1 Large scale biogeography and environmental regulation of
2 methanotrophic bacteria across boreal inland waters

3 running title : Methanotrophs in boreal inland waters

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Abstract

Aerobic methanotrophic bacteria (methanotrophs) use methane as a source of carbon and energy, thereby mitigating net methane emissions from natural sources. Methanotrophs represent a widespread and phylogenetically complex guild, yet the biogeography of this functional group and the factors that explain the taxonomic structure of the methanotrophic assemblage are still poorly understood. Here we used high throughput sequencing of the 16S rRNA gene of the bacterial community to study the methanotrophic community composition and the environmental factors that influence their distribution and relative abundance in a wide range of freshwater habitats, including lakes, streams and rivers across the boreal landscape. Within one region, soil and soil water samples were additionally taken from the surrounding watersheds in order to cover the full terrestrial-aquatic continuum. The composition of methanotrophic communities across the boreal landscape showed only a modest degree of regional differentiation but a strong structuring along the hydrologic continuum from soil to lake communities, regardless of regions. This pattern along the hydrologic continuum was mostly explained by a clear niche differentiation between Type I and Type II methanotrophs along environmental gradients in pH, and methane concentrations. Our results suggest very different roles of Type I and Type II methanotrophs within inland waters, the latter likely having a terrestrial source and reflecting passive transport and dilution along the aquatic networks, but this is an unresolved issue that requires further investigation.

Keywords: boreal inland water, large-scale spatial patterns, methane cycle, methanotroph community composition, methanotrophs ecology, microbial biogeography.

Introduction

Methane is currently the second most abundant greenhouse gas in the atmosphere and has a much higher warming potential than carbon dioxide (IPCC, 2013). Although a significant amount of atmospheric methane is produced by anthropogenic sources, much of the methane in the atmosphere originates from natural environments (Nisbet, Dlugokencky, & Bousquet, 2014). Amongst them, wetlands are the highest contributors. However, there is increasing evidence that lakes and rivers contribute significantly to natural methane emissions, yet are seldom considered in global greenhouse gas (GHG) budgets (Bastviken, Tranvik, Downing, Crill, & Enrich-Prast, 2011). In aquatic systems, the amount of methane that is ultimately released to the atmosphere is strongly modulated by the activity of aerobic methanotrophic bacteria (hereafter methanotrophs). For example, methanotrophs can consume from 60 to 98% of the methane produced in wetlands (Le Mer & Roger, 2001; Chowdhury & Dick, 2013; Dean et al., 2018), and up to 98% of the methane produced in lake sediments (Kankaala, Huotari, Peltomaa, Saloranta, & Ojala, 2006a; Rahalkar, Deutzmann, Schink, & Bussmann, 2009; Thottathil, Reis, del Giorgio, & Prairie, 2018). Likewise, in rivers and small streams, which are generally super-saturated with methane (Campeau, Lapierre, Vachon, & Del Giorgio, 2014; Stanley et al., 2016), methanotrophy can oxidize up to 70% of the methane produced during summer (de Angelis & Cranton, 1993). Some natural systems are in fact sinks of methane, as it is the case for emergent oxic soils, where methanotrophs can oxidize methane at atmospheric concentration levels (Kolb, 2009), consuming up to an estimated 10% of the atmospheric methane (Smith et al., 2000; Le Mer & Roger, 2001).

Methanotrophs typically account for a small proportion of the total bacterial community in surface layers of aquatic ecosystems (Eller, Känel, & Krüger, 2005; Rahalkar et al., 2009; Samad & Bertilsson, 2017), although they may reach significant densities within certain oxic/anoxic interfaces (Schiff et al., 2017, Rissanen et al., 2018), but regardless they appear to play a disproportionately important role in the environment. First because of the control they exert on net methane emissions to the atmosphere (Hanson

& Hanson, 1996), but also as a potential food source for other microbial and metazoan grazers within the food web (Kankaala et al., 2006b; Shelley, Grey, & Trimmer, 2014; Morana et al., 2015). Methanotrophs comprise a functional guild of bacteria distributed across different phylogenetic groups. The two most commonly studied groups belong to the Alpha- (Type II) and Gamma- (Type I) proteobacteria (Bowman, 2006). Type I methanotrophs belong to the *Methylococcaceae* family and typically comprise genera such as *Methylomonas*, *Methylobacter*, *Methylomicrobium*, *Methylocaldum* and *Methylococcus*, although the latter two compose a clade that is evolutionary more distant, sometimes referred to as Type X (Bowman, 2006). Type II methanotrophs include the genera *Methylosinus* and *Methylocystis* in the *Methylocystaceae* family, and *Methylocapsa* and *Methylocella* in the *Beijerinckiaceae* family (Borrel et al., 2011). *Methylocella* genera were actually found to be facultative methanotrophs and able to grow on a multitude of other carbon compounds (Dedysh, Knief & Dunfield., 2005). The application of molecular techniques has also unravelled new groups of uncultured methanotrophs, including taxa outside the Proteobacteria phylum (Knief, 2015), such as the Verrucomicrobia (*Methylacidiphilales*) and the NC10 phylum (Dunfield et al., 2007; Ettwig, van Alen, van de Pas-Schoonen, Jetten, & Strous, 2009).

Methanotrophs are thought to be generally influenced by the ambient concentration of methane, oxygen, or nitrogen, as well as pH and temperature (Conrad, 2007). However, the differential environmental regulation of the abundance and activity of Type I and Type II methanotrophs is still under debate, since experimental and environmental studies have yielded ambiguous and sometimes contrasting results. For example, although it has been hypothesized that Type I are favoured by low methane concentrations compared to Type II (Amaral & Knowles, 1995; Henckel, Roslev, & Conrad, 2000), some studies have reported Type I dominating in high methane environments (Duan et al., 2017; Krause et al., 2012) and Type II favoured under low methane concentrations (Knief, Lipski, & Dunfield, 2003). In addition, whereas most methanotrophs tend to grow better at neutral pH (Dunfield, Knowles, Dumont, & Moore, 1993; Semrau, DiSpirito, & Yoon, 2010), some Type II methanotrophs seem to be better adapted

to acidic environments such as peatbogs (Dedysh et al., 2000, 2002, 2004; Chen et al., 2008a,b), and dominance of Type I methanotrophs in acidic environments or co-dominance of Type I and Type II has also been observed (Kip et al., 2011; Esson et al., 2016). Further, increases in temperature have been associated with shifts from Type II to Type I dominance (He et al., 2012), yet a meta-analysis found Type I being preferentially associated with cold boreal lakes and Type II with warm tropical lakes (Borrel et al., 2011). In contrast, Liebner and Wagner (2007) found no correlation between *in situ* temperature and the distribution of Type I and Type II methanotrophs in permafrost soils. The different environmental preferences and tolerances of Type I and Type II methanotrophs are likely to influence their niche differentiation and therefore their spatial distributions (Conrad, 2007). However, there is still a lack of consensus regarding the regulation of the structure of the methanotrophic communities in inland waters, which stems perhaps from the fact that most studies on methanotrophs are focused on specific habitats or limited to individual types of ecosystems (Zheng, Zhang, Zheng, Di, & He, 2008; Barbier et al., 2012; Crevecoeur, Vincent, Comte, & Lovejoy, 2015; Lau et al., 2015; Samad & Bertilsson, 2017), and do not span sufficiently wide ranges of environmental gradients, geographic scales and types of ecosystems to determine robust patterns in community composition and their underlying environmental drivers at the landscape scale.

