1 Introduction

Permafrost is widespread in Arctic and boreal regions (Schuur et al., 2008) and is estimated to contain ca. 1700 Pg of organic carbon (McGuire et al., 2009; Tarnocai et al., 2009). Permafrost thawing and erosion is evident by the northward retreat of the permafrost boundary (Thibault and Payette, 2009). In some northern regions this has led to the expansion of permafrost thaw ponds and lakes (thermokarst systems; Grosse et al., 2013), whereas in other regions there has been a contraction and loss of these waterbodies (e.g., Andresen and Lougheed, 2015). These thermokarst systems are part of circumpolar and global biogeochemical cycles (Abnizova et al., 2012; Walter et al., 2007). Although some are carbon sinks (Walter Anthony et al., 2014), others are net sources of carbon dioxide (CO_2) and methane (CH_4) to the atmosphere due to the mobilization of ancient carbon stored in permafrost (Laurion et al., 2010; Negandhi et al., 2013; Walter et al., 2008).

Bacterial communities are among the main drivers of key biogeochemical processes (Ducklow, 2008), and in thermokarst systems are composed of functionally diverse taxa (Crevecoeur et al., 2015; Rossi et al., 2013). In particular, these systems are favorable for bacterial methanotrophs (Crevecoeur et al., 2015) as well as archaeal methanogens (Mondav et al., 2014), and the relative activity of these two groups will affect methane balance and the net emission of greenhouse gases. Identifying factors that shape bacterial communities in these aquatic systems is therefore essential for understanding the functional significance of these permafrost thaw systems in the global carbon budget.

Aquatic bacterial communities are thought to be selected by a combination of bottom-up (resource availability) and top-down (viral lysis, grazing) controls. Less studied are bacteria-bacteria interactions (facilitation, competition), which may further contribute to non-random distributions observed among microbial taxa (e.g., Horner-Devine et al., 2007). Examining co-occurrence patterns has the potential to unveil ecological processes that structure bacterial communities. Specifically, patterns of co-occurrence may reveal to what extent groups of microbes share habitat preferences, to what extent there may be ecological linkages among bacterial taxa and with other planktonic organisms, and the extent of phylogenetic closeness of co-occurring bacterial taxa given that closely related taxa may share life strategies and ecological traits.

Résumé des commentaires sur Ms Comte track mode IL_bgd-12-C7646-2015-supplement.pdf

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the argument behind mentionning this is unclear: is it because this stock of OC once dissolved in lake water can drive the bacterial communities as evidenced by your study?

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this part of sentence is only supported by Walter et al. 2008, not by Laurion et al. 2010 neither by Negandhi et al 2013. And in fact I don't think it's right to say they are net sources of GHG to the atmosphere DUE to the mobilization of ancient C. There is still a lot of work to be done to quantify to which degree the old stock is fueling microbes. And see Bouchard et al. in the special issue related to this question.

Across northern landscapes, both regional (e.g., climate and the degradation state of permafrost) and local (e.g., nutrients, dissolved organic carbon and oxygen) conditions are likely to influence the distribution and bacterial community composition of thaw ponds and lakes. Thaw ponds and lakes show a high degree of limnological (Deshpande et al., 2015) and bacterial heterogeneity (Crevecoeur et al., 2015), making them suitable models to investigate the co-occurrence patterns among bacterial taxa as well their network relationships within microbial consortia. The main objectives of this study were to characterize the ecological linkages within microbial communities as a response to permafrost thawing. Our hypotheses were that (i) bacterial communities follow co-occurrence patterns along the permafrost degradation gradient, due to distinct habitat preferences among bacteria, and (ii) these habitat preferences relate to differences in the phylogenetic structure of bacterial communities.

To test the above hypotheses, we employed high-throughput sequencing of the 16S rRNA gene to determine the composition of bacterial communities in thaw ponds and lakes of Nunavik (Quebec, Canada) along a North-South permafrost degradation gradient. In addition, we sampled rock-basin lakes that were under the same regional climate but whose formation was not related to climate change. We investigated the relationships among bacterial taxa and local environmental conditions by means of network analysis, which has been applied with success elsewhere to evaluate microbial distribution patterns (Barberan et al., 2012; Peura et al., 2015; Steele et al., 2011) and responses to environmental perturbation (Araújo et al., 2011). We then examined the potential linkages between the bacteria and phytoplankton, autotrophic picoplankton and zooplankton biomass in the ponds.

