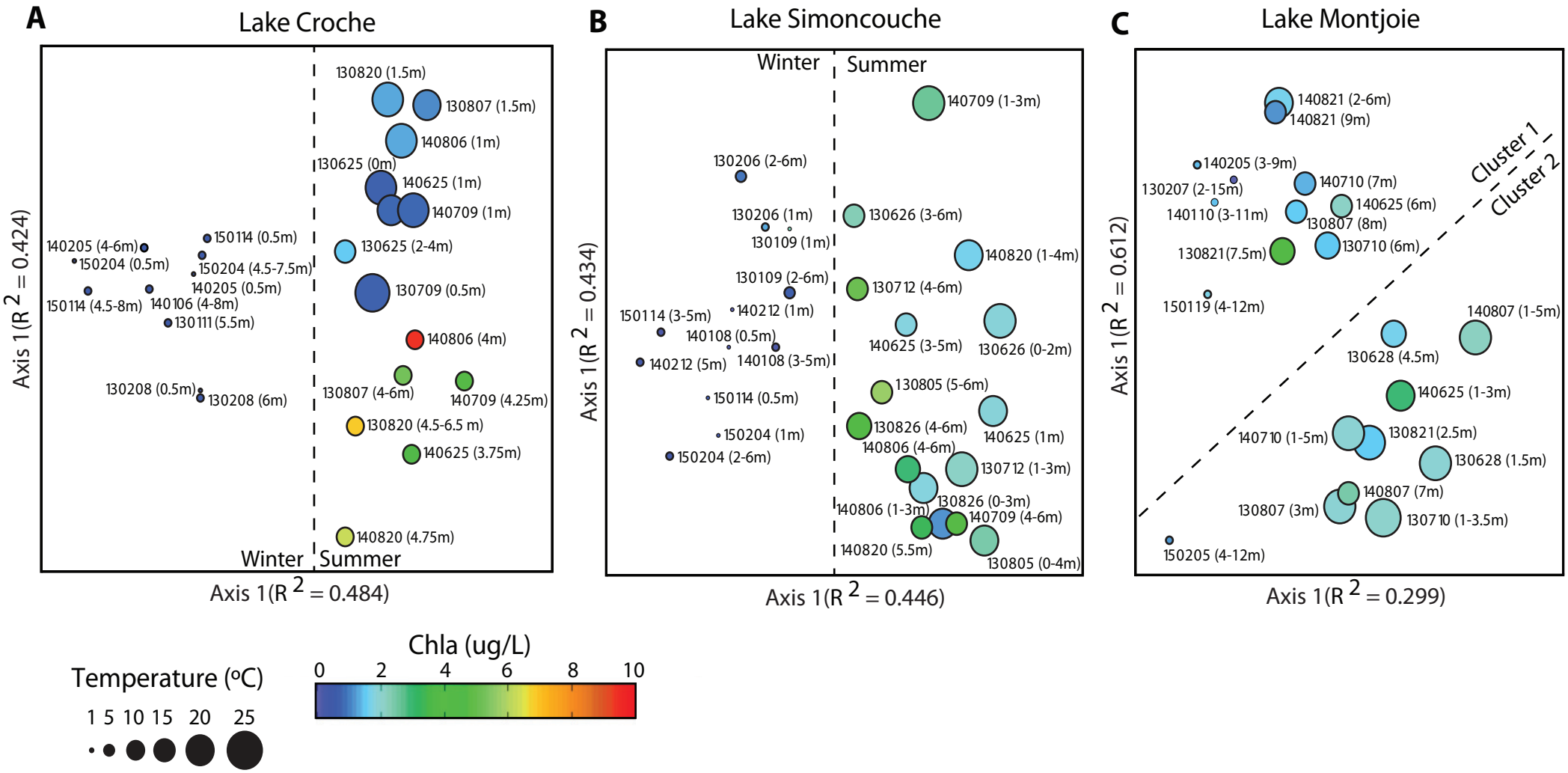
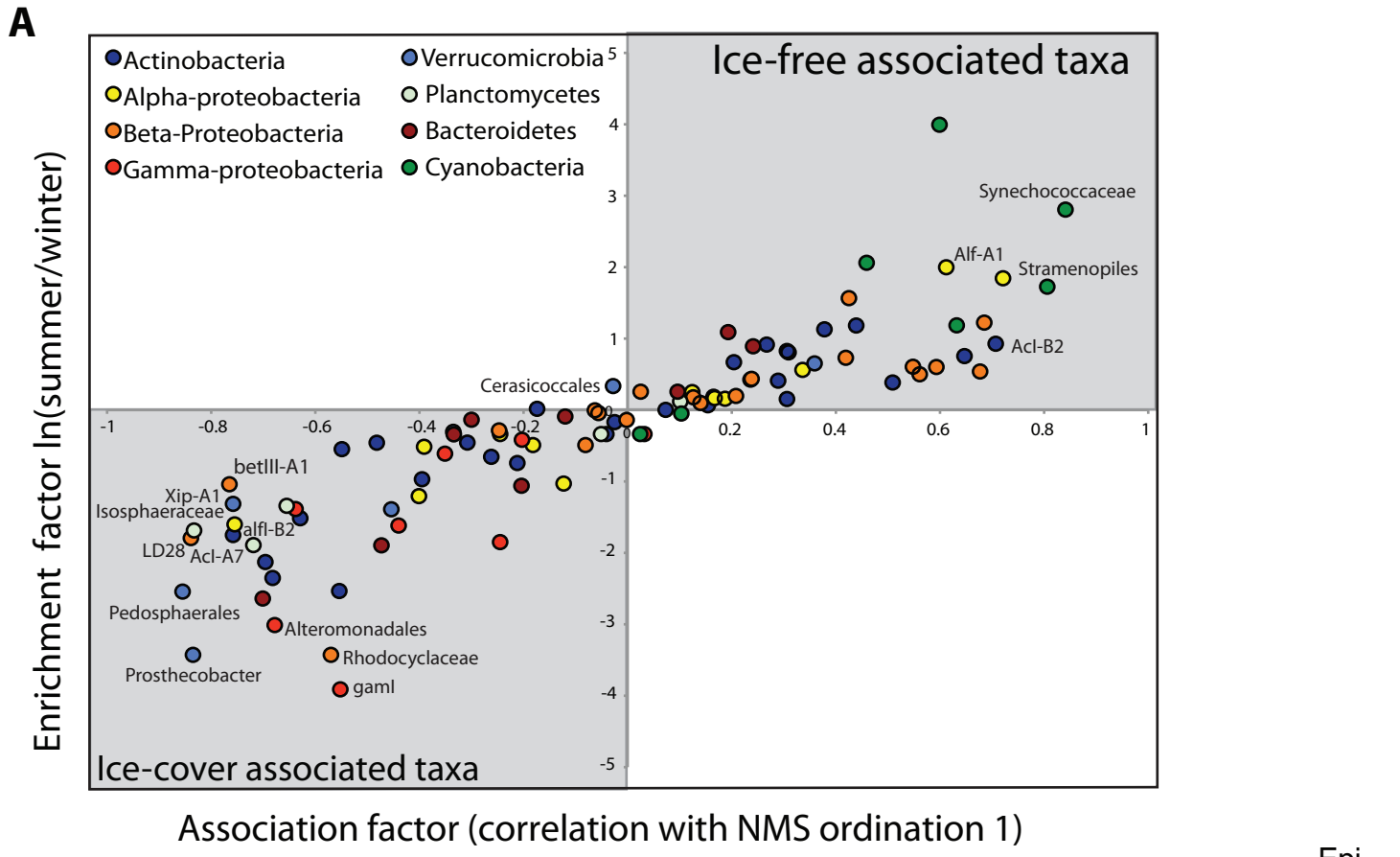
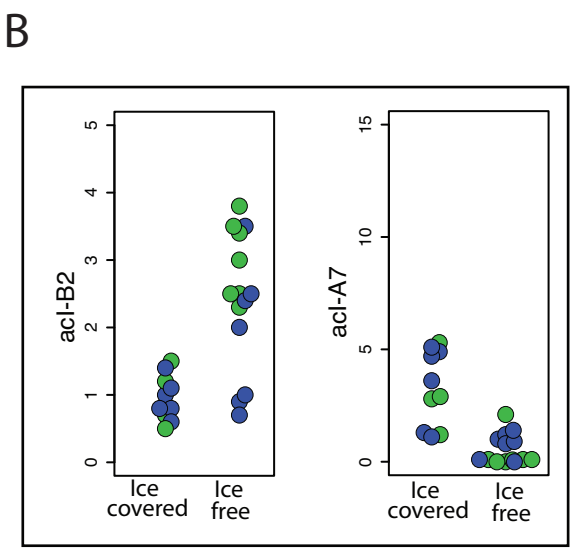
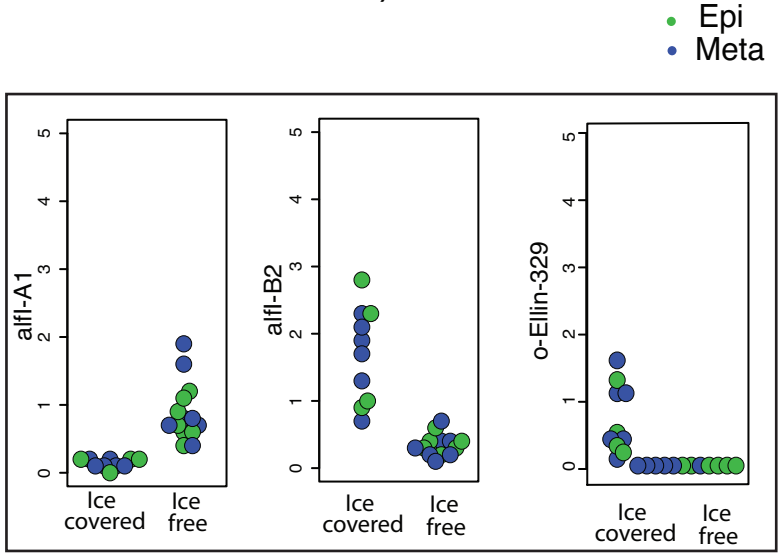
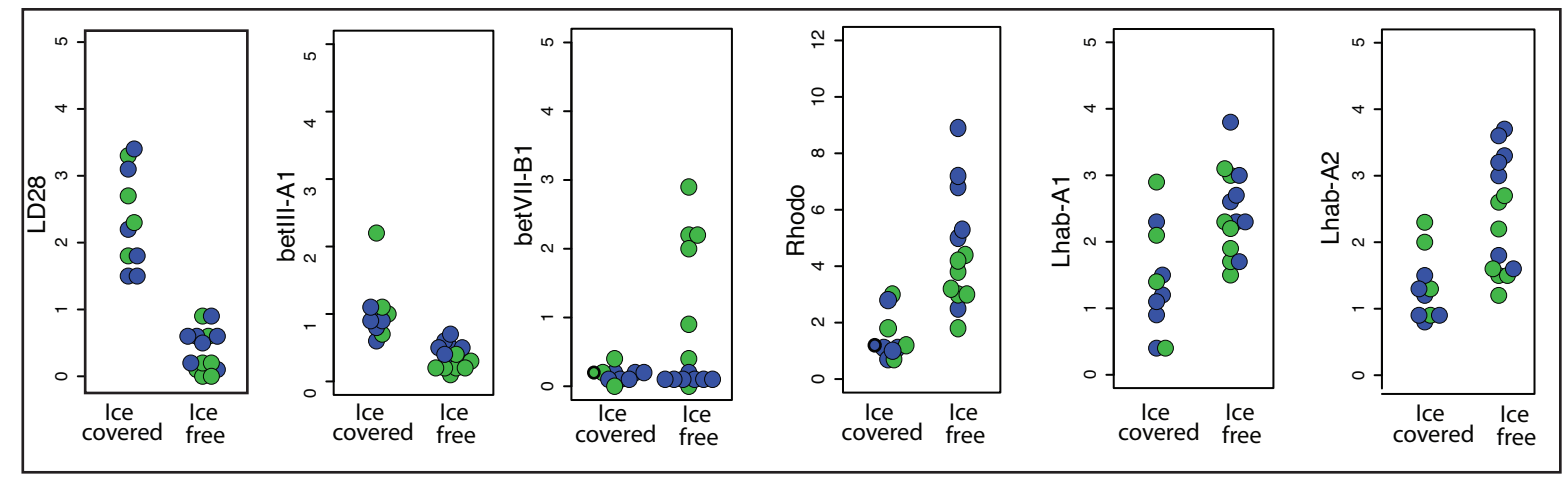