Here we assess the large-scale biogeography of methanotrophic bacteria in surface inland waters across the boreal biome of Québec (Canada). We sampled more than 500 lakes, rivers and streams located in seven major regions of Québec, spanning extremely wide environmental and climatic gradients. In addition, within one region, soils and soil water samples were also collected to assess the changes in methanotrophic community composition along the entire terrestrial-aquatic continuum. Previous studies of methanotrophic community composition have often been based on the use of specific gene markers, such as *pmoA* (Knief, 2015) and while these yield unequivocal identification of target organisms, they have the disadvantage of having limited taxonomic coverage, because some methanotrophs carry enzyme variants

that are not detected by conventional primers (Stoecker et al., 2006), or simply lack one of the targeted enzymes (Vorobev et al., 2011). Here we have used 16S rRNA gene sequences to characterize the methanotrophic community in these ecosystems. Although different 16S rRNA gene primer pairs can fail to detect certain bacterial groups (Klindworth et al., 2012), the 16S marker has the advantage of being ubiquitous and allows the detection of a substantial fraction of the extant methanotrophic taxa (Lau et al., 2015). The use of this marker renders sequencing results directly comparable, within the limits of sequencing depth and the uncertainty associated to taxonomic assignment (see Methods below), which is essential in the context of determining the large-scale biogeographical patterns of the entire methanotrophic community and the underlying environmental drivers. In this context, we address the following questions: (1) Does the methanotrophic community structure vary as a function of geographic region, type of ecosystem and network position? (2) What are the main environmental factors shaping methanotrophic community structure and geographical distribution? (3) Do Type I and Type II show different habitat preferences or regional organization across the boreal biome, and what are the factors that influence their relative abundances?

Methods

Study sites and physico-chemical parameters

In this study, we combined the datasets previously published in Niño-García, Ruiz-González and del Giorgio (2016a) and in Ruiz-González, Niño-García, Kembel and del Giorgio (2017), and Ruiz-González, Niño-García and del Giorgio (2015a). This combined dataset consists of 705 environmental samples (302 lakes, 316 rivers, 43 soil waters and 44 soils) collected between 2009 and 2013 from 7 different regions in Québec (Canada): Abitibi, Baie-James, Chibougameau, Saguenay, Côte-Nord, Laurentides and Schefferville (Fig. 1). Table 1 summarizes the main features of the regions and systems sampled, and further details are provided in Lapierre and del Giorgio (2014) and Rasilo et al. (2015).

Water samples from lakes were taken 0.5 m below the surface and near the shore for river samples as described in Ruiz-González et al. (2015b). Soil water and soil samples were all collected in the Côte-Nord region. Soil water samples were collected with piezometers deployed near streams, and soil samples from the top layer (0-20 cm) of the soil around the streams by pooling samples from five different locations as described in Ruiz-González et al. (2015a). For all sampling sites, temperature, dissolved oxygen, pH and conductivity were measured with a YSI probe, and water samples for measurement of DOC concentration were filtered through a 0.45 µm filter and analysed with an OI1010 TOC analyser; cDOM was quantified with a Ultrospec3100 spectrophotometer as explained in Ruiz-González et al. (2015a). Chlorophyll *a* was extracted with ethanol and concentrations were measured with a spectrophotometer. Total phosphorus (TP) and nitrogen (TN) samples were digested with persulfate and alkaline persulfate respectively and then analysed as described in Rasilo et al. (2015). Methane and carbon dioxide partial pressure ($p\text{CH}_4$ and $p\text{CO}_2$, in µatm) were measured using the headspace technique as detailed in Rasilo et al. (2015). Samples for water isotopes (^2H and ^{18}O) were analysed as in Niño-García et al. (2016a) in order to calculate the deuterium excess (*d*-excess), which provides an indication of the degree of evaporation relative to precipitation and decreases as water temperature and evaporation increase (Gibson, Prepas, & McEachern, 2002).

Methanotrophic community composition

Soil (0.25 g) or 300–500 mL of water filtered onto 0.22 µm filters were used for DNA extraction with the MoBio PowerSoil and PowerWater extraction kits, respectively (Mo Bio, Carlsbad, CA, USA). The V3–V4 regions of the 16S rRNA gene was amplified with the primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') and sequenced on an Illumina MiSeq2000 (Illumina, San Diego, CA, USA) following a pair-end approach. Details of bioinformatics analysis are provided in Niño-García et al. (2016a). In brief, sequences were assembled with FLASH (Magoč & Salzberg, 2011) and analysed following the steps of the QIIME pipeline

(Caporaso et al., 2010), with quality reads being binned into operational taxonomic units (OTUs, $\geq 97\%$ similarity) using UCLUST v1.22q (Edgar, 2010) and taxonomically assigned with RDP classifier (Wang, Garrity, Tiedje, & Cole, 2007). Sequences with less than 10 reads or in less than 10 samples were discarded, which resulted in an OTU table composed of 202,669 OTUs. This OTU table was then rarefied at 40,421 reads per samples with the command “rarefy_even_depth” of the R package Phyloseq (McMurdie & Holmes, 2013). The rarefaction process discarded 91 samples. Raw sequences have been deposited in the European Nucleotide Archive under the accession numbers PRJEB11530 and PRJEB17975. The methanotrophic OTU table was constructed from this final table. We tested the efficiency of the primer set used here in detecting the main methanotroph groups using the TestPrime tool (Klindworth et al., 2012), and this procedure confirmed that our primer recovers more than 90% of the sequences belonging to the families *Methylococcaceae*, *Methylocystaceae* and *Beijerinckiaceae*, 90% of the NC10 (now Rockubacteria) phylum, and 76% of the *Methylacidiphilales* family. However, we cannot rule out that our primers failed to detect candidate phyla. In order to select only obligate methanotrophic taxa, phylogenetic trees were built with representative sequences of the families *Methylococcaceae*, *Methylocystaceae* and *Beijerinckiaceae* downloaded from GenBank (Lipman et al., 2012). Sequences were first aligned with the MUSCLE algorithm available in the software MEGA-X, then the best molecular model was tested with MEGA, and a consensus neighbour joining tree was build based on 1000 trees with associated bootstrap values, with the best fitted model Kimura 2 with gamma parameter. Only sequences that represented monophyletic clusters with known obligate methanotrophic taxa were kept. Representative sequences of those clusters were submitted to a BLAST on GenBank to verify they shared more than 95% of similarity with known methanotrophs. This procedure recovered 265 OTUs belonging to the families *Methylococcaceae* (Type I), 42 OTUs belonging to the *Methylocystaceae* (Type II) and 4 OTUs belonging to the family *Beijerinckiaceae* (Type II) for a total of 22,838 sequences. No taxa belonging to the Verrucomicrobia or NC10 phylum were recovered from the original OTU table. 90 out of the 614 samples kept after rarefying did not contain any methanotrophic sequences.

