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Role of organic matter and microbial communities in mercury retention and methylation in sediments near run-of-river hydroelectric dams

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Highlights

- The St. Maurice watershed was impacted by logging, wildfire and hydropower dams
- Sediment Hg levels were higher in the pondages created by run-of-river dams
- Sediment Hg and methylHg levels were strongly correlated to organic matter
- Hg methylation potential was related to the C/N ratio in sediments
- Hg-methylating community included sulfate reducers, methanogens and fermenters

Abstract

Run-of-river power plants (RoRs) are expected to triple in number over the next decades in Canada. These structures are not anticipated to considerably promote the mobilization and transport of mercury (Hg) and its subsequent microbial transformation to methylmercury (MeHg), a neurotoxin able to biomagnify in food webs up to humans. To test whether construction of RoRs had an effect on Hg transport and transformation, we studied Hg and MeHg concentrations, organic matter contents and methylating microbial community abundance and composition in the sediments of a section of the St. Maurice River (Quebec, Canada). This river section has been affected by the construction of two RoR dams and its watershed has been disturbed by a forest fire, logging, and the construction of wetlands. Higher total Hg (THg) and MeHg concentrations were observed in the surface sediments of the flooded sites upstream of the RoRs. These peaks in THg and MeHg were correlated with organic matter proportions in the sediments (r^2 = 0.87 and 0.82, respectively). In contrast, the proportion of MeHg, a proxy for methylation potential, was best explained by the carbon to nitrogen ratio suggesting the importance of terrigenous organic matter as labile substrate for Hg methylation in this system. Metagenomic analysis of Hg-methylating communities based on the *hgcA* functional gene marker indicated an abundance of methanogens, sulfate reducers and fermenters, suggesting that these metabolic guilds may be primary Hg methylators in these surface sediments. We propose that RoR pondages act as traps for sediments, organic matter and Hg, and that this retention can be amplified by other disturbances of the watershed such as forest fire and logging. RoR flooded sites can be conducive to Hg methylation in sediments and may act as gateways for bioaccumulation and biomagnification of MeHg along food webs, particularly in disturbed watersheds.

1. Introduction

Hydroelectricity accounts for over 16% of total electricity production and is the world's predominant renewable energy source (Zarfl et al., 2015). Flooding of soils associated with construction of large hydroelectric dams and their reservoirs promotes the mobilization of inorganic mercury and its transformation into methylmercury (MeHg) by anaerobic microbial communities. MeHg is a neurotoxin that can transfer along trophic chains, ultimately bioaccumulating in predatory fish that may be consumed by humans (Bilodeau et al., 2017; Willacker et al., 2016). In consequence, hydroelectric development also comes with social conflicts regarding local populations and mostly indigenous communities living nearby rivers (Calder et al., 2016; Jiménez et al., 2015; Orellana, 2005).

Interaction between the quantity and composition of organic matter (OM) and Hg mobilization and transformation in impounded freshwaters has been recognized as important in controlling Hg dynamic (De Bonville et al., 2020; Mucci et al., 1995; Teisserenc et al., 2014). Indeed, OM is an important vector for the transport of Hg from catchments to river systems (Bravo et al., 2017; Grigal, 2002; Meng et al., 2016) as well as a major player in the bioavailability of inorganic mercury (Chiasson-Gould et al., 2014; T. D. French et al., 2014). In boreal ecosystems, organic-rich soils contribute to the sequestration of Hg (Grigal, 2003). Indeed, Hg has a high affinity for OM and particularly thiol groups (Bravo et al., 2017; Grigal, 2002) that may be transported to sediments (Meng et al., 2016). Moreover, as an energy source for anaerobic microbial communities inhabiting sediments of hydroelectric dam reservoirs, OM can fuel microbial Hg methylation. With the recent characterization of the genetic basis of microbial methylation (presence of *hgcAB* genes) (Gionfriddo et al., 2020; Jones et al., 2019; McDaniel et al., 2020; Parks et al., 2013; Podar et al., 2015), genomic

approaches have implicated a diversity of taxonomic groups and metabolic guilds in MeHg production, including sulfate reducers, methanogens, iron reducers, and fermenters (Bravo et al., 2018a; Gilmour et al., 2013; Parks et al., 2013; Podar et al., 2015). Further insights require studies that integrate information on Hg quantity and speciation, OM origin and quality, and the underling microbial communities responsible for MeHg production along the riverscapes where hydroelectric dams are present.