Figure 6. NMS ordination of samples collected during 2013-2015 from each of the studied lakes. (A) Samples collected from Croche. (B) Samples collected from Simoncouche. (C) Samples collected from Montjoie. Colour is an index of chlorophyll-a concentration (ug/L). Size of the circle is an index of water temperature.


A
Actinobacteria
Alpha-proteobacteria
Beta-Proteobacteria
Gamma-proteobacteria
Verrucomicrobia
Planctomycetes
Bacteroidetes
Cyanobacteria
Ice-free associated taxa
Ice-cover associated taxa
Synechococcaceae
Alf-A1
Stramenopiles
AcI-B2
Cerasicoccales
betIII-A1
Xip-A1
Isosphaeraceae
alfI-B2
LD28
AcI-A7
Pedosphaerales
Alteromonadales
Rhodocyclaceae
Prosthecobacter
gamI
Enrichment factor ln(summer/winter)
Association factor (correlation with NMS ordination 1)


B
acI-B2
acI-A7
Ice covered
Ice free


C
Epi
Meta
alfI-A1
alfI-B2
o-Ellin-329
Ice covered
Ice free


D
LD28
betIII-A1
betVII-B1
Rhodo
Lhab-A1
Lhab-A2
Ice covered
Ice free

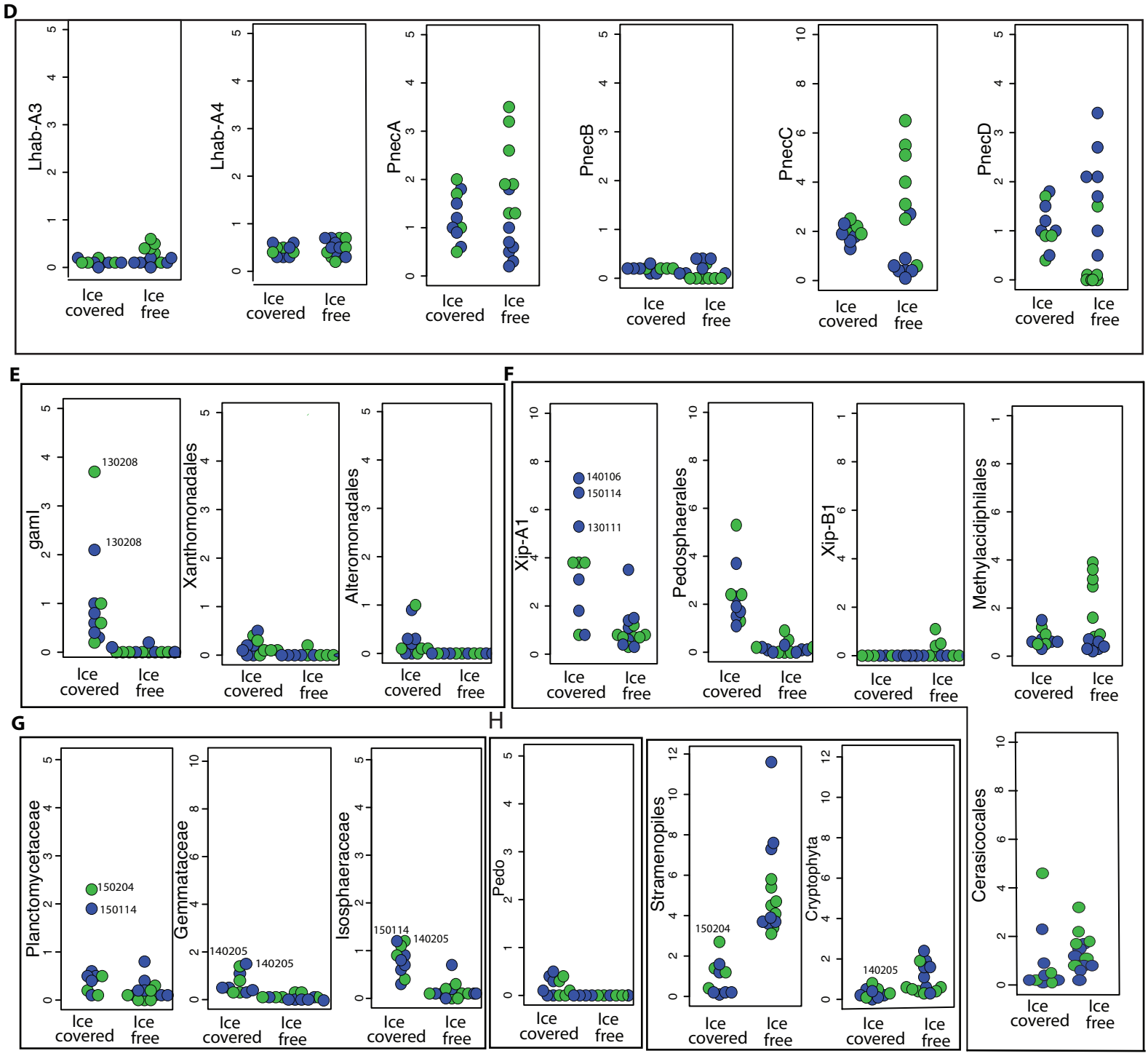
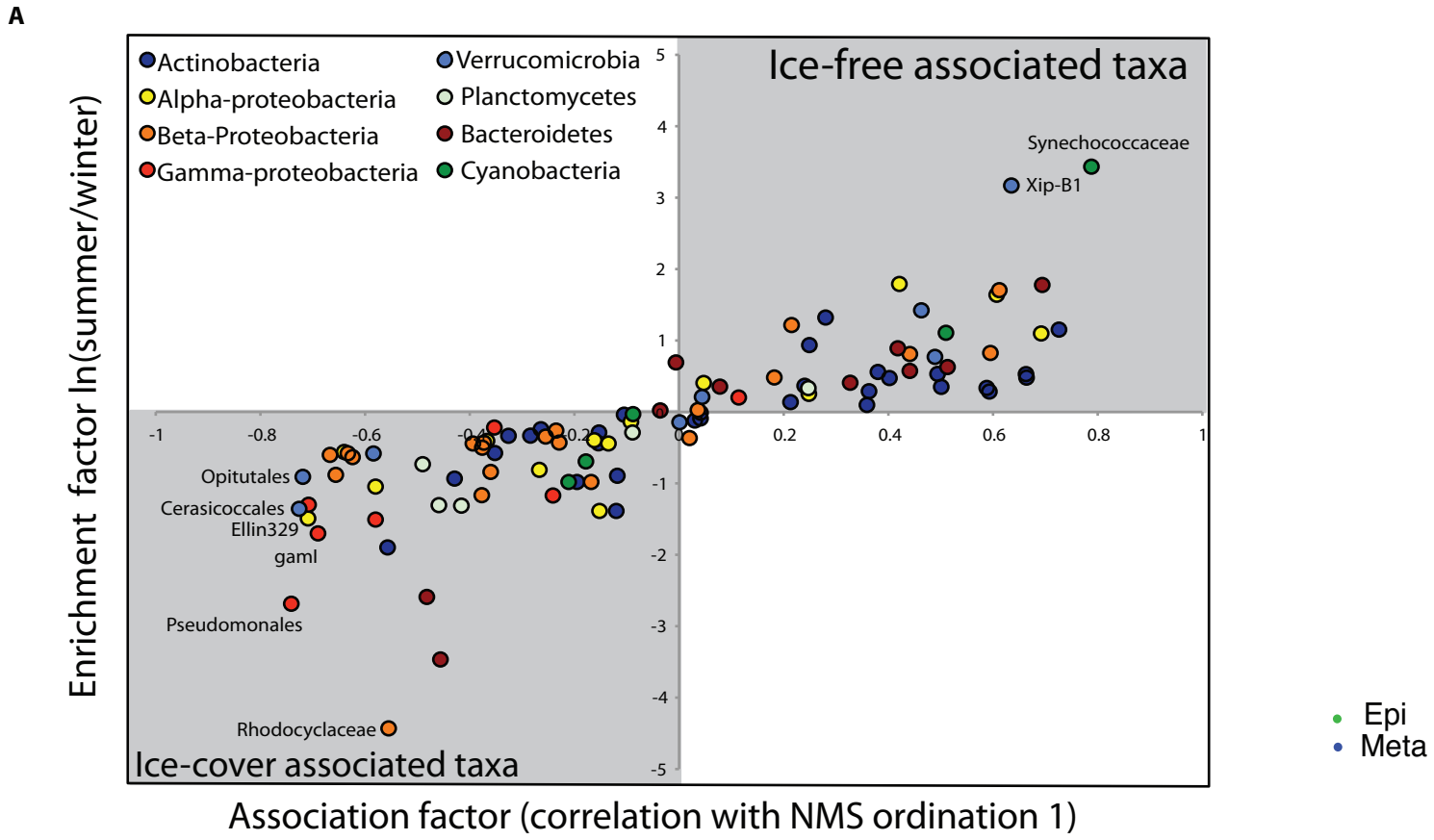
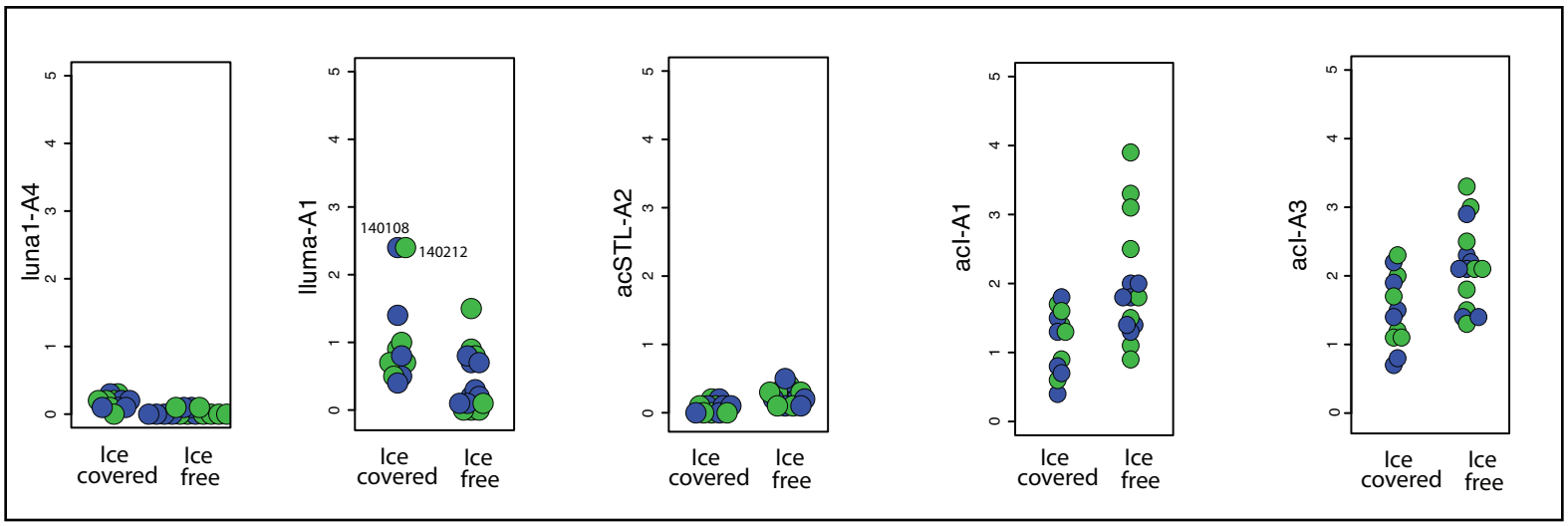
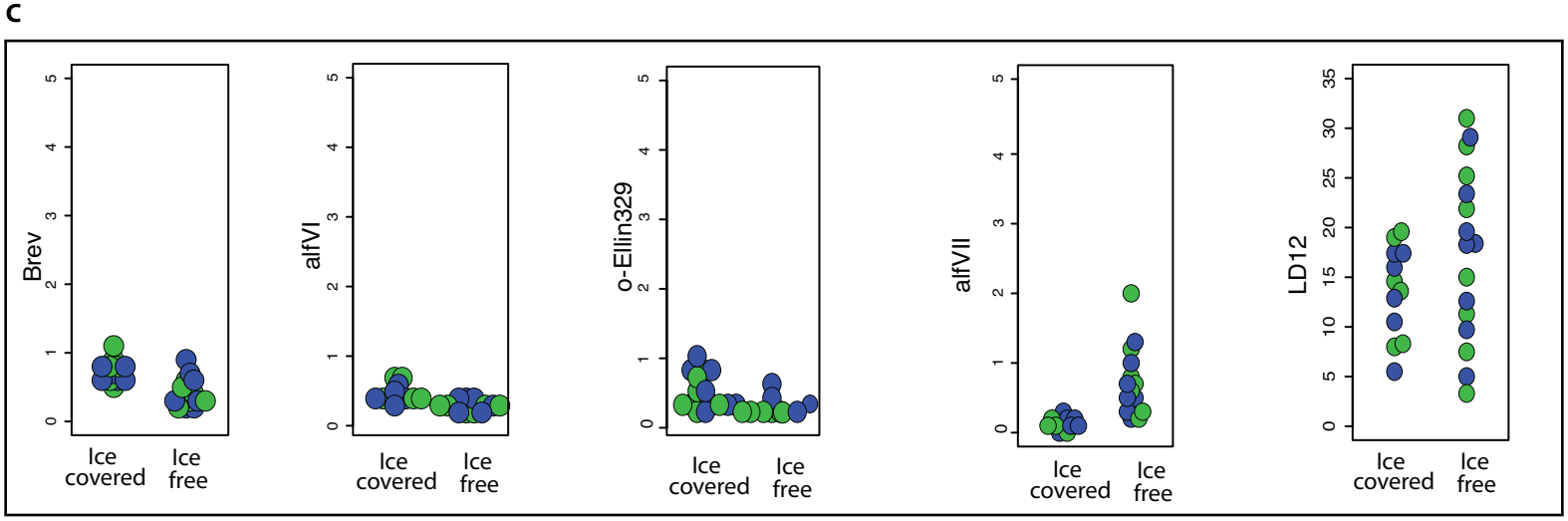


Figure 7. Bacterial seasonal association in Lake Croche. X axis (association factor) represents the correlation strengths of each taxon along ordination axis 1. Y axis (enrichment factor) calculated from the relative abundance ratio (ln (average relative abundances during summer/average relative abundance during winter)) for each taxon.