Statistical analysis

All the statistical analyses were performed in R (R Core Team, 2014). The physico-chemical properties of samples with or without methanotrophic sequences were compared by means of non-parametric Kruskal-Wallis test because the data did not follow normal distribution and variances were not homogenous. Differences in methanotrophic community structure across the sampled sites (all the methanotrophs together, or Type I and Type II separately) were visualised by means of principal coordinates analysis (PCoA) using Bray-Curtis dissimilarities on the squared root of the community matrix with the command `cmdscale` in the package `vegan` (Oksanen et al., 2015). Rivers were separated into small streams (Strahler order ≤ 2) and larger rivers (Strahler order > 2). A separate analysis was done on the Côte-Nord samples that include soil and soil water communities, in order to explore changes in methanotrophic communities across the terrestrial-aquatic continuum. The environmental variables most strongly influencing the methanotrophic community structure were selected with multivariate regression trees (MRT) with the package `mvpart` (De'ath, 2002). For each analysis, 100 iterations of a decision tree were run and the best tree within 1 standard error of the overall best tree was selected. The variables selected were then plotted on the PCoA with the command `envfit` of the `vegan` package (Oksanen et al., 2015). Standard and partial Mantel tests were used to investigate correlations between geographical location and distance, methanotrophic community composition (all the methanotrophs together, and Type I and Type II separately) and the most influential environmental variables while accounting for spatial autocorrelation. Different distance metrics were used depending on the nature of the variables: Euclidean distance for the geographic coordinates and of the most influential environmental variables (squared-root transformed), and Bray-Curtis dissimilarities of the methanotrophic community composition data (squared-root transformed), both calculated with the command `vegdist` in the `vegan` package (Oksanen et al., 2015). Differences in methanotrophic community composition between ecosystem types and regions were tested with an analysis of similarities (ANOSIM) on Bray-Curtis dissimilarity matrix. The

relationship between the environmental variables and the relative abundance of Type I and Type II methanotrophs were visualised by binning the data into 6 to 12 groups of balanced observation based on normal scale for pH, DOC, nitrogen, phosphorus, oxygen and temperature, and on logarithmic scales for $p\text{CH}_4$. Means and standard error of each group as well as the ratio between the relative abundance of Type II and Type I methanotrophs were represented on a scatter diagram with a squared-root y axis with the package ggplot2 (Wickham, 2016). In order to evaluate the best relationship between environmental variables and relative abundance of methanotrophs, we fitted a linear and a quadratic model with the lm command in R for each variable and tested the significance of the model with the lowest AIC.

Results

Physico-chemical parameters

The different systems sampled here covered a wide range of physico-chemical, climatic and landscape variables (Table 1). In general, sites from the same region had similar characteristics and tended to group as a function of water chemistry (pH, DOC) and trophic status (details not shown). For example, inland waters from Abitibi and Bay-James were in general more productive, while systems from Saguenay and Côte-Nord were more oligotrophic and acidic; Schefferville sites were the most oligotrophic and acidic (for further details see Niño-García et al. (2016a) and Ruiz-González et al. (2015a)). Within the ensemble of sites, we detected a total of 311 OTUs that could be unambiguously identified as obligate methanotrophs. Presence of methanotrophic OTUs was detected in 524 out of the 614 samples kept after rarefaction (258 rivers, 204 lakes, 31 soil waters and 31 soils). In the majority of the samples, these methanotroph OTUs represented less than 1% of the total number of sequences recovered within any given site. The highest proportion of methanotrophs was detected in a river sample in Abitibi, where they accounted for 4.4% of the total microbial community. On average, the highest proportions of methanotrophs were detected in soil waters (0.22% of total 16S rRNA gene sequences), followed by soils

(0.19%), rivers (0.11%) and lakes (0.07%). The 90 samples containing no methanotroph sequences were mostly from Lakes (65) and Rivers (24), with only 1 soil sample. These sites had significantly lower methane, DOC, nitrogen and phosphorus concentration (all Kruskal-Wallis $p < 0.01$), and significantly higher pH and temperature (all Kruskal-Wallis $p < 0.01$) than the samples where methanotrophs were detected (Supplemental Fig. S1). No significant differences in oxygen concentration were observed (Kruskal-Wallis $p = 0.06$).

Phylogeny of the 16S rRNA gene sequences related to methanotrophs

According to the phylogenetic analysis (Supplemental Fig. S2), the majority of the Type I OTUs clustered with sequences of the genus *Methylocaldum* (115) and *Methylomonas* (33). A large number of OTUs (96) did not cluster clearly with any genus but still fell markedly inside the *Methylococaceae* family. Smaller clusters containing 1 to 4 OTUs contained the genera *Methylovulum*, *Methylospira*, *Methylosoma*, *Methylobacter*, *Methyloglobulus*, *Methylococcus*, and *Crenothrix* (Supplementary Fig. S2). For the Type II *Methylocystaceae* family, 24 OTUs clustered with sequences of the genus *Methylocystis* while 3 OTUs with the genus *Methylosinus* (Supplementary Fig. S3). 15 OTUs fell into clusters that contained both genera *Methylocystis* and *Methylosinus*. Two OTUs clustered with the non-methanotrophic genera *Hansschlegelia* and *Pleomorphomonas* and were therefore removed. Finally, inside the Type II *Beijerinckiacaceae* family, 4 OTUs clustered closely with sequences of genus the *Methylocapsa* and shared 98% homology with the methanotroph *Methylocapsa paslarum* (NR_137418) (Supplemental Fig. S4). *Methylocella* OTUs were removed from the final methanotrophic OTU table due to the facultative methanotrophic nature of this genus (Dedysh et al., 2005).