Run-of-river hydroelectric plants (RoRs) may represent environmentally sustainable alternatives to large hydroelectric dams. Indeed, RoRs are small facilities assumed to have minor environmental footprint due to a reduction in reservoir storage time, flooded areas and water level fluctuations (Abbasi & Abbasi, 2011; Anderson et al., 2015; Bakis, 2007). As such, the number of small hydropower plants including RoRs may triple in the next decades (Abbasi & Abbasi, 2011; Couto & Olden, 2018; Kelly-Richards et al., 2017). It is thus important to adequately assess the environmental impact of RoRs in order to improve their construction and management (IPCC, 2012; Maavara et al., 2020). The construction of RoRs may generate cumulative impacts on stream patchiness, water and habitat quality, and composition of periphyton, invertebrate and fish communities (Ellis & Jones, 2013; Gibeau et al., 2016; Kibler & Tullos, 2013; Poole, 2002; Saulino et al., 2017; Vadnais et al., 2012). In addition, there is no clear consensus on the impact of RoRs on upstream sediment retention (Fuller et al., 2016; Pearson et al., 2016; Vörösmarty & Sahagian, 2000). With respect to Hg cycling, studies are very scarce (Cebalho et al., 2017; Silverthorn et al., 2018) but since RoRs generate rather small pondages, the potential impact of RoRs on Hg accumulation is thought to be negligible.

Compounding potential environmental issues, RoRs are often found on small rivers with disturbed watersheds due to urbanization, logging and forest fires (Aguiar et al., 2016; Couto & Olden, 2018; Gauthier et al., 2015). Such disturbances can facilitate the remobilization,

transformation and transfer of Hg and OM that was previously stored in surrounding soils into freshwater ecosystems (Abraham et al., 2017; Skyllberg et al., 2009). For example, logging activities and forest fires can mobilize Hg from biomass, litter and organic soils (Burke et al., 2010; Friedli et al., 2001; Olsson et al., 2017). In both cases, the soil is destabilized due to loss of vegetation cover, which can increase runoff and erosion (Eklöf et al., 2014; Kronberg et al., 2016). Soils may also become hydrophobic, which reduces infiltration and promotes anaerobic conditions favourable to Hg methylation (Eklöf et al., 2018). No study has yet considered the combined effect of watershed disturbances and RoRs construction on Hg cycling.

Moreover, dam construction often reduces wetland areas along small rivers (Gardner & Finlayson, 2018). Since these wetlands have an essential value for the entire ecosystem (Galloway & Branfireun, 2004; St. Louis et al., 1994; Tjerngren et al., 2012), artificial wetlands are often constructed to restore fish spawning habitats, increase biodiversity and to conform to the "no net loss principle" policies for habitat conservation both in Canada and the United States. These constructed wetlands are sites of OM transformation and may act as new sources of microbially-produced MeHg near RoRs (Harper & Quigley, 2005; He, 2019; Hermoso et al., 2019; Kennedy & Mayer, 2002).

While the influence of landscape perturbations such as logging, forest fires and wetland creation on the Hg cycle is well documented in the literature (Hsu-Kim et al., 2018; Obrist et al., 2018), understanding the effect of RoRs and the concomitant interaction with watershed perturbations on Hg transformation is necessary. To address this issue, we focused on a specific river located in a riverscape disturbed by a forest fire, logging, constructed wetlands and the construction of two RoR hydroelectric dams close to the Atikamekw community of Wemotaci (Quebec, Canada). By using a combination of geochemical and metagenomic approaches, this study addresses three research questions: (1) Do RoR pondages

in disturbed riverscapes act as accumulation sites for sedimentary MeHg and OM? (2) Can OM of allochthonous or autochthonous origin be a good predictor of Hg methylation potential in such systems? (3) Which microbial taxonomic groups and metabolic guilds are associated with Hg methylation along the altered riverscape? This study will help decision makers in improving their prediction models by providing new insight related to the construction RoRs in disturbed riverscapes in the context of a constant growth of small hydroelectric plants.