A
Actinobacteria
Alpha-proteobacteria
Beta-Proteobacteria
Gamma-proteobacteria
Verrucomicrobia
Planctomycetes
Bacteroidetes
Cyanobacteria
Ice-free associated taxa
Synechococcaceae
Xip-B1
Opitutales
Cerasicoccales
Ellin329
gaml
Pseudomonales
Rhodocyclaceae
Ice-cover associated taxa
Enrichment factor ln(summer/winter)
Association factor (correlation with NMS ordination 1)
Epi
Meta


B
luna1-A4
lluma-A1
140108
140212
acSTL-A2
acI-A1
acI-A3
Ice covered
Ice free


C
Brev
alfVI
o-Ellin329
alfVII
LD12
Ice covered
Ice free

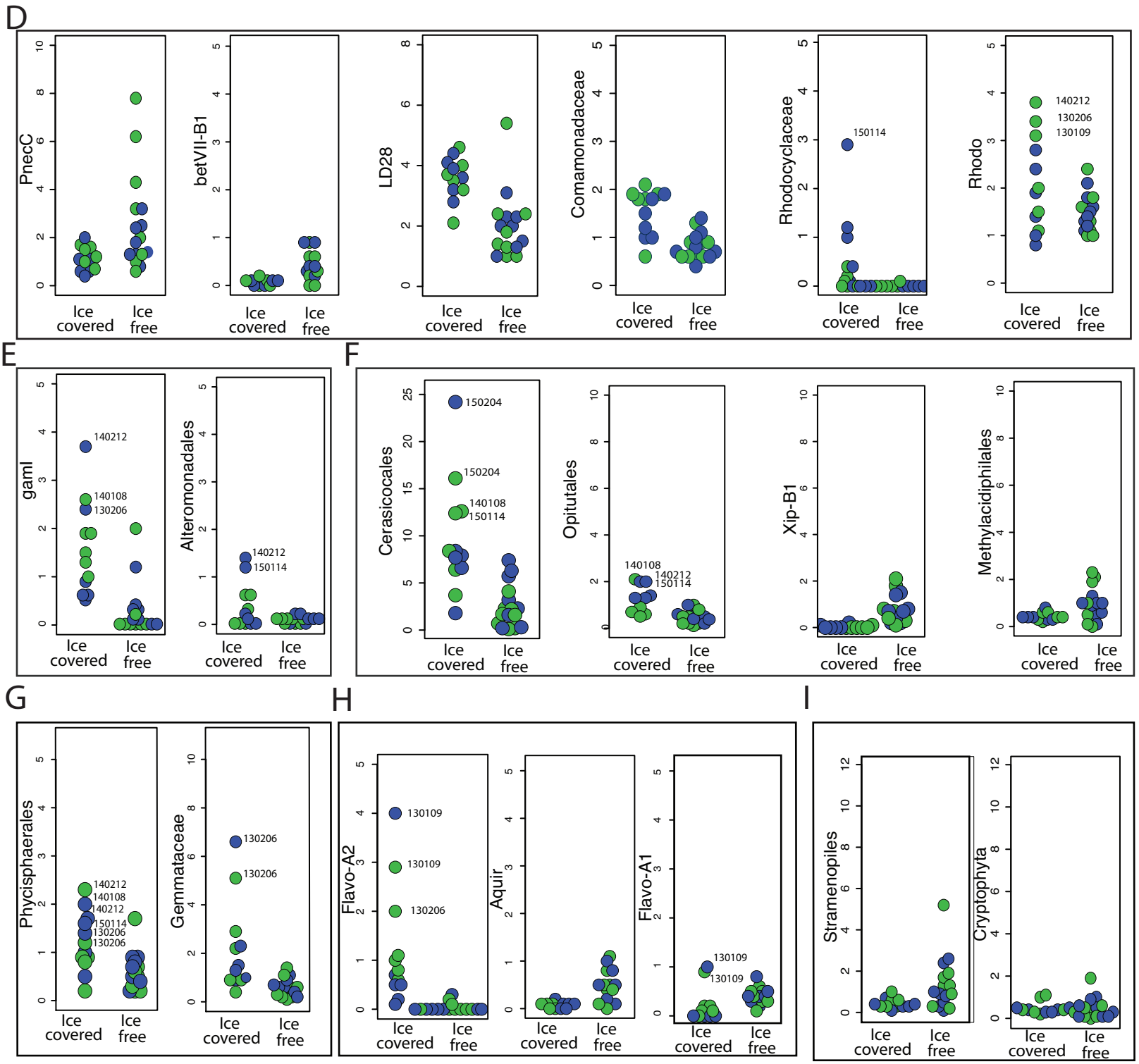
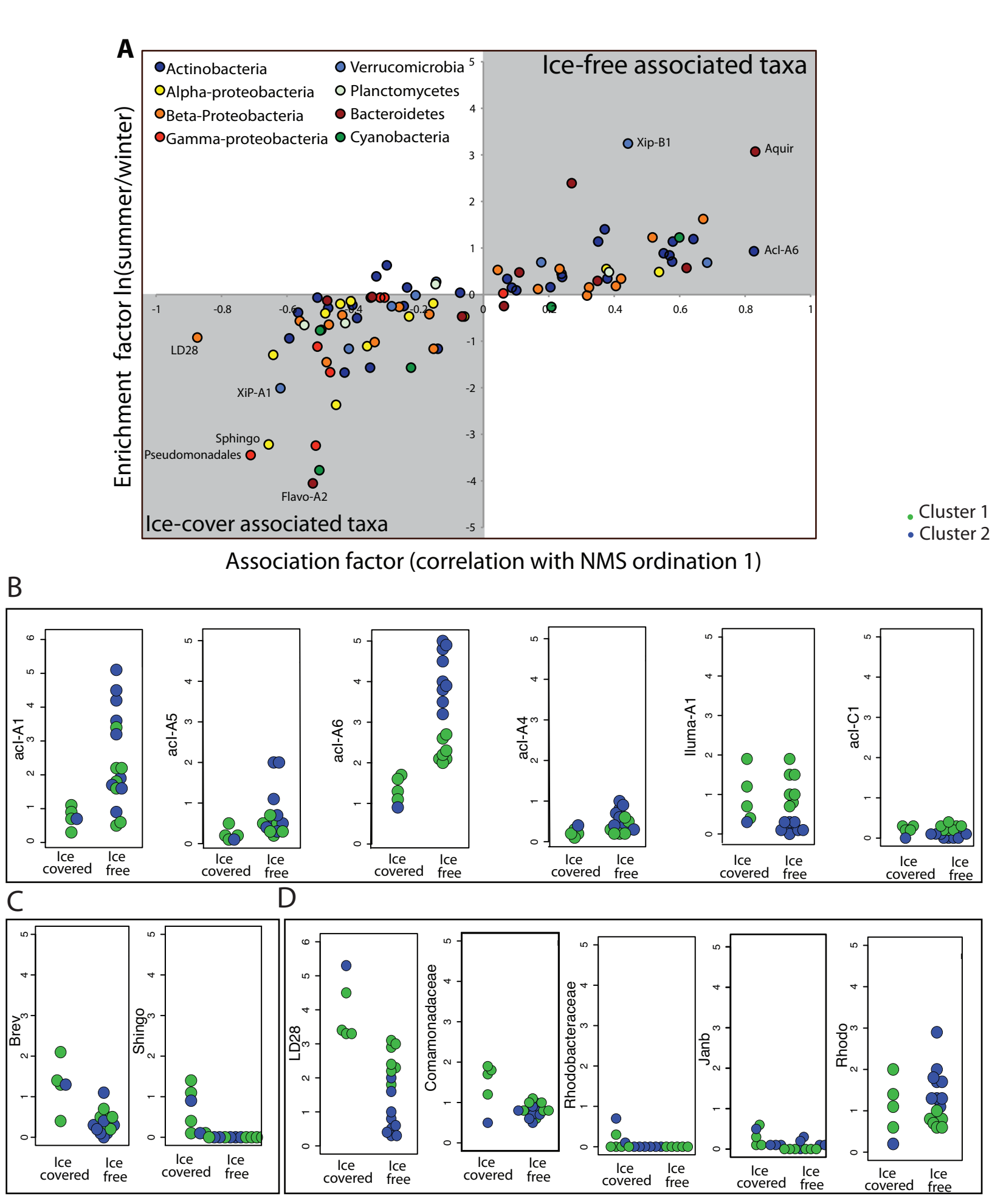


Figure 8. Bacterial seasonal association in Lake Simoncouche. X axis (association factor) represents the correlation strengths of each taxon along ordination axis 1. Y axis (enrichment factor) calculated from the relative abundance ratio (ln (average relative abundances during summer/average relative abundance during winter)) for each taxon.