Spatial patterns of methanotrophic communities across the boreal landscape

Methanotrophic community composition showed a weak but significant segregation as a function of type of system (i.e. rivers, small streams and lakes; Fig. 2a, ANOSIM_{byECOSYSTEM} $R = 0.05$, $p < 0.01$) with PCoA axis 1 reflecting to some extent a gradient from the smallest headwater streams to rivers and finally

lakes. Some degree of regional segregation was also observed (Fig. 2b), with small but significant differences in composition across regions (ANOSIM_{byREGION} $R=0.16$, $p<0.01$), reflected in the weak but significant correlation between community dissimilarity and geographical location (Mantel $R=0.13$, $p<0.01$). This large-scale spatial structuring of methanotrophic community composition was mostly influenced by pH, and nitrogen concentration (MRT $R^2=0.115$), even after accounting for geographical location, suggesting that the environmental variables had a true effect beyond any geographical structuring that they might have (partial Mantel $R=0.04$, $p<0.01$). When considering lakes or rivers separately, pH emerged as the strongest environmental predictor of methanotroph community composition in lakes (MRT $R^2=0.03$), although with a low explanatory power, whereas pH and nitrogen emerged in rivers (MRT $R^2=0.31$).

In order to explore the taxonomic changes of methanotrophic communities along the terrestrial-aquatic continuum, we repeated the analysis focusing only on the Côte-Nord watershed, which is the only region in which we had sampled the full hydrologic soil/soil water/river/lake continuum. The methanotrophic communities of the Côte-Nord region differed significantly between the different habitat types (ANOSIM_{byECOSYSTEM} $R=0.14$, $p<0.01$), and changed gradually along the hydrologic continuum, from soils to lakes (Fig. 3). Although *d*-excess (which reflects the degree of evaporation of the water and is therefore a proxy of the “age” of water within the network) was not selected as one of the most influential variables by the MRT analysis, it was nevertheless significantly related to the methanotrophic community in la Côte-Nord (envfit $p<0.01$) and loaded heavily on Axis 1 of the PCoA, reflecting the strong hydrological control of the methanotroph spatial structure at the network scale. The most influential environmental variable of this pattern was DOC (MRT $R^2=0.06$), which was higher in soil, soil water and headwaters samples, and decreased towards downstream sites (Fig. 3).

When Type I and Type II methanotrophs were considered separately, Type II methanotrophs (Alphaproteobacteria) showed a spatial distribution similar to that of the whole methanotrophic

community (Fig. 4a), displaying a directional shift from small streams to lakes. The most influential factors on the large-scale spatial structuring of Type II methanotrophs was pH (MRT $R^2=0.05$). Type I methanotrophs (Gammaproteobacteria), on the other hand, showed a different spatial pattern from that of Type II and that of the whole methanotrophic community (Fig. 4B), wherein Type I methanotrophic community from small stream and river samples tended to closely overlap in the ordination, and differed from those in lakes and some rivers. The partial pressure of CO₂ in the water (pCO_2) was selected as the most influential variable on Type I methanotroph community composition (MRT $R^2=0.09$). Despite these differences in spatial structure, both Type I and Type II methanotrophic communities shared a pattern of significant differences between ecosystem types and regions (ANOSIM_{byECOSYSTEM} $R=0.11$ and 0.04 , respectively, $p<0.01$ for both types). Moreover, the two groups showed a significant but weak correlation between community dissimilarity and geographical location (Mantel $R=0.08$, $p<0.01$ for both types), with pCO_2 as the most influential variable for Type I (partial Mantel $R=0.04$, $p<0.01$), and pH for Type II (partial Mantel $R=0.1$, $p<0.01$).

Relative contribution and niche differentiation of Type I and II methanotrophs

The relative abundance of Type I and Type II methanotrophs showed contrasting trends along the hydrologic continuum: Type II methanotrophs were clearly dominant in soils and soil waters, whereas Type I methanotrophs became increasingly dominant towards large rivers and lakes (Fig. 5). Consequently, the ratio of Type II to Type I decreased pronouncedly from soil to lake habitats. Note that for this analysis, samples from soil and soil water came from the Côte-Nord region only, while the rest of the hydrological continuum integrated samples from all the other regions.

In order to assess the degree of community turnover of Type I and Type II OTUs along the hydrologic continuum, we assigned each OTUs to the environment type where they were first detected assuming a directionality from soils towards lakes, as conceptualized in Crump et al. (2012) and Ruiz-González et al. (2015a). This allows to determine the origin of OTUs (terrestrial or aquatic), as well as to

establish whether changes in taxonomic composition are mostly due to shifts in the abundance of methanotrophic OTUs that are present throughout the networks or by the appearance of new OTUs along the continuum. Interestingly, Type I and Type II OTUs showed markedly different patterns (Fig. 6). In the case of Type I methanotrophs (Gammaproteobacteria), many new OTUs were recruited along the hydrological continuum (Fig. 6a) such that the lake Type I community represented an ensemble of OTUs with very diverse network origins. Of these, Type I OTUs derived from soils represented a very small proportion of the total number of reads detected in downstream ecosystems (Fig. 6b). In contrast, soil-derived taxa overwhelmingly dominated Type II methanotrophs (Alphaproteobacteria) across the entire continuum in terms of OTUs (Fig. 6c) and percentage of reads (Fig. 6d).

We further explored how variations in the measured environmental parameters related to changes in the relative abundances of Type I and II, and this analysis revealed largely contrasting environmental preferences of these two groups (Fig. 7). The relative abundance of Type I methanotrophs showed a quadratic (hump-shaped) relationship with pH, that was albeit not significant, while Type II relative abundance decreased significantly with pH ($R^2=0.91$, $p<0.01$) as did the Type II/I abundance ratio (Fig. 7a). The relative abundance of both Type I and II followed a quadratic relationship with the ambient partial pressure of methane (pCH_4 , log-transformed), but whereas Type I peaked at intermediate pCH_4 concentrations ($R^2=0.48$, $p=0.02$), Type II relative abundance significantly increased with pCH_4 ($R^2=0.93$, $p<0.01$), as did the Type II/Type I ratio (Fig. 7b). Both Types tended to increase with DOC concentration following a quadratic relationship (Fig. 7c), that was only marginally significant for Type II ($R^2=0.48$, $p=0.05$). Only Type II relative abundance had a significant relationship with temperature ($p=0.03$) (Fig. 7d) that was unimodal, resulting in the hump-shaped relationship of the Type II/I ratio. The relative abundance of the two groups increased significantly with nitrogen concentration (Fig. 7e), but the linear relationship was stronger for the Type II ($R^2=0.95$, $p<0.01$) than for Type I ($R^2=0.56$, $p=0.05$). The same trend was observed for phosphorus with a linear relationship that was only significant for Type II

($R^2=0.79$, $p=0.01$) (Fig. 7f). The relative abundance of both groups and their ratio decreased with oxygen concentration following a significant quadratic relationship for Type II ($R^2=0.85$, $p<0.01$) and a significant linear relationship with Type I ($R^2=0.55$, $p=0.03$) (Fig. 7g).