2. Material and Methods

2.1. Sites description

The St. Maurice River takes its main source from Reservoir Gouin flooded in 1918 and flows south until the St. Lawrence River. The studied zone is located in the Haute-Mauricie region (Quebec, Canada), in a subsection (about 40 km long) of the St. Maurice River between the Atikamekw native reserve of Wemotaci and an old reservoir called Reservoir Blanc (upstream of the town of La Tuque). In this subsection, two run-of-river hydroelectric power plants have been constructed in 2008. Chute Allard (CA- 47°53'34.321" N; 73°43'5.919" W) and Rapides-des-Coeurs (RDC- 47°47'16.92" N; 73°22'28.152" W). The two resulting pondages flooded 2 km^2 and 3.7 km^2, respectively, and caused the loss of 2.4 km^2 of wetlands. In order to compensate the loss of important ecological processes taking place in wetlands, such as fish spawning and nursery, Hydro-Quebec (a major public utility for electricity in Eastern Canada) constructed artificial wetlands by excavation of water channels of about 0.08 km^2. The river section has undergone two additional landscape perturbations in the last decade: a wildfire of more than 180 km^2 in 2010 and continuous logging activities (Supplementary Information (SI), **Fig S1**). Five years after impoundment of the RoR dams, elevated concentrations of

MeHg in fish muscle, causing a modification of the fish consumption guidelines, have been reported.

2.2. Sampling

Two sampling campaigns were performed in late August 2017 and 2018 along a 40 km subsection of the river. Over this period, bulk sediments or undisturbed sediment cores were collected at 18 sites (**Table S1).** Water temperature ranged from 16 to 21 °C. These sites were classified according to their distance to the RoRs and to the concomitant disturbances (forest fire and logging) (**Fig. S1)**. Each sampling location corresponds to either flooded terrestrial soils upstream of the CA dam (CAUP sites) or the RDC dam (RDC sites), new constructed wetland channels (CC sites), sites downstream of the RDC dam which are located in the Reservoir Blanc (BR sites), sites downstream of CA (CADW site) or a reference site (RDC-REF) which is a flooded site not impacted by logging activities nor forest fire (**Table S1**). CAUP sites are surrounded by watersheds impacted by forest fire while RDC and BR sites are impacted by logging. CAUP, RDC and BR sites are all located in shallow bays (< 2 m, but mostly < 1 m) with a reduced river flow.

All plastic containers for Hg measurements were acid-washed (HCl 10% volume/volume (v/v)) and rinsed with ultrapure water before sampling. Bulk sediments were sampled in triplicates at 1 to 3 m from the river edge and at less than 2 m depth. We used an Ekman Grab (1.52x1.52x1.52 cm or 3.5 L) in 2018 (n = 15 sites), except for CAUP-12 (n = 2), BR-2 (n = 2), and CAUP-10 where bulk and cores were pooled for logistical reasons (n = 5). A subsample (around 10-20 grams) of the 5 first cm was sampled with a plastic spoon and stored in a plastic bag at -20°C until Hg and OM analyses. A gravimetric hand corer was used to collect three cores per site in 2017 (5 sites) and four cores in 2018 (8 sites). Cores were sealed at the bottom using a cap and the overlying water was removed by piercing the plastic

core in the field. The remaining 2-3 cm of water were jellified using sodium polyacrylate (cross linked, Sigma-Aldrich®) to preserve the water-sediment interface (Tomkins et al., 2008). The core was then sealed with floral foam and a cap was placed on top. In the laboratory, sediment cores were carefully cut longitudinally with a rotating saw and split into two halves. The sediment surfaces of the half-cores were cleaned using a plastic ruler in order to smooth the sediment surface (parallel to the laminations). The half core was immediately sliced every cm in order to have depth profiles. Every cm was split into one sample for chemical analysis and one sample for metagenomic sequencing (around 5-10 grams). The sample for chemical analysis were frozen at -20°C and genomic samples were immersed in liquid nitrogen and then kept in a -80°C freezer.