A
Actinobacteria
Alpha-proteobacteria
Beta-Proteobacteria
Gamma-proteobacteria
Verrucomicrobia
Planctomycetes
Bacteroidetes
Cyanobacteria
Ice-free associated taxa
Ice-cover associated taxa
Xip-B1
Aquir
Acl-A6
LD28
XiP-A1
Sphingo
Pseudomonadales
Flavo-A2
Enrichment factor ln(summer/winter)
Association factor (correlation with NMS ordination 1)
Cluster 1
Cluster 2
B
acl-A1
acl-A5
acl-A6
acl-A4
Iluma-A1
acl-C1
Ice covered
Ice free
C
Brey
Shingo
D
LD28
Comamonadaceae
Rhodobacteraceae
Janb
Rhodo

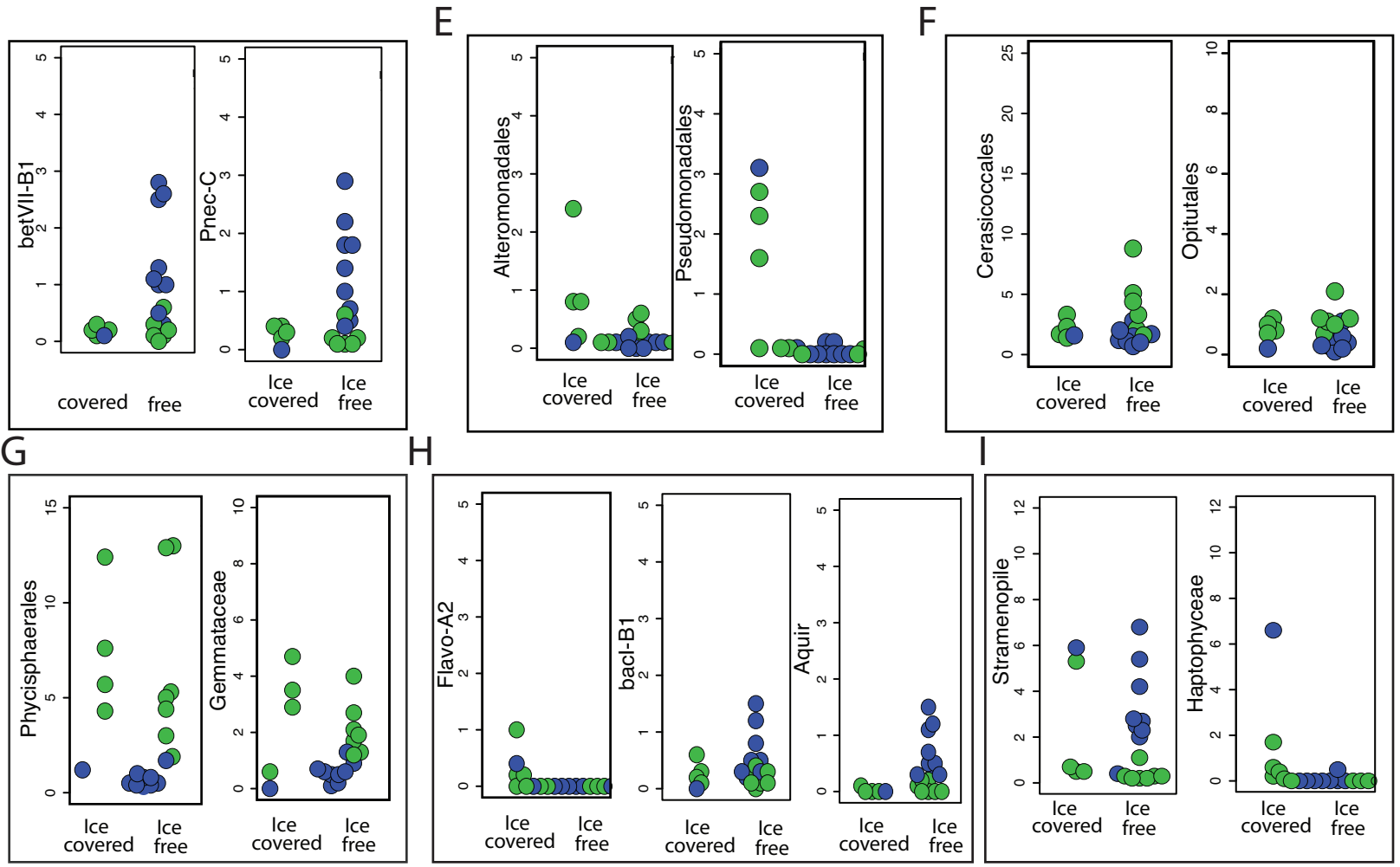


Figure 9. Bacterial seasonal association in Lake Montjoie. X axis (association factor) represents the correlation strengths of each taxon along ordination axis 1. Y axis (enrichment factor) calculated from the relative abundance ratio (ln (average relative abundances during summer/average relative abundance during winter)) for each taxon.