Discussion

Methanotroph sequences were recovered in more than 500 of the total 705 samples that we analyzed for this study, suggesting that this functional guild is widespread across surface layers of inland waters and also in soils and soil waters in the boreal biome. There may be hotspots of methane oxidation at the oxic/anoxic interface in sediments, and in lakes that develop steep water column oxyclines, populated perhaps by consortia of methanotrophs that may differ somewhat from what we observe in surface waters. In this regard, we should point out that all of the rivers, as well as a large portion of the lakes (around 60%) were not stratified at the time of sampling, the latter being typical shallow boreal lakes, and therefore the surface water samples do integrate to some extent the vertical heterogeneity that may exist. In the lakes that were stratified, the composition of the epilimnetic methanotroph communities still reflects the ambient conditions and environmental selection occurring not only in the surface water itself, but also surely carry traces of bacterial from littoral sediments as well as those that inhabit the base of the epilimnion that is in contact with the thermocline, where there is typically a peak of methane oxidation due to a combination of lower oxygen and higher methane; these surface communities therefore do carry a local environmental imprint that is relevant from a biogeographic perspective. It is also important to point out that, beyond these discrete potential hot spots of methanotrophic activity, there is evidence of presence and activity of methanotrophs, as well as vigorous and widespread methane oxidation also occurring in the epilimnion of lakes and surface waters of rivers (Samad & Bertilsson, 2017; Crevecoeur et al., 2017; Stanley et al. 2016; and from our own group, Reis, Thottathil, Ruiz-González & Prairie, in prep), and we emphasize that these layers ultimately represent the last barrier before the aquatic methane diffuses to the

atmosphere. From this point of view, it is still important to understand what is happening in terms of methanotrophy in surface waters.

Importantly, the patterns in composition in the surface waters not only reflect local environmental selection, they also reflect the connectivity with the hydrologic network and the surrounding terrestrial catchment, because these communities include taxa that may have been transported by rivers and groundwater from adjacent soils. This transport and connectivity dimension is another important aspect that we set out to explore in this study and this actually requires a comparison of surface water sites. Our own previous work we have shown that microbial communities from these boreal aquatic systems are strongly influenced by hydrology and connectivity with the surrounding terrestrial landscape (Niño-García et al., 2016a; Ruiz-González et al., 2015a). The patterns shown here seem to suggest that methanotrophic communities are also influenced by these processes, likely driven by the transport of cells that are flushed out from soils and wetlands and onto rivers and eventually to lake surface waters, some eventually being positively selected at oxic/anoxic interfaces or in the surface layers.

The apparent absence of methanotrophs in some samples could be due to extremely low methanotroph abundance combined with the rarefaction threshold, as at least one or two reads of methanotrophic OTUs could be detected in several of the samples that did not contain any methanotrophs after rarefaction. More likely, this absence could be caused by specific environmental conditions, as our analysis suggests that samples where no methanotrophs were detected significantly differed in terms of methane, DOC and nutrient concentration, pH and temperature from those sites where methanotrophs were present. Their relative abundance, however, remained systematically below 1% of the total number of community reads, rendering this functional guild consistently rare, and in accordance with previous results from temperate lakes where methanotrophs were found to contribute no more than 2% of total number of community sequences (Oswald et al., 2015; Samad & Bertilsson, 2017). Rare taxa, however, might actually represent a very responsive part of the community and may have exert a disproportionate

influence on biogeochemical cycles (Pedrós-Alió, 2012). For example, the experimental rewetting of soil communities resulted in a net reduction of methane emissions driven by the resuscitation of rare methanotrophic bacteria (Aanderud, Jones, Fierer, & Lennon, 2015), and efficient methane consumption from a wetland was driven by methanotrophs that constituted less than 0.1% of the communities (Bodelier et al., 2013). Consequently, the low relative abundance of methanotrophs may not imply lack of activity or a minor biogeochemical role. In addition, methanotrophs have been shown to be an important food resource for protist and zooplankton (Kankaala et al., 2006b; Shelley et al., 2014; Morana et al., 2015). Selective top down regulation may be in fact one of the reasons why this group is systematically in low abundance within inland waters, as has been shown for other functional bacterial groups such as the aerobic anoxygenic phototrophic bacteria (AAP) (Garcia-Chaves et al., 2015). Indeed, the samples containing the lowest number of sequences, or where no methanotrophic sequences were detected, were mostly lake samples, where grazing and top-down control are most likely to be intense.

The most ubiquitous Type I OTU in our dataset was associated to the genus *Methylocaldum*. We found this genus throughout the boreal landscape and in different types of ecosystems, supporting that this group may be composed of generalists (Knief, 2015). This contrasts with reports of *Methylocaldum* strains that appear to occupy very specific habitats, such as hydrothermal vents or landfill soils (Takeuchi et al., 2014; Zhang, Kong, Xia, Su & He, 2014), implying thermotolerant and thermophilic properties (Bodrossy, Holmes, Holmes, Kovacs & Murrell, 1997). We identified OTUs belonging to the genus *Methylomonas*, which has been described as an acid-tolerant genus inhabiting acidic peatlands (Knief, 2015). Interestingly, we detected relatively few sequences that clustered with the genus *Methylobacter*, although this genus has previously been identified as dominant in northern freshwater ecosystems (Crevecoeur et al., 2015; Samad & Bertilsson, 2017), comprising psychrotolerant (Wartianen et al., 2006) and psychrophilic species (Omelchenko et al., 1996). Most of the Type II methanotrophs, on the other hand, fell into clusters linked to the genera *Methylocystis* and *Methylosinus*, which are prevalent in soil and

groundwater (Bowman, 2006) and have also been isolated in acidic peatlands (Knief, 2015). This agrees with the observation of dominance of Type II methanotrophs across our studied soil and soilwater samples and at low pH sites.