2 Methods

2.1 Study sites and sampling

Surface water (0.2 m) from 29 thermokarst ponds was collected from 1 to 13 August 2012 in two types of permafrost landscapes. Thaw ponds were located in the vicinity of Whapmagoostui-Kuujjuarapik (W-K: lat. 55° 15' N, long. 77° 45' W) and Umiujaq (lat. 56º 32’ N, long. 76º 33’ W), within four valleys in the eastern Canadian subarctic,

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reverse so that we can understand you mean bacterial distribution, otherwise it becomes the distribution of lakes and ponds

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why autotrophic and not phototrophic?

Nunavik along a North-South permafrost degradation gradient as described in Comte et al. (2015): the Sasapimakwananisikw River valley (SAS) and the Kwakwatanikapistikw River valley (KWK), in sporadic, highly degraded permafrost landscapes (< 10% permafrost coverage; see Bhiry et al. 2011 for details); and the Sheldrake River valley (BGR) and Nastapoka River valley (NAS) that are in discontinuous permafrost landscapes (10-50% permafrost coverage). In addition, we sampled 5 rock-basin lakes as 'reference lakes' (RBL) in catchments near the W-K village as a fifth 'valley'; these waters occupy glacially scoured basins, and their origin is not related to permafrost degradation.

At each site, temperature, conductivity, dissolved oxygen and pH were measured using a 600R multiparametric probe (YSI, Yellow Springs, OH, USA). Water for dissolved organic carbon (DOC) and Chlorophyll-*a* (Chl-*a*) was filtered through a MilliQ water pre-rinsed 47-mm diameter, 0.22-µm pore size acetate filters and GF/F filters respectively (Whatman, GE Healthcare Life Sciences, Little Chalfont, Buckinghamshire, UK). Water samples for total phosphorus (TP) and total nitrogen (TN) were preserved with H_2SO_4 (0.15% final concentration) until further analyses.

Samples for zooplankton were collected using a 35 µm net ~~and~~ fixed in ethanol (final concentration: 75%, v/v), and stored in cold (4 °C) dark conditions until analysis by inverted microscopy. Microbial abundance samples for flow cytometry (FCM) analysis were further collected and fixed with glutaraldehyde (final concentration: 2%, v/v) and stored frozen at -80 °C until analysis.

2.2 Chemical and plankton analyses

DOC and nutrient analyses were conducted at the Institut National de la Recherche Scientifique, Centre Eau Terre Environnement (INRS-ETE, Quebec City, QC, Canada). DOC concentrations were analyzed on a Shimadzu TOC-5000A carbon analyzer and nutrients were analyzed using standard methods (Stainton et al., 1977). Colored dissolved organic matter (CDOM) was measured by spectrophotometric analysis of absorption at 254 nm by water filtered through 0.2 µm pore-size filters and the dissolved aromatic carbon content was determined using the $SUVA_{254}$ index (Weishaar et al., 2003).

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strictly speaking, water for DOC is filtered THROUGH a filter, and water for Chla is filtrered ONTO a filter… but I understand you were trying to condense...

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,

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strictly speaking this should be absorbance, that is used in the SUVA calculation (not absorption coefficient)

Phytoplankton biomass was estimated as Chlorophyll *a* concentrations (Chl-*a*), which were determined using high performance liquid chromatography (ProStar HPLC system, Varian, Palo Alto, CA, USA) following the procedures described in (Bonilla et al., 2005). Zooplankton, specifically copepods, rotifers and cladocerans, were enumerated following the Utermöhl procedure (1958) and inverted microscopy (Zeiss Axiovert, Carl Zeiss Microscopy GmbH, Jena, Germany). Bacteria, picocyanobacteria and autotrophic picoeukaryotes were enumerated using a FACScalibur flow cytometer (BD, Mississauga, ON, Canada), equipped with an argon laser, at the lowest flow rate (12 µl min^{-1}), using 1 µm yellow green microspheres (Polysciences Inc, Warrington, PA, USA) in suspension as an internal standard. Bead concentration was controlled using Truecount Absolute counting tubes (BD, Mississauga, ON, Canada). Bacteria were stained by adding 20 µl of a 50X SYBR Green I (Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA) to 500 µl of sample for 10 min in the dark. Bacterial cells were then discriminated on the basis of their green fluorescence (FL1) and side scatter signals (SSC) while excited at 488 nm, whereas autotrophic picoeukaryotes and picocyanobacteria were discriminated on the basis of their red fluorescence (FL3) with a threshold in orange (FL2) and SSC. The resulting data were analyzed using the CellQuest Pro software with manual gating.