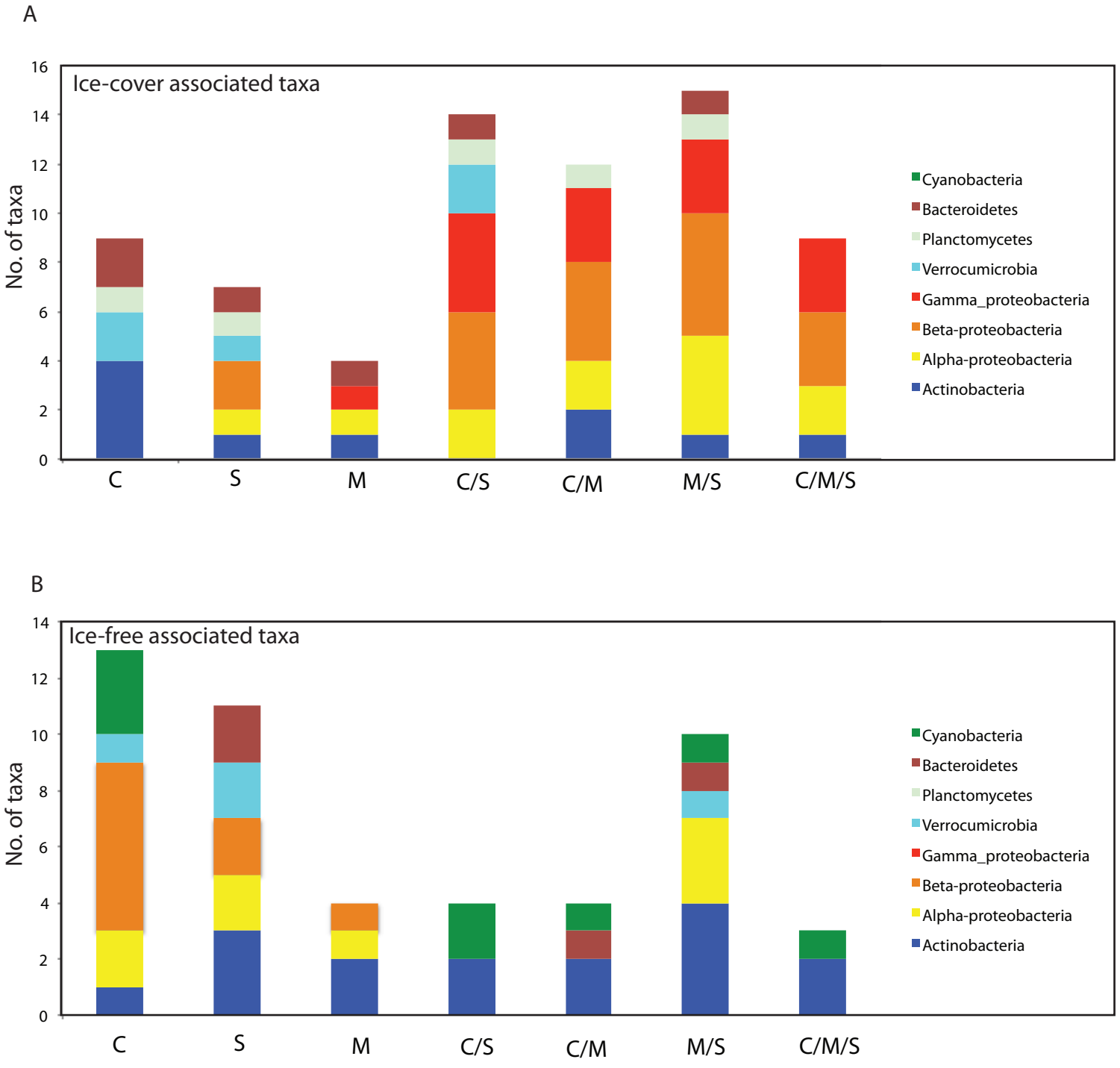


Figure 10. Subset of bacterial tribes exhibiting strong seasonal association across lakes.
A: subset of bacterial tribes exhibiting strong association during the ice-covered season. B: subset of bacterial tribes exhibiting strong association during the ice-free season. C: subset of bacterial tribes exhibiting strong seasonal association only in Lake Croche. S: subset of bacterial tribes exhibiting strong seasonal association only in Lake Simoncouche. M: subset of bacterial tribes exhibiting strong seasonal association only in Lake Montjoie. C/S: subset of bacterial tribes exhibiting strong seasonal association in Croche and Simoncouche. C/M: subset of bacterial tribes exhibiting strong seasonal association Croche and Montjoie. S/M: subset of bacterial tribes exhibiting strong seasonal association in Simoncouche and Montjoie. C/M/S: core taxa (subset of bacterial tribes exhibiting strong seasonal association in the three lakes).

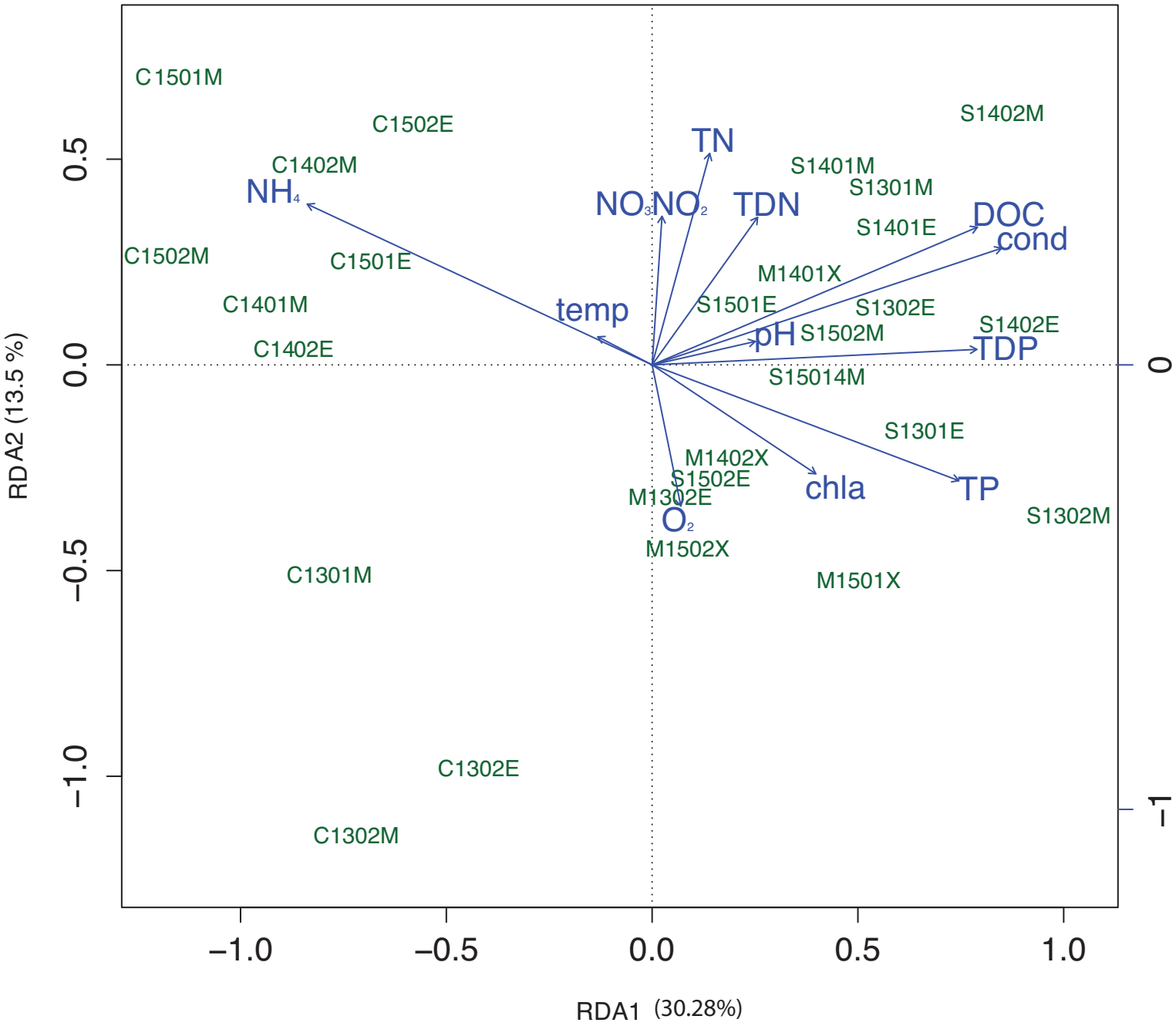


Figure 11. Redundancy-analysis (RDA) plot, illustrating the percent of the community variation caused by the environmental factors during the ice-covered season in Lake Croche, Lake Simoncouche and Lake Montjoie. The RDA explained 59 % of the total variation of the bacterial community during the ice-covered season.

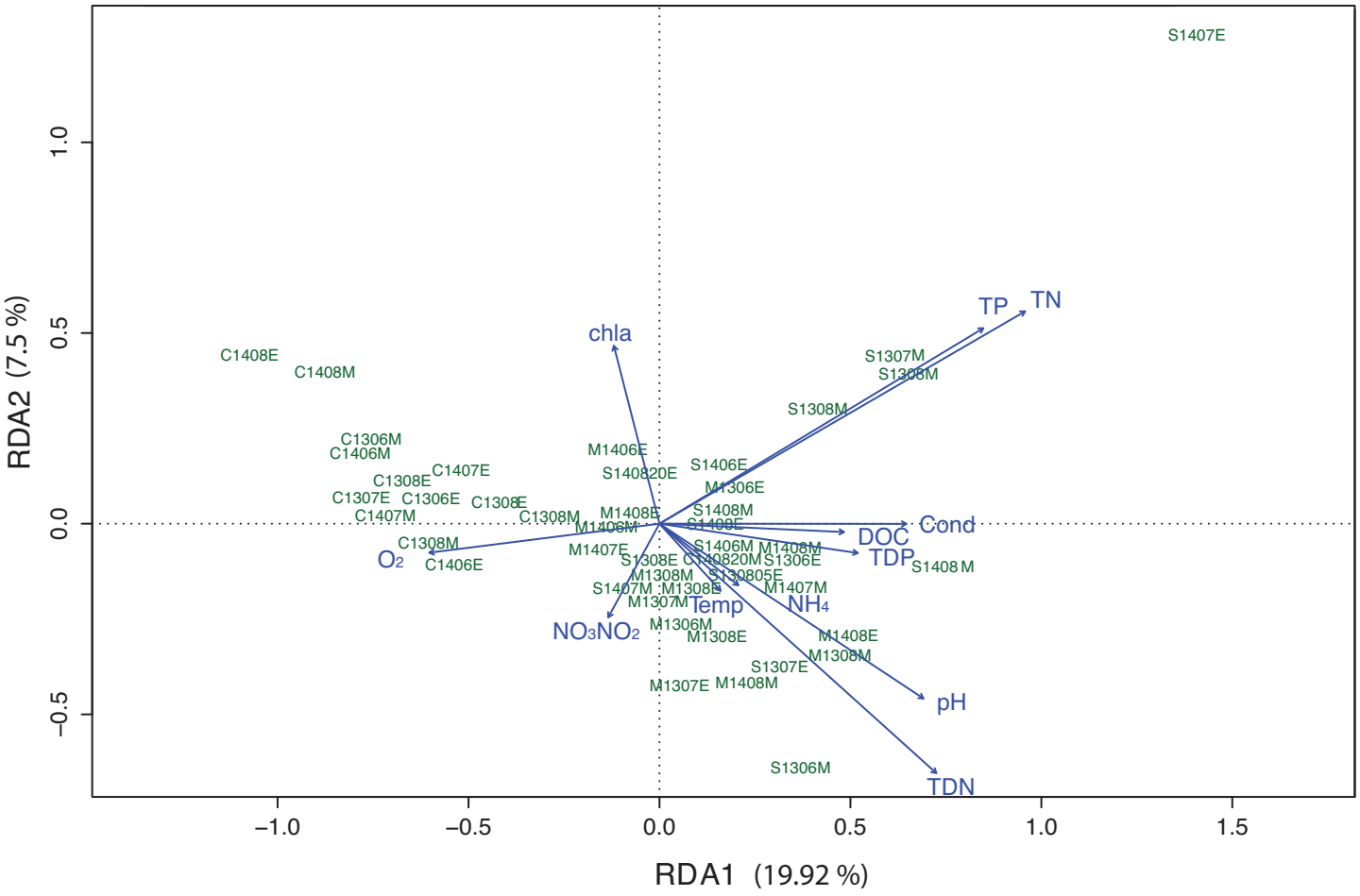


Figure 12. Redundancy-analysis (RDA) plot, illustrating the percent of the community variation caused by the environmental factors during the ice-covered season in Lake Croche, Lake Simoncouche and Lake Montjoie. The RDA explained 36 % of the total variation of the bacterial community during the ice-free season.