Large-scale biogeography and spatial structure of methanotrophic community composition

Although freshwater methanotrophic bacteria have received increasing attention in the past decade, there have been few comparative studies of methanotrophic community composition across large geographic gradients (Lücke et al., 2010), ecosystem types or along the hydrologic continuum (Siljanen et al., 2011). Our results indicate that across the boreal landscape, the methanotrophic communities from inland waters have only a weak degree of differentiation as a function of the latitude, region or geographic distance, and that these differences were mostly driven by rearrangement of the relative abundance of existing taxa and not by dispersal limitation, since we only found a very small number of endemic taxa (i.e., taxa detected only in a single region, data not shown). Rather, we observed a significant differentiation of communities between streams, lakes, and rivers, although ANOSIM R was low and there was still a large degree of overlap, suggesting a continuous transition between these systems. When focusing on the region (Côte-Nord) that included samples from soils and soil waters, we observed a clear directional structuring of methanotrophic communities along the hydrologic continuum, with community structure shifting sequentially from terrestrial and headwater streams towards large rivers and lakes (Fig. 3). The fact that the terrestrial landscape clearly acts as a source of methanotrophic diversity for rivers and lakes, as has been reported before for whole bacterial communities (Crump et al., 2012; Besemer et al., 2013; Ruiz-González et al., 2015a; Niño-García et al., 2016a), suggests that the mechanisms underlying community assembly of methanotrophs are similar to those of the bulk bacterial community. The variable δ -excess, which is linked to the provenance of the water and its degree of evaporation, was significantly correlated to taxonomic shifts in methanotrophs from the Côte-Nord watershed (Fig. 3), reinforcing the

notion of a progressive methanotrophic community shift along the hydrologic continuum, rather than the existence of discrete methanotrophic communities between ecosystem types.

The methanotrophic community structure as a whole of boreal inland waters appears to be mostly influenced by pH, DOC and TN concentrations, and sites where no methanotrophs were detected had significantly higher pH and lower methane, DOC and TN concentration. pH may affect methanotrophic community composition either directly or may act as an integrative factor of multiple landscape properties, as previously suggested for whole bacterial communities (Fierer & Jackson, 2006; Logue & Lindström, 2008; Ren et al., 2015; Niño-García et al., 2016a). DOC also emerged as one of the strongest predictors of methanotrophic community composition, especially when soils and soilwaters were considered, supporting the strong link between the dynamics of methane oxidation and DOC concentration observed across boreal lakes (Thottathil et al., 2018). This link, which has been previously hypothesized (Crevecoeur et al., 2017), has only been explained by indirect causes so far, for example, via the inhibitory effect of light on methanotrophy (Murse & Sugimoto, 2005) with higher DOC environments providing protection against light inhibition (Thottathil et al., 2018). Alternatively, DOC may also favour methanogenesis by influencing the resources available to methanotrophs, and also by promoting bottom water anoxia in lakes (Thottathil et al., 2018). Other studies, however, have reported a negative relationship between DOC and methane concentration in surface waters of lakes (Bastviken, Cole, Pace & Tranvik, 2004), and also with methanotrophic abundance (Samad & Bertilsson, 2017). Although more work is needed to better understand the link between DOC concentration and methane dynamics, our results do support a role of DOC in structuring the methanotrophic communities across northern inland waters.

In lakes, nutrient and trophic status are known to influence microbial community structure (Lindström, 2000), but less is known about the influence of nutrients on methanotrophic communities. Nitrogen can be a strong driver of trophic status (Bogard et al., 2017), suggesting a connection with

ecosystem productivity. In our case, although methanotrophs seem to have ways to overcome nitrogen limitation through potential nitrogen fixation (Auman, Speake & Lidstrom, 2001; Vile et al., 2014), nitrogen concentration was still an important variable explaining methanotrophic community distribution and relative abundance, suggesting that trophic status plays an important role on methanotrophic community structuring. It is interesting that TN, and not TP, emerged as the strongest proxy of trophic status when considering the entire methanotrophic community. This result in no way diminishes the role of P in determining aquatic productivity, but underlines the importance of N in these boreal inland waters, as has been highlighted in a recent study (Bogard et al., 2017). Despite the fact that those environmental factors were selected statistically, their explanatory power remained generally low in both the partial Mantel and MRT analyses. It is possible that other factors not considered in this study, for example biotic interactions such as grazing or viral lysis, might play a role in structuring the observed spatial patterns in boreal methanotrophic communities.

Niche differentiation between Type I and Type II methanotrophs

The spatial patterns in overall community structure of methanotrophs described above were underlain by very different spatial behaviours of subgroups within the community. In particular, Type I and Type II methanotrophs showed different patterns along the hydrological continuum, and different relationships to major environmental drivers (Fig. 4 a-b). Type I methanotrophs (Gammaproteobacteria) showed a more defined segregation between ecosystem types, with small streams separated from large river and lakes. There was still a small regional segregation for the two groups, reflected in the weak but nevertheless significant Mantel correlations with geographic distance. This suggests that there may be climatic or regional landscape drivers influencing methanotrophic community composition operating at large scales, but that these play a minor role in the overall community assembly of these communities, relative to local environmental drivers. The relative abundance of Type I and Type II also followed a clear spatial shift from soils to lakes with small rivers acting as transition sites, with Type II being the dominant

methanotrophs in soils and soil waters and Type I dominating in large rivers and lakes (Fig. 5). The decline of the ratio of abundances between Type II and Type I methanotrophs along the hydrological continuum suggests a systematic replacement of Type II for Type I from soils to lakes, which could result from the selective growth of the latter and/or the loss of the former. In addition, the spatial behaviour and patterns of assembly of Type I and Type II along the hydrologic continuum seemed to be governed by different processes (Fig. 6). The vast majority of the Type II OTUs that we detected in rivers and lakes could be retraced to soils, and the fact that most of these tended to decline in relative abundance along the hydrological continuum suggests passive transport from the surrounding landscape as the main driver of their community assembly (Adams, Crump, & Kling, 2014). It is also interesting to note that most of the Type II methanotrophs from all the sampled lakes and rivers across the different regions of Québec could be retraced to the terrestrial samples from a single region (Côte-Nord), suggesting that there must be a pan-boreal pool of soil Type II methanotrophs. In contrast, relatively few Type I OTUs detected in rivers and lakes originated from soils, and most of the dominant aquatic Type I OTUs were recruited within the hydrological continuum, which would suggest that Type I methanotrophs are preferentially aquatic and change gradually along the continuum due to local species sorting by environmental conditions, in support of previous hypothesis (Van der Gucht et al., 2007; Langenheder & Székely, 2011). Our results support and further link previously unconnected system-specific reports from the literature. Indeed, Type I methanotrophs have been reported to dominate lake ecosystems (Sundh, Bastviken, & Tranvik, 2005; Crevecoeur et al., 2015; Samad & Bertilsson, 2017), where they supposedly play a more active role in methane cycling than Type II (Hanson & Hanson, 1996). Type II, on the other hand, have been shown to dominate in soils (Kolb, Knief, Stubner, & Conrad, 2003; Steenbergh, Meima, Kamst, & Bodelier, 2010), although in permafrost and Arctic soils Type I methanotrophs were also abundant (Liebner & Wagner, 2007). The role of Type II methanotrophs in lake waters needs to be further evaluated, since this does not seem to be their preferred habitat and yet they are still ubiquitously found there. We did detect, however, a small subset of Type II taxa that increased in abundance from soils to lakes, suggesting a planktonic

lifestyle for this subset (details not shown). Collectively, these patterns have implications for our understanding of the functioning of freshwater methanotrophic communities, since it is possible that a significant fraction of the Type II (Alphaproteobacteria) diversity detected in lakes by DNA sequencing techniques is merely accidental (*sensu* Niño-García et al., 2016a), and thus may play little or no role in lake methanotrophy. This large niche differentiation was further supported by the observation that Type I and Type II methanotrophs show different ecophysiological preferences, based on their patterns of distribution along key environmental gradients (Fig. 7). Relationships between relative abundance and all the other environmental variables tested were always significant for Type II, whereas only methane, nitrogen and oxygen concentrations were significantly correlated with variations in Type I abundance.