2.3 Bacterial community composition

Bacterial community composition (BCC) was determined by 454-pyrosequencing of the V6-V8 regions of the 16S rRNA gene. In brief, water was sequentially filtered through a 20 µm mesh net to remove larger organisms, a 47-mm diameter, 3 µm pore size polycarbonate filter (Whatman) and a 0.2 µm pore size Sterivex unit (EMD Millipore, Billerica, MA, USA) using a peristaltic pump. The filters were preserved with 1.8 ml of RNA*later* (Life Technologies) and stored at -80°C until further processing. For this study, DNA was extracted from cells collected onto Sterivex units using the PowerWater Sterivex DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA USA) following the manufacturer instructions. Extracted DNA was amplified in three separate 20 µl PCR reactions using 1 µl of template (3 concentrations: 1, 0.5, and 0.2X) and a Phusion high-fidelity DNA polymerase kit (New England Biolabs, Whitby, ON, USA), and reverse 1406R and forward 969F primers with sample specific tags as in Comeau et al. (2011).

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remove parenthesis in front of author

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but I beleive this is done on UNstained samples right? (no SYBR Green)

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I suggest that you make this choice more explicit, which means that you excluded bacterial communities associated to particles (collected on the 3um filters), and this can be a large fraction in thermokarst lakes… You might want to briefly acknowledge this in the discussion as well.

GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify the lowest level of classification possible. A heatmap was produced to examine the variability in the ecological preference among the 30 most abundant OTUs.

2.6 Co-occurrence patterns

Co-occurrence analyses were performed using the overall dataset and each of the datasets for the 5 individual valleys. The data were filtered by using only those OTUs with a minimum of 20 reads and that were detected in at least three different ponds. This filtering step removed poorly represented OTUs and reduced the network complexity, resulting in a core community of 294 OTUs.

Randomness in co-occurrence of OTUs in the regional and individual valley datasets was tested in a null model using the quasiswap algorithm (Miklós and Podani, 2004) and *C*-score metric (Stone and Roberts, 1990) under 50000 simulations. SES (standardized effect size) was used as a measure of OTU segregation as described in Heino and Grönroos (2013) in order to determine whether this may relate to the overall environmental heterogeneity, the heterogeneity in biotic and abiotic variables separately, or to specific environmental variables. Environmental heterogeneity was determined using homogenization of group dispersion (Anderson et al., 2006) and defined as the mean distances of ponds to the centroid (central point) of each valley. Analyses were conducted on Euclidean distances on standardized variables and based on 1000 permutations. Similarly, the homogenization of group dispersion method was used to determine whether communities among ponds within a given valley were more similar than within other valleys.

Network analyses were conducted on the filtered OTU dataset. In addition, a total of 8 physicochemical variables (DOC, TP, TN, pH, $SUVA_{254}$, COND: conductivity, T: water temperature, DO: Dissolved oxygen concentration) and 7 biotic variables (Chl-*a*: phytoplankton biomass, BA: bacterial abundance, PC: picocyanobacteria, PE: autotrophic picoeukaryotes, Rot: rotifers, Clad: cladocerans, Cop: copepods) data were also included in the network. For each environmental variable, any missing data were estimated as the mean for the corresponding valley and all data were then normalized by subtracting the mean value for the overall study and dividing by the corresponding standard deviation.

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these are also abundances (not only bacterial)

Analysis of the network hubs further showed that the top 24 were mainly composed of Actinobacteria OTUs, in particular members of Actinomycetales and Acidimicrobiales. In addition, OTUs assigned to Betaproteobacteria represented a large fraction of these highly connected OTUs including the typical freshwater *Limnohabitans*, whereas Verruccomicrobia and Bacteroidetes were represented by only a few highly connected OTUs. Interestingly, the anaerobic photosynthetic sulphur bacterium Chloroflexi was also identified as a hub in the overall network (SI Table 3).