Supplementary Table 1: Environmental factors

Samples	Temp °C	conductivity µS/cm	pH	mean O2 mg L-1	NH4 µg L-1	DOC mg L-1
C_130111_MF	3.94	20.00	6.40	8.47	50.15	4.09
C_130208_EF	1.23	21.00	6.63	13.67	35.75	5.28
C_130208_MF	4.04	21.00	6.51	9.00	46.58	4.30
C_140106_MF	4.04	27.00	6.28	7.61	68.22	4.86
C_140205_EF	1.18	27.00	5.69	10.28	46.59	5.07
C_140205_MF	4.11	28.00	6.23	6.41	47.06	4.98
C_150114_EF	1.40	24.00	6.47	11.84	60.04	6.08
C_150114_MF	3.54	23.00	6.53	7.54	109.92	5.68
C_150204_EF	1.23	25.00	7.48	11.40	62.30	5.29
C_150204_MF	3.62	26.00	6.53	6.20	91.45	4.97
C_130625_EF	22.29	17.00	6.28	10.24	15.03	4.85
C_130625_MF	12.83	20.00	6.00	9.47	11.94	4.84
C_130709_EF	24.14	16.00	6.62	8.68	8.69	5.08
C_130807_EF	20.50	16.00	7.17	8.34	11.32	4.75
C_130807_MF	11.70	20.00	6.52	7.78	14.30	4.78
C_130820_EF	21.51	17.00	7.51	8.19	5.11	3.57
C_130820_MF	12.17	21.00	6.79	7.12	9.86	4.99
C_140625_EF	20.21	18.00	6.24	8.58	8.33	5.54
C_140625_MF	11.09	21.00	4.86	8.51	10.10	4.15
C_140709_EF	22.00	19.00	6.42	8.20	9.35	4.91
C_140709_MF	11.00	21.88	5.16	8.64	6.80	4.34
C_140806_EF	21.50	19.00	6.40	8.50	4.45	5.47
C_140806_MF	11.60	21.90	5.80	9.30	8.22	4.53
C_140820_MF	11.00	23.70	5.50	6.30	4.54	4.39
M_130207_EF	2.48	46.69	7.17	11.29	22.88	6.18
M_140110_XF	2.31	41.00	6.95	11.52	24.81	6.56
M_140205_XF	2.65	42.00	6.41	10.84	22.52	6.46
M_150119_XF	2.10	70.47	7.04	12.48	29.18	6.27
M_150205_XF	2.24	75.32	6.19	12.43	17.39	5.94
M_130628_EF	21.94	62.96	7.64	8.58	6.15	6.29
M_130628_MF	16.25	55.78	7.04	8.26	11.99	6.52
M_130710_EF	24.79	70.07	7.59	8.03	19.14	5.82
M_130710_MF	16.00	58.52	6.75	6.98	6.23	6.70
M_130807_EF	21.44	65.94	7.30	8.42	7.55	0.53
M_130807_MF	13.29	54.50	6.23	4.81	12.49	5.64
M_130821_EF	21.49	61.24	7.49	8.41	4.57	6.53
M_130821_MF	17.71	56.50	6.91	6.52	9.44	5.88
M_140625_EF	20.06	66.00	7.48	8.94	7.14	5.51
M_140625_MF	14.10	66.00	6.31	8.37	5.97	5.15

Samples	Temp °C	conductivity µS/cm	pH	mean O2 mg L-1	NH4 µg L-1	DOC mg L-1
M_140710_EF	21.19	65.68	6.62	8.38	3.68	6.02
M_140710_MF	14.60	65.10	6.10	7.15	5.87	5.85
M_140807_EF	21.70	66.04	7.63	8.40	4.84	5.88
M_140807_MF	12.70	65.28	6.39	4.76	4.31	5.69
M_140821_EF	20.09	66.20	7.35	8.29	5.22	5.92
M_140821_MF	13.29	66.00	6.51	3.77	2.78	5.77
S_130109_EF	1.33	117.81	7.41	11.50	21.06	5.61
S_130109_MF	4.23	187.71	7.11	7.30	54.40	6.17
S_130206_EF	1.81	147.76	7.88	11.06	18.23	6.44
S_130206_MF	4.36	245.20	7.06	5.65	15.08	6.13
S_140108_EF	0.81	124.00	7.55	11.42	21.66	6.93
S_140108_MF	3.46	179.00	7.08	6.56	42.85	6.20
S_140212_EF	0.96	243.00	5.27	11.19	34.75	7.50
S_140212_MF	3.87	374.00	5.88	5.08	22.07	6.54
S_150114_EF	0.96	119.00	6.33	16.44	20.01	7.61
S_150114_MF	4.11	179.00	6.57	9.22	25.80	7.05
S_150204_EF	0.86	122.00	6.17	10.90	23.40	5.40
S_150204_MF	4.00	207.69	6.19	4.08	24.34	6.66
S_130626_EF	21.01	110.00	7.71	8.75	9.84	6.78
S_130626_MF	14.85	191.10	7.06	6.34	45.85	6.60
S_130712_EF	20.88	109.00	7.61	8.50	11.19	6.60
S_130712_MF	13.63	254.22	6.75	3.75	41.78	6.27
S_130805_EF	20.33	121.92	7.55	7.94	9.09	6.57
S_130805_MF	13.74	320.17	6.69	2.22	15.33	6.11
S_130826_EF	19.93	127.00	7.45	8.67	12.73	13.08
S_130826_MF	15.36	230.80	6.58	3.28	6.45	14.05
S_140625_EF	19.98	133.00	6.72	8.71	5.05	5.48
S_140625_MF	14.47	371.00	6.51	6.91	22.99	5.47
S_140709_EF	21.58	137.00	7.03	7.62	3.03	5.95
S_140709_MF	13.50	508.04	6.61	5.32	184.62	5.83
S_140806_EF	20.70	283.00	7.13	8.56	6.32	5.88
S_140806_MF	15.53	906.00	6.53	4.71	90.69	6.12
S_140820_EF	19.33	151.00	7.09	8.90	2.36	6.02
S_140820_MF	14.83	559.00	6.65	3.93	152.91	6.02