Type II seemed to be more sensitive to pH and were dominant in acidic soil waters, whereas Type I methanotrophs showed no significant relationship with pH, but their relative abundances seemed to peak around pH of 6-7, which is the average pH in most lakes and larger rivers. Type II seemed to dominate at high methane concentration, whereas Type I relative abundance was highest at intermediate $p\text{CH}_4$. This pattern agrees with what was previously hypothesized by Amaral and Knowles (1995), and Henckel et al. (2000), but contrasts with previous studies reporting a preference of Type I for high methane concentrations (Qiu, Noll, Abraham, Lu, & Conrad, 2008; Dumont, Pommerenke, Casper, & Conrad, 2011; Krause et al., 2012; Duan et al., 2017), although in very different types of habitats and environmental conditions than those covered in our study. Albeit the relative abundance of both types increased with DOC, Type II methanotrophs might be more sensitive to light inhibition due to their significant response to DOC. The nutrient gradient did not lead to differential responses between both types, although it has been hypothesized the Type II perform better under nutrient limitation because of their ability to fix nitrogen (Graham, Chaudhary, Hanson, & Arnold, 1993; Dedysh et al., 2002).

Temperature has been shown to play a major role in regulating aquatic methane dynamics (Börjesson, Sundh, & Svensson, 2004; Wagner, Lipski, Embacher, & Gatteringer, 2005; Warttinen,

Hestnes, McDonald, & Svenning, 2006; Mohanty, Bodelier, & Conrad, 2007; Graef, Hestnes, Svenning, & Frenzel, 2011; He et al., 2012), and our results support these findings. Previous studies that have explored the seasonal patterns in methanotrophic communities have reported dominance of Type I methanotrophs during cold seasons (Ricão Canhelas, Denfeld, Weyhenmeyer, Bastviken & Bertilsson, 2016; Samad and Bertilssen, 2017; Vigneron et al., 2019). However, we only found a significant and unimodal relationship between Type II methanotrophs relative abundance and temperature, perhaps due to fact that most of the samples were taken during the summer and the temperature gradient was relatively modest. Oxygen is also reported as a highly influential variable in the literature (Reim, Lüke, Krause, Pratscher, & Frenzel, 2012; Oswald et al., 2015; Crevecœur et al., 2017; Martinez-cruz et al., 2017) and in our dataset we observed declining trend of both types as a function of oxygen concentration, in spite of the relatively narrow range oxygen gradient present in our data, since samples were all taken in the well mixed surface layers of rivers and lakes. Overall, variation in pH and $p\text{CH}_4$ seem to be leading to different responses of Type I and Type II in our dataset, suggesting that those variables are amongst the ones that could be responsible for the niche partition between Type I and Type II methanotrophs at large scales. This apparent reactivity to aquatic environmental parameters of Type II might seem contradictory to the hypothesis that they are mostly passively transported within the aquatic network. These two observations may be reconciled if one considers that the environmental footprint of this group might be reflecting the conditions of the terrestrial source habitats, which persists within the aquatic network, rather than environmental sorting per se within the network, as hypothesized by Niño-García et al. (2016b).

In conclusion, the composition of methanotrophic communities across the boreal landscape shows only a modest degree of regional differentiation but a strong structuring along the hydrologic continuum from soil to lake communities, regardless of regions. The observed structural shifts were mostly driven by the changes in pH, DOC, methane and nitrogen concentration. The large-scale patterns of community structure and assembly of Type I and Type II methanotrophs, however, were drastically different. Whereas

Type I (Gammaproteobacteria) dominated in larger rivers and lakes and seemed mostly structured by local recruitment and environmental species sorting, Type II methanotrophs (Alphaproteobacteria) were dominant in soil and soil water and then decreased in abundance along the hydrologic continuum, reflecting passive transport and dilution along the networks. This group may thus be less active in aquatic systems than Type I, perhaps playing a lesser role in surface water methanotrophy relative to their contribution to total methanotrophic richness and sequence number, but this is an unresolved issue that requires further investigation. This is important to consider when seeking or interpreting connections between methanotrophic community composition and ambient methane oxidation dynamics in aquatic ecosystems. Indeed, global estimates and models on methane emissions seldom incorporate aspects of methanotroph community structure, even though these are the main controllers of methane evasion from inland waters. Since major methanotroph functional groups that appear to differ in their intrinsic performance may also differ in their response to environmental conditions, including this information might improve our capacity to understand or predict changes in methane oxidation upon changes in the environment.

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Data Accessibility

Raw sequences have been deposited in the European Nucleotide Archive under the accession number PRJEB11530 and PRJEB17975.

Author Contribution

SC, CRG and PdG designed study. CRG and PdG collected data. CRG and SC analysed sequenced data. SC and YP performed statistical analyses. SC, CRG, YP and PdG performed research and wrote the paper.

Tables

Table 1. Average (standard deviation in brackets) of the environmental variables for each region and each ecosystem type: pH, Temperature (T), conductivity (Cond), dissolved oxygen (DO), chlorophyll a (Chla), total phosphorus (TP), total nitrogen (TN), dissolved organic carbon (DOC), CO₂ (*p*CO₂) and CH₄ (*p*CH₄) partial pressure.