4 Discussion

The main goal of the present study was to identify co-occurrence patterns among bacterial communities in thaw ponds and lakes in the changing subarctic landscape. Consistent with our first hypothesis, there was a non-random distribution of bacterial taxa across the distinct valleys sampled in this study. The results showed that thaw ponds communities from the same valley, especially those located in the sporadic permafrost landscape, tended to be more similar in terms of bacterial community composition than communities originating from ponds located in other valleys. Furthermore, the thaw ponds differed taxonomically from the rock-basin reference lakes, with specific bacterial OTUs associated with a particular valley or permafrost landscape type. Contrary to our second hypothesis, that differences in habitat preferences among bacterial communities were related to distinct phylogenetic structure, we found no evidence for differences in the community phylogenetic relatedness between the different valleys. The same bacterial phyla occurred throughout the region, and variability among ponds in the same valley was greater than the differences among valleys.

4.1 Local community composition uniqueness and habitat preference among bacterial communities

Non-random distribution patterns among bacterial taxa were detected, indicating that bacterial taxa in our study region tended to co-occur more than expected by chance. Non-random assembly patterns indicate the dominance of deterministic processes such as environmental filtering in shaping community composition (Horner-Devine et al., 2007). The bacterial communities of freshwater ecosystems elsewhere (Eiler et al., 2011), as

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Here and below down to the end of conclusion: I understand that when you talk in general (intro), you want to include both lakes and ponds, but in your dataset, can't you decide if your water bodies are lakes or ponds according to your classification (freezing to bottom?, connected to hydrographic network? size?) instead of carying this unprecise/large qualification?

well as in certain terrestrial (Barberan et al., 2012) and marine (Steele et al., 2011) ecosystems, have also been reported to have distributional patterns that relate to the environment. Such patterns may depend on niche breadth and competitive abilities (Székely et al., 2013), grazing and viral lysis susceptibilities (Chow et al., 2014; Miki, 2008) and dispersal capabilities (Fahlgren et al., 2010; Hervas and Casamayor, 2009).

No significant relationship was found between distribution patterns and environmental heterogeneity. This was unexpected, as previous studies have shown that thaw ponds and lakes are heterogeneous environments with marked differences in community composition across the different valleys associated with distinct environmental variables (Crevecoeur et al., 2015; Comte et al. 2015). In agreement with Heino and Grönroos (2013), we suggest that the relationship between distribution pattern and environmental heterogeneity may be scale-dependent such that environmental heterogeneity may have effects on the bacterial taxa distribution patterns at the overall study region scale and not at the valley scale as tested here. The results did show differences in the phylogenetic composition of bacterial communities among the different valleys, which highlight distinct habitat preferences among taxa (Fig. 2, SI Fig. 4). In particular, the combination of LCBD and regression analyses indicated that the compositional uniqueness of thaw ponds and lakes was positively related to DOC concentrations, a well known determinant of bacterial communities and processes (Kritzberg et al., 2006; Ruiz-González et al., 2015). Along with the variations in permafrost degradation state across the study region, there were also differences among valleys in terms of availability and origin of carbon subsidies. The northern sites are located within the discontinuous permafrost area where most of the soil remains frozen and is thus not available for microbial degradation, while in the southern sporadic area, permafrost is highly degraded (Bouchard et al., 2014) and large amounts of ancient permafrost carbon may be available for microbial processes. Consistent with this pattern, elevated concentrations and high rates of CO_2 and CH_4 emission to the atmosphere have been reported among the southern sites within the most degraded area of permafrost (Laurion et al., 2010; Deshpande et al. 2015). In addition, SAS sites originated from palsas (organic permafrost mounds) and were likely different in DOC composition relative to other valleys, where the ponds were formed by the thawing of lithalsas (mineral permafrost mounds). This is consistent with recent

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I am searching for a description of this specificity in DOC (or you mean in DOM i.e. DOC and SUVA?) at SAS, but cannot find it in the results.

observation of a direct link between community composition and the degradation of terrestrially derived DOM (Logue et al. 2015) and may in turn explain the significantly higher bacterial richness and diversity observed in SAS thaw ponds communities and why OTUs assigned to methanotrophic bacteria such as *Methylobacter* and *Methylotenera* were amongst the most abundant detected in this valley (Fig. 2).