Samples	TN µg L-1	TDN µg L-1	NO3NO2 µg L-1	TP µg L-1	TDP µg L-1	Chla µg L-1
C_130111_MF	418.24	262.96	18.69	8.91	1.72	0.37
C_130208_EF	253.26	253.28	34.57	5.10	2.24	0.51
C_130208_MF	247.32	268.43	24.02	4.38	1.59	0.24
C_140106_MF	419.93	312.11	21.96	3.52	1.65	0.30
C_140205_EF	332.93	207.04	32.67	3.22	1.02	0.30
C_140205_MF	309.18	192.86	26.61	3.24	1.32	0.13
C_150114_EF	340.64	287.34	24.40	1.94	1.94	0.31
C_150114_MF	378.79	338.67	42.97	2.08	2.08	0.37
C_150204_EF	317.71	269.62	29.33	1.71	1.71	0.22
C_150204_MF	405.75	351.43	84.18	2.04	2.04	0.16
C_130625_EF	291.76	211.60	18.65	4.18	2.09	0.73
C_130625_MF	290.43	187.73	22.42	5.14	1.81	1.43
C_130709_EF	229.86	180.32	2.66	4.77	3.53	0.75
C_130807_EF	202.27	155.39	1.04	4.99	3.51	0.97
C_130807_MF	235.15	222.54	14.23	6.28	3.19	5.39
C_130820_EF	230.64	153.36	0.00	4.65	2.92	1.30
C_130820_MF	289.40	223.62	15.04	8.04	3.28	6.81
C_140625_EF	224.49	226.08	2.18	6.50	2.26	0.78
C_140625_MF	293.82	231.14	26.35	8.34	1.91	3.72
C_140709_EF	210.07	189.94	2.77	12.12	1.43	0.81
C_140709_MF	203.73	159.33	10.72	5.65	0.93	4.17
C_140806_EF	197.51	177.00	0.00	3.59	1.57	1.19
C_140806_MF	233.13	184.63	1.33	6.82	0.96	9.41
C_140820_MF	216.78	150.89	0.00	5.80	0.71	6.22
M_130207_EF	288.25	255.02	23.93	6.40	2.51	0.00
M_140110_XF	476.42	266.75	10.07	11.02	3.67	1.52
M_140205_XF	390.03	263.00	15.74	7.95	3.38	1.36
M_150119_XF	310.08	289.44	8.29	6.18	3.71	1.70
M_150205_XF	404.36	272.89	10.56	6.73	2.59	1.32
M_130628_EF	341.51	239.40	0.00	6.39	2.63	1.95
M_130628_M	435.49	272.15	0.00	7.36	2.80	1.79
M_130710_EF	299.51	256.09	9.63	6.30	3.88	2.00
M_130710_M	296.78	294.94	0.45	8.66	4.70	1.57
M_130807_EF	292.76	233.25	0.00	10.15	4.45	1.90
M_130807_M	284.40	252.85	40.77	8.94	3.79	1.50
M_130821_EF	305.44	248.74	0.00	7.55	4.69	2.11
M_130821_M	286.22	256.18	24.64	9.46	4.67	4.64
M_140625_EF	411.77	318.27	0.00	8.89	3.04	2.81
M_140625_M	379.74	228.03	10.59	9.05	2.74	2.13

Samples	TN µg L-1	TDN µg L-1	NO3NO2 µg L-1	TP µg L-1	TDP µg L-1	Chla µg L-1
M_140710_EF	310.77	235.79	0.00	6.31	1.78	1.96
M_140710_M	277.48	236.42	24.49	7.90	1.81	1.51
M_140807_EF	371.80	262.59	0.00	7.95	1.98	2.12
M_140807_M	266.69	200.14	2.24	8.21	2.13	2.37
M_140821_EF	311.32	286.47	0.00	6.93	1.96	1.67
M_140821_M	294.99	245.55	41.98	7.57	1.94	1.20
S_130109_EF	360.79	262.42	27.84	7.36	3.03	2.22
S_130109_MF	352.24	312.60	28.59	7.01	4.00	0.67
S_130206_EF	322.31	281.40	67.89	5.63	2.37	1.22
S_130206_MF	331.41	327.06	23.93	7.03	2.62	0.80
S_140108_EF	376.07	309.61	32.57	5.96	2.84	0.25
S_140108_MF	368.50	313.16	17.51	5.97	2.64	0.10
S_140212_EF	411.45	339.54	37.88	7.48	5.75	0.00
S_140212_MF	450.68	328.52	81.47	6.97	2.72	0.26
S_150114_EF	412.16	293.90	29.69	6.70	2.88	0.52
S_150114_MF	325.52	282.09	42.97	7.77	2.73	0.22
S_150204_EF	287.13	216.41	29.33	5.68	2.87	0.40
S_150204_MF	343.01	291.21	84.18	5.75	2.90	0.35
S_130626_EF	370.63	263.63	0.00	9.72	3.61	1.85
S_130626_MF	339.88	374.66	4.57	10.06	3.22	2.25
S_130712_EF	331.23	322.39	0.00	13.02	6.61	2.10
S_130712_MF	445.43	279.08	0.56	25.98	7.00	5.11
S_130805_EF	368.32	275.82	0.00	9.70	8.95	2.30
S_130805_MF	348.93	232.75	0.00	19.51	8.09	5.76
S_130826_EF	339.67	303.55	0.00	9.99	9.27	1.32
S_130826_MF	362.82	255.33	0.00	11.01	9.68	4.42
S_140625_EF	351.37	254.02	0.00	14.41	2.62	1.90
S_140625_MF	309.78	266.17	5.58	8.54	2.42	1.85
S_140709_EF	834.49	201.44	0.00	21.59	2.29	2.66
S_140709_MF	470.96	438.81	37.55	18.87	2.51	4.07
S_140806_EF	265.82	242.76	0.96	6.38	1.60	1.87
S_140806_MF	285.05	385.47	8.95	11.31	3.11	2.92
S_140820_EF	294.50	213.21	1.45	7.27	2.28	1.72
S_140820_MF	456.57	380.47	14.18	11.47	2.34	3.49

Supplementary Table 2: Diversity indices

Group	Chao-1	Shannon
Ice-coverd period		
Sampeles	**chao**	**shannon**
C_130111_MF	3233.49528	0.826917
C_130208_EF	5330.33036	0.864151
C_130208_MF	5675.27248	0.88325
C_140106_MF	3575.23305	0.843073
C_140205_EF	3446.80169	0.847608
C_140205_MF	3148.99115	0.842434
C_150114_EF	4134.47584	0.843562
C_150114_MF	4203.35556	0.860847
C_150204_EF	4330.44622	0.860793
C_150204_MF	4332.28947	0.846959
S_130109_EF	6037.81395	0.824971
S_130109_MF	5526	0.818603
S_130206_EF	4652.03587	0.82509
S_130206_MF	6239.97266	0.84608
S_140108_EF	4524.34818	0.800213
S_140108_MF	5238.52113	0.812018
S_140212_EF	6141.18548	0.828464
S_140212_MF	4808.9823	0.810932
S_150114_EF	4552.37643	0.80712
S_150114_MF	4704.47059	0.826066
S_150204_EF	4832.34657	0.792486
S_150204_MF	4287.89243	0.769654
M_130207_EF	3370.73391	0.832096
M_140110_XF	3718.90435	0.858888
M_140205_XF	3782.26506	0.863739
M_150119_XF	4142.31395	0.853041
M_150205_XF	3210.89202	0.740917

Ice-Free period		
Sampeles	**chao**	**shannon**
C_130625_EF	3301.74725	0.785763
C_130625_MF	3996.80569	0.804416
C_130709_EF	3516.71963	0.816863
C_130807_EF	3063.27228	0.77299
C_130807_MF	3182.22816	0.831884
C_130820_EF	4223.40845	0.812009
C_130820_MF	3902.89952	0.849662
C_140625_EF	3961.66292	0.810822
C_140625_MF	2865.06061	0.829148
C_140709_EF	3623.06115	0.852902
C_140709_MF	3411.58333	0.814342
C_140806_EF	2860.75431	0.79355
C_140806_MF	3177.28947	0.815602
C_140820_MF	3377.8984	0.825455
S_130626_EF	3571.43612	0.81697
S_130626_MF	3929.30592	0.856622
S_130712_EF	3741.00797	0.762435
S_130712_MF	5524.65041	0.813829
S_130805_EF	3398.34061	0.722047
S_130805_MF	4854.12632	0.802613
S_130826_EF	4074.78161	0.753012
S_130826_MF	4899.83696	0.817452
S_140625_EF	4330.23849	0.800548
S_140625_MF	4299.10728	0.832317
S_140709_EF	5901.24521	0.831976
S_140709_MF	3699.45622	0.743206
S_140806_EF	4584.46224	0.770692

	Ice-Free period	
Sampeles	**chao**	**shannon**
S_140806_MF	4834.11745	0.78293
S_140820_EF	4272.43946	0.826373
S_140820_MF	4254.64876	0.78587
M_130628_EF	3011.61039	0.751037
M_130628_MF	3743.82589	0.814268
M_130710_EF	2377.96209	0.726912
M_130710_MF	3166.97951	0.818421
M_130807_EF	3326.35983	0.722483
M_130807_MF	3537.4918	0.836533
M_130821_EF	3848.73643	0.760606
M_130821_MF	4271.22603	0.8214
M_140625_EF	3296.02395	0.774192
M_140625_MF	3095.21005	0.82723
M_140710_EF	3559.25581	0.763381
M_140710_MF	3583.89568	0.840743
M_140807_EF	3970.06787	0.818792
M_140807_MF	3037.6747	0.74664
M_140821_EF	3346.36364	0.827328
M_140821_MF	3612.71548	0.820009

	Chao-1(average)		
	Croche	**Montjoie**	**Simoncouche**
Ice-covered	4141.07	3645.02	5128.83
Ice-Free	3461.74	3424.09	4385.58

Shannon (average)			
	Croche	**Montjoie**	**Simoncouche**
Ice-covered	0.85	0.83	0.81
Ice-Free	0.82	0.79	0.79