Region	System	pH	T (°C)	Cond (µS cm ⁻¹)	DO (mg l ⁻¹)	Chla (µg l ⁻¹)	TP (µg l ⁻¹)	TN (mg l ⁻¹)	DOC (mg l ⁻¹)	<i>p</i> CO ₂ (µatm)	<i>p</i> CH ₄ (µatm)
Abitibi	Rivers	7.3 (0.9)	13.2 (6.3)	135 (128)	9 (1.8)	2.3 (2.7)	33.7 (21)	0.5 (0.22)	20.1 (18)	2776 (1779)	1267 (2477)
	Lakes	7.4 (0.6)	18.8 (4.9)	135 (220)	9 (1.1)	4.7 (7)	26.9 (34)	0.39 (0.19)	11.2 (4.3)	684 (384)	198 (205)
Baie-James	Rivers	7 (0.8)	11.1 (5.1)	75 (60)	10.1 (1.05)	2.7 (1.1)	26.5 (25)	0.47 (0.16)	29 (15)	2081 (1294)	231 (334)
	Lakes	7.2 (0.6)	15.6 (4.5)	48 (55)	9.6 (1.2)	1.2 (2.1)	16.2 (13)	0.28 (0.13)	13.6 (7.4)	546 (122)	170 (367)
Chibougamau	Rivers	6.8 (0.2)	18.7 (3.3)	84 (81)	7.7 (1.3)	2.4 (1.8)	12.1 (1.9)	0.38 (0.12)	15.5 (4.7)	3951 (3450)	1984 (2292)
	Lakes	6.9 (0.5)	15.7 (4.3)	26 (14)	9.4 (1.2)	1.7 (0.5)	9.1 (2.1)	0.19 (0.03)	9.1 (2.6)	676 (148)	34 (32)

Saguenay	Rivers	6.8 (0.6)	13 (3.7)	61 (76)	9.8 (1.2)	1.3 (0.8)	20.3 (12.2)	0.28 (0.16)	10.1 (6.8)	1443 (1107)	160 (174)
	Lakes	6.7 (0.5)	17.4 (3.2)	33 (31)	9 (0.8)	2 (0.7)	12.7 (4.6)	0.23 (0.12)	7.8 (2.2)	921 (337)	243 (221)
Laurentides	Lakes	7.1 (0.6)	22.9 (0.8)	41 (42)	8.6 (0.6)	3.4 (1.5)	10.4 (8.2)	0.27 (0.13)	6.2 (3.4)	474 (442)	1099 (335)
Schefferville	Rivers	6.7 (1.2)	13 (2.6)	37 (21)	8.2 (2)	0.4 (0.3)	8.6 (6.7)	0.17 (0.11)	4.3 (4.1)	2076 (1799)	58 (140)
	Lakes	7.1 (0.8)	15 (1.1)	32 (36)	9.4 (0.4)	0.8 (0.6)	7.3 (3.8)	0.2 (0.11)	4 (1.5)	500 (104)	145 (297)
Côte-Nord	Soil	5.1 (0.6)	15 (2.5)	-	-	-	-	-	21.1 (18.8)	-	-
	Soil water	6 (0.5)	15 (2.7)	371 (998)	6.2 (1.5)	-	-	-	17 (13.4)	5758 (6320)	66074 (75779)
	Rivers	6.2 (0.8)	15 (4.5)	32 (26)	9.3 (1.7)	2.5 (8.8)	30 (35)	0.4 (0.29)	12.8 (13.8)	2342 (2135)	1149 (2704)
	Lakes	5.9 (0.7)	17 (2.4)	15 (4.6)	9.3 (0.7)	1 (0.3)	9.1 (3.4)	0.23 (0.06)	9 (2.5)	840 (294)	290 (384)

869

870 -: no data.

871

872 Figures legends

873 **Figure 1.** Location of the sampling sites across the boreal landscape in northern Quebec, Canada, colored
874 by geographic region. Map created in R with the open-access databases "worldHires"
875 <https://www.evl.uic.edu/pape/data/WDB/>

876 **Figure 2.** Principal coordinate analysis (PCoA) of aquatic methanotrophic communities based on Bray-
877 Curtis distances, coloured as a function of ecosystem type (a) and region (b), overlaid with the most
878 influential environmental parameters.

879 **Figure 3.** Principal coordinate analysis (PCoA) of the methanotrophic communities from the Côte-Nord
880 region, where soil and soil water assemblages were also considered, colored by ecosystem type and
881 overlaid with the most influential environmental parameters.

Figure 4. Principal coordinate analysis (PCoA) of the type II (Alphaproteobacteria) (A) and type I (Gammaproteobacteria) (b) methanotrophic communities in boreal inland water separately, overlaid with the most influential environmental parameters.

Figure 5. Relative abundance of the Type I and Type II methanotrophs and their ratio along the hydrologic continuum. Note that soil and soil waters were only sampled at La Côte-Nord, while the rest include samples from all regions. The middle line inside each box plot indicates the median, the box delimits the 25th and 75th percentile, the whisker the range and the individual dots are outlier beyond the range of the whisker.

Figure 6. Proportion of Type I OTUs (a) and reads (b) and Type II OTUs (c) and reads (d) along the whole hydrologic continuum, considering all samples together. The colors indicate the farthest upslope environment where each OTU was first detected along the continuum assuming a directionality from soils towards lakes (e.g. light blue indicates the proportion of OTUs -or sequences belonging to OTUs- detected in soils across all the sampled sites, see Results for further details).

Figure 7. Relationship between Type I and Type II methanotroph relative abundances as well as their ratio with pH (a), methane (b), DOC (c), temperature (d), Total nitrogen (TN, e), Total phosphorus (TP, f) and Oxygen (g). Data were binned into 6 to 12 groups based on normal scale for pH, temperature, DOC, temperature, nutrients and oxygen, and logarithmic scales for methane. Dots and error bar represent the means and the standard error of the binned data, respectively. Dashed lines correspond to the regression fit of the binned data with the lowest AIC.

Supplemental Figure S1. pH (a), methane (b), DOC (c), temperature (d), Total nitrogen (TN, e), Total phosphorus (TP, f) and Oxygen (g) in samples containing methanotrophic sequences (Yes) or not (No). The middle line inside each box plot indicates the median, the box delimits the 25th and 75th percentile, the whisker the range and the individual dots are outlier beyond the range of the whisker.

Supplemental Figure S2. Neighbour-Joining phylogenetic tree of 265 OTUs belonging to the *Methyloccocaceae* family detected in this study in comparison with their close relatives and representatives downloaded from GenBank. Only bootstrap values above 50 % from 1000 replicates are indicated at the nodes of branches. Scale bar represent the number of base substitutions per site.

Supplemental Figure S3. Neighbour-Joining phylogenetic tree of 44 OTUs belonging to the *Methylocystacea* family detected in this study in comparison with their close relatives and representatives downloaded from GenBank. Only bootstrap values above 50 % from 1000 replicates are indicated at the nodes of branches. Scale bar represent the number of base substitutions per site.

Supplemental Figure S4. Neighbour-Joining phylogenetic tree of 167 OTUs belonging to the *Beijerinckiacaceae* family detected in this study in comparison with their close relatives and representatives downloaded from GenBank. Only bootstrap values above 50 % from 1000 replicates are indicated at the nodes of branches. Scale bar represent the number of base substitutions per site.