4.2 Bacterial phylogenetic structure

The mean NRI across all communities was significantly greater than zero. This provides evidence for a dominant role of environmental filtering on community composition (Kembel, 2009). The corollary is that a set of environmental variables constrained community composition, resulting in taxa that were closer phylogenetically and more ecologically similar than if stochastic processes (including dispersal) drove community assembly. In fact, there is no corridor such as streams that connects the ponds, and thus local dispersal processes are unlikely to explain the local phylogenetic structure of the thaw pond communities. Similar results were obtained for microbial community studies in the ocean (Monier et al., 2015) and on groundwater communities (Stegen et al., 2012).

No significant difference in NRI was found among the different valleys, but this result likely reflects the high variability within individual valleys. In particular, two ponds in the NAS valley had higher values of NRI in comparison to their neighboring ponds. These two ponds had specific environmental characteristics including high concentrations of suspended clay particles and low phytoplankton concentrations, which may have favored certain environmental specialists. The rock-basin waters had higher NRI values than the thaw ponds, indicating that their assemblages were more ecologically similar to each other than those originating from thaw ponds and lakes. This could relate to their respective histories in that the rock- basin lakes originate from deglaciation followed by retreat of the Tyrell Sea ca. 8000 years ago and have thus been exposed to longer term ecological processes. In contrast, the high environmental heterogeneity in the permafrost landscape is consistent with the higher degree of community relatedness observed among thaw ponds.

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I think you are not explicit enough on why you make a link to methanotrophs; I suggest this part makes is own sentence with the link to specificity of SAS DOM and CH4 (more CH4 brings more CH4 consumers I suppose?).

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is this bringing lower diversity in general/elsewhere?

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is this equivalent in saying lower diversity, i.e. more similar? I am confused now cause you said above rock ponds had more similar communities (than thaw ponds right?)

The extent of permafrost erosion (permafrost landscape type) appeared to influence phylogenetic structure. When controlling for the two outliers mentioned above (NAS-A and NAS-B), the northern communities (BGR, NAS) had a greater phylogenetic distance among co-occurring taxa than expected by chance (lower NRIs) than communities from the thaw ponds located in valleys from sporadic permafrost (KWK, SAS). This suggests that taxa from SAS valley (and to a lesser extent KWK), tend to be more ecologically similar to each other than those from northern valleys. These findings are in line with studies elsewhere that showed that clustered communities are mainly retrieved from environments that have constrained environmental conditions (Monier et al., 2015).

4.3 Network associations

The extent to which closely related bacterial taxa may coexist is still a subject of considerable discussion (Mayfield and Levine, 2010). Previous studies on aquatic microbial communities have shown that closely related taxa have coherent temporal dynamics and share similar ecological niches (Andersson et al., 2009; Eiler et al., 2011). Co-occurrence networks enable the depiction and visualization of co-occurrence patterns among OTUs, and they provide a way of identifying potential ecological niches within microbial consortia. Network analyses have recently been applied to a wide range of microbial communities and biomes, and specific associations among bacterial OTUs and with environmental variables have been reported (Barberan et al., 2012; Chow et al., 2014; Eiler et al., 2011; Steele et al., 2011).

Our results point toward the importance of environmental filtering for community assembly in thaw ponds and lakes. In co-occurrence networks, correlations between OTUs and environmental variables highlight the conditions that may favor particular assemblages. Specifically, our co-occurrence networks identified two abiotic variables (DOC and conductivity) to be among the most connected nodes (SI Fig. 5B), and these variables separated according to landscape type: the northern ponds located in the discontinuous permafrost landscape had high conductivity and low DOC, whereas southern sites within the sporadic permafrost landscape had high DOC and lower conductivity (SI Table 2; further details are given in Comte et al. 2015). The analysis of the DOC subnetwork showed that only a few OTUs were significantly and directly

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the 2 "than" makes the comparison confusing

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please specify what constrains are you referring to for SAS?