2.3. Total mercury and methylmercury analyses

For THg and MeHg analyses of the 2017 sediment samples, 0.05 to 0.1 g of freeze-dried sediments were weighed and digested using 1 mL of diluted HNO_3 (5.3 M, grade Omnitrace Ultra) at 60°C overnight. MeHg was analyzed in an aliquot by cold vapor atomic fluorescence spectrophotometer (CVAFS; series 2700, Tekran™, Canada) according to the EPA method 1630 (USEPA, 2001). Subsequently, the first digestate underwent a second digestion using 270 µL of HNO_3 (16 M) at 120 °C in an industrial pressure cooker (125^{o}C; 1.054 kg cm^{-2} ; All American Canner, Red Hill General Store) followed by the addition of 150 µL of pure HCl (12.1 M, grade OmniTrace Ultra, MilliporeSigma™) and 250 µL of hydrogen peroxide (9.8 M, grade Optima™, Fisher Scientific) for a final digestion overnight at room temperature. Samples were diluted to 15 mL with ultrapure water and one mL of this digestate was used to quantify total mercury (THg) using a CVAFS (series 2600, Tekran™, Canada) according to the EPA method 1631 (USEPA, 2002). Analytical quality was checked

using two certified reference materials, IAEA-405 with a certified value of 5.49 ± 0.53 ng g^{-1} and TORT-2 with a certified value of 152 ± 13 ng g^{-1} (± confidence interval).

Sediment MeHg was also measured for 2018 samples by CVAFS (series 2700, Tekran™) according to the EPA method 1630, but the digestate volume was higher (5 mL of HNO_3; 5.3 M, grade OmniTrace Ultra, MilliporeSigma™). The analytical quality was checked using the certified reference material, IAEA-158 for MeHg with a certified value of 1.4 ± 0.4 ng g^{-1}. Sediment THg from 2018 samples was measured using a Direct Mercury Analysis system (DMA-80). The analytical quality was checked using the certified reference material TORT-2 and SO-2 with certified values for Hg of respectively 270 ± 60 ng g^{-1} and 82 ± 9 ng g^{-1}. All values are reported in dry-weight (dw) concentrations. Details on quality controls are available in SI.

2.4. Organic matter proportion and C/N ratio

Organic matter proportions (%) were assessed using loss on ignition (LOI) after heating for 2h at 550°C (Dean, 1974). Carbon (C) to nitrogen (N) ratio (C/N) was calculated from the measurements of the proportion of atomic carbon and nitrogen on total weight of the sample. About 7-10 mg of sediments were analyzed using a CHNS-O Element Analyzer (EA-1108 model, Fisons©) calibrated with acetanilide. Quality control was conducted with internal standards, sulphanilamide (N = 16.26%, C = 41.85%) and atropine (N = 4.84%, C = 70.56%). Recovery percentage for both standards ranged between 95 and 105% and the blanks were undetectable.

2.5. Metagenomic analysis

DNA extraction and sequencing

DNA was extracted from 0.25 ± 0.01 g of wet sediment using the PowerSoil®DNA Isolation Kit (Mo Bio Laboratories inc.) according to the manufacturer's recommendations. DNA quantification was performed using Qubit™ fluorometer with a Qubit™dsDNA BR Assay Kit (Invitrogen™). Shotgun DNA sequencing was performed using Illumina NovaSeq 6000 S4 PE150 technology at Genome Québec (McGill University, QC, Canada).

Metagenome assembly and annotation

Raw metagenomic reads were quality-trimmed and filtered with Trimmomatic v0.32 (with a minimum length of 36 bp and a quality threshold for bases of 15). The filtered paired-end reads were assembled using Megahit v.1.0.6 (--k-list 23, 43, 63, 83, 103, 123) (Li et al., 2016). Reads were mapped to the assembly with the Burrows-Wheeler Alignment Maximum Exact Matches (BWA-MEM) tool using the option –bwtsw (Li & Durbin, 2010). The scaffolds and average scaffold coverage files (generated with jgi_summarize_bam_contig_depth script) were submitted to the Integrated Microbial Genomes and Microbiomes platform available at the Joint Genome Institute (JGI IMG/M) for gene identification and functional annotation (Markowitz et al., 2008).

Analysis of *hgcA*, *dsrB,* and *mcrA* gene diversity and abundance

The predicted protein sequences generated by the JGI-IMG/M pipeline were screened for genes encoding HgcA by a hidden Markov model (hmm) search (Eddy, 1992). Protein sequences were screened against a previously generated profile hmm model containing 155

diverse HgcA sequences (Bravo et al., 2018b) using HMMER (Eddy, 1992). HgcA hits were selected using an alignment length threshold of 0.5 and a bitscore cutoff of 100. Taxonomic assignment of HgcA sequences was based upon the closest related sequences available in a custom HgcA database (McDaniel et al., 2020) using the Genome Taxonomy Database (GTDB; Parks et al., 2018). Closest-related sequences were identified base on the top hit using Diamond V.0.9.30 (Buchfink et al., 2015) with the following settings: e-value ≤1E-30 and ≥ 60% sequence similarity across 80% of the full length protein. The top hit for each query sequence meeting these criteria was used for representative taxonomic assignation. The *hgcA* gene abundance was estimated by summing the average coverage of *hgcA*-containing scaffolds assigned to a taxonomic group. Gene abundance was normalized across metagenomes by dividing the gene coverage by the coverage of the total number of reads in a metagenome, and values are reported as *hgcA* copies per million metagenomic reads. All the sequences can be found in **Table S2**. The functional marker genes for methanogens (*mcrA*; EC: 2.8.4.1) and sulfate reducers (*dsrB*; EC: 1.8.99.5) were identified in the annotated metagenomic data from JGI-IMG/M (11/2019) using their respective EC numbers. The *mcrA* and *dsrB* gene abundances were calculated in an equivalent manner as described for *hgcA*.

2.6. Statistical analysis

MeHg, THg, LOI and C/N ratio measurements were presented as means and standard deviations (means ± SD). The proportions of MeHg (%MeHg) was calculated by dividing THg concentrations from MeHg concentrations ([MeHg]/[THg]×100). To compare the means for THg, OM, MeHg, and %MeHg in bulk sediments, we first performed a normality (Shapiro-Wilk) and equal variance tests (Brown-Forsythe) followed by an analysis of variance (ANOVA) and a posthoc Holm-Sidak Test (all groups paired). If normality was not respected

(e.g. MeHg), the Kruskal-Wallis Test on rank was performed. Linear regression models between THg, MeHg, %MeHg and OM were performed with the packages *car* and *tidyverse* in RStudio® (RStudio Team, 2020). Linear regression models between %MeHg and C/N ratio and between total *hgcA* abundance and *hgcA* assigned to sulfate reducers and methanogens were performed in the same manner. A Linear Mixed Model (LMM) was used to take into account the sites as a random effect when performing regression analysis between *hgcA* abundance assigned to Methanomicrobia and *mcrA* abundance (package *lme4* in Rstudio®).

3. Results

3.1. Mercury, methylmercury and organic matter along the river section

THg and MeHg concentrations, %MeHg and %OM in bulk sediments co-varied spatially along the river section (**Fig. 1**), and significant differences between sites was observed (ANOVA; $p < 0.05$). Specifically, CAUP-10, CAUP-12, RDC-10/11/13 had significantly higher THg and OM than other sites (**Fig. 1.A-B**). These sites were affected by forest fires and logging activities and were also associated with peaks for MeHg and %MeHg (CAUP and RDC sectors). When considering all sites, THg concentrations in bulk sediments varied from 9 to 201 ng g^{-1} (**Fig. 1A**; average = 57.8 ± 46.8 ng g^{-1}) and from < 0.02 to 19 ng g^{-1} for MeHg (**Fig. 1B;** average = 2.2 ± 3.9 ng g^{-1}). MeHg concentrations represented from 0.04 to 12% of THg (**Fig. 1D**) and the proportion of organic matter ranged from 3 to 73% (**Fig. 1C**).

Peaks for all these variables were observed in the RoR pondages (CAUP and RDC). Lows were observed in the reference site (RDC-REF), the constructed wetlands (CC) and the

area downstream from the first RoR (CADW), which are all sites not associated with concomitant disturbances. Compared with the sites of lowest THg concentration (CC-3; 17.8 ± 8.7 ng g^{-1}), peaks associated with sites CAUP-12, RDC-11 and BR-2 were 10.1, 6.9 and 4.2 times higher, respectively. When sites were grouped per sector, the RDC and CAUP sectors stood out for their higher MeHg concentrations and %MeHg (5.3 ± 3.5% for CAUP; 2.9 ± 2.5% for RDC). Indeed, compared to the sectors with the lowest MeHg concentrations (average of CC, RDC-REF and CADW), CAUP, RDC and BR sectors were 11.7, 6.7 and 2.1 times higher, respectively. %MeHg was 3.0 times higher in CAUP and 1.7 times higher in RDC compared to the other sectors. The BR sector had intermediate THg concentrations (54 ± 30 ng g^{-1}) and MeHg concentrations (1.0 ± 0.7 ng g^{-1}) but lower %MeHg (1.7 ± 0.4%) and OM content (14 ± 7 %). Given the similar spatial patterns observed for HgT, MeHg and %MeHg, we performed correlation analyses to confirm their relationship.

3.2. Relationship between organic matter, mercury retention and methylation

We observed a strong significant correlation ($p < 0.0001$) between OM proportions and the different Hg variables (THg, MeHg and %MeHg) when considering all sites (**Fig. 2)**. OM proportion explained 87 and 82% of the observed variability for THg and MeHg concentrations, respectively, and 46% for the MeHg proportions. The strong relationship between OM and THg is consistent with the spatial co-variations that we observed in **Fig. 1**. In general, the sites located in RDC and CAUP sectors had higher THg concentrations and higher OM proportions in sediments. Sites with high OM accumulation are also those with higher methylation potential as estimated with %MeHg (**Fig.S2**).

The C/N ratio, used as an indicator for the origin of organic carbon, was measured at each cm of the sediment cores for both 2017 and 2018 field campaigns. The sites CAUP-03,

CAUP-06 and RDC-14 were characterized by C/N ratios over 20 (**Fig. 3**) indicative of sediments mostly composed of terrigenous OM (Kaushal & Binford, 1999; Meyers, 1994). In contrast, CAUP-10 and CC-4 sediments had the lowest C/N ratios (< 15) and are thus more likely dominated by river autochthonous primary production. Indeed, both sites were characterized by abundant periphyton growth on branches and macrophytes. The C/N ratio explained 87% of the observed variability in methylation potential (%MeHg), when averaging the five-upper cm of each core (**Fig. 3**). Further, when looking at depth profiles for each site (**Fig. S3**), C/N ratios covaried with the %MeHg. Hence sediments characterized by more terrigenous OM had a higher methylation potential.

3.3. Abundance and composition of the mercury-methylating community

A subgroup of 15 sediment samples representing the upper 10 cm of the sediment cores including most %MeHg peaks (> 5%, **Fig. S3**), was selected for metagenomic analyses based on inflection points of the [THg] and [MeHg] profiles in sediment cores from 2017. The *hgcA* gene was identified in all sediment samples, and *hgcA* abundance ranged between 15 and 90 *hgcA* gene copies per million total reads (*hgcA*/M). The lowest *hgcA* abundances (< 20 copies *hgcA*/M) were generally observed in the upper first cm, except for the constructed wetlands where similar *hgcA* gene abundances were identified throughout all core layers (**Fig. S4**). No significant correlation ($p > 0.05$) was found between *hgcA* gene abundance and THg concentration, MeHg concentration, or %MeHg (**Fig. S5)**.

Given the detection of *hgcA* in all samples, we next explored the taxonomic composition of the *hgcA*-containing community. We identified a broad taxonomic diversity of Hg methylators among sites and depths. In total, *hgcA* genes were assigned to 35 distinct bacterial and archaeal classes but only nine classes were present in at least 50 % of the

samples **(Fig. 4**). Among all sequences (all samples pooled), 27 ± 9 % of the sequences remained unassigned (**Fig. 4-A**). Among the nine most abundant classes found in our samples, were Methanomicrobia (16%), Syntrophia (13%), Bacteroidia (10%), Anaerolinaea (9%), BSN_033 (6%), Spirochaetia (3%), Thermodesulfovibrionia (3%), UBA10030 (2%) and Desulfobacteria (2%). Syntrophia, BSN_033 and Desulfobacteria are putative sulfate