positive co-occurrence (Fig. 5C), consistent with bacterial network relationships. Although other examples of positive co-occurrence among bacterial OTUs were identified in the dataset (Fig. 5A), there was also evidence of ‘non co-existence’ (*sensu* Reshef et al. (2011)) among certain OTUs: In the northern, less degraded permafrost valley (BGR), OTU 1242 (Betaproteobacteria *Limnohabitans*) dominated, whereas in the southern highly degraded permafrost valleys (SAS, KWK), OTU 14 (Actinobacteria *ACK-M1*) dominated (Fig. 5B). These trade-offs among OTUs were partially explained by the geographic location of the valleys, suggesting that environmental variables not only drive the composition of the bacterial assemblages within the individual valleys but may also determine the ecological associations within microbial consortia. Furthermore, the positive relationship found between the connectivity and the habitat specificity among the most abundant OTUs is most likely driven by the dominance of highly connected OTUs in the southern high degraded permafrost valleys in comparison to the northern less degraded permafrost valleys. In addition, the OTUs retrieved from the southern thaw ponds were closely related to specific bacterial functional groups such as methanotrophs and nitrogen fixing bacteria (Fig. 5).

The microbial networks for the thaw ponds had ‘small world’ properties, with only a few, highly connected nodes, which can be viewed as keystone species. This property would render the networks more resilient to environmental change, but vulnerable to the loss of these nodal species (Montoya et al., 2006). The bacterial hubs were identified as typical freshwater, terrestrial and marine taxa (SI Table 3), and some of them were closely related to taxa that are involved in key biogeochemical processes such as nitrogen fixation and degradation of complex polymers, or that are known to be restricted in niche breadth, for example to cold environments. In accordance with Peura et al. (2015), the importance of a taxon in a microbial network may be less associated with its abundance, but instead determined by its connectivity, as represented by node degree for example. Thus many of the hub taxa identified in this study could be defined as a keystone microbial species (SI Table 3). For example, the nitrogen-fixing bacterium *Beijerinckia* was among the most connected node in the co-occurrence network despite its low relative abundance.

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highly?

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what do you mean? less likely driven by this in northern site?

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is this an expression used in other context or by Montoya? If so, please define further what you mean, otherwise useless

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it would be interesting to discuss why N-fixing bacterium would be important in an environment relatively rich in N

Conclusions

The thaw ponds and lakes sampled in the present study showed large variability in their bacterial community structure, even among sites in a single valley. This underscores the heterogeneous nature of permafrost aquatic environments, and is consistent with their known limnological variability. A small number of taxa occurred in high abundance and dominated many of the communities; these northern dominants included members of the betaproteobacterial order Burkholdiales and the Actinobacterial family ACK-M1; other dominants included members of the Bacteroidetes family Chitinophagaceae and Verrucomicrobia. Despite this variability and the existence of common taxa, there were taxonomic differences among different valleys and between permafrost landscape types, implying some degree of habitat selection.

The bacterial networks further showed that DOC and conductivity played an important role in the co-occurrence patterns of bacterial OTUs, corresponding at least in part to differences in these two environmental variables among valleys (SI Table 2). Strong positive associations as well as non-coexistence among OTUs were detected, and the resultant networks were composed of a limited number of highly connected OTUs. This ‘small world network’ property would render these communities more resilient to environmental change, but sensitive to the loss of their hub OTUs, which themselves showed some degree of habitat specificity. With ongoing global warming, these waters are likely to experience the effects of increased permafrost erosion and associated changes in their chemical environment, including shifts in DOC and conductivity. If such changes eventually cause the loss of keystone species that form the hubs of the present microbial networks, there would be a major disruption of thaw pond community structure, with potentially large biogeochemical consequences.

Acknowledgements

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you mean among lakes?

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All first names could be abreviated (as above) (same for Marie-Josée and Isabelle)

pigment analyses, Isabelle Laurion (INRS-ETE) for flow cytometry and INRS-ETE for chemical analyses. Computing support from CLUMEQ/Compute Canada, aid from A. Monier for bioinformatics and phylogenetic analyses, advice from A. Eiler for network analyses, and insightful comments from two anonymous reviewers were also greatly appreciated. We acknowledge the Natural Sciences and Engineering Council (NSERC) of Canada funding for Discovery grants to WFV and CL and Discovery Frontier (ADAPT) grant to WFV, the support from the Network of Centres of Excellence program ArcticNet to WFV and CL, and the Canadian Research Chair Program to WFV. Additional support from Fonds de Recherche du Québec Nature et Technologies (FRQNT) to CEN is acknowledged. JC was partially supported by a FRQNT postdoctoral fellowship and the EnviroNorth CREATE program from NSERC.

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I think there is no need to thank INRS for analyses that were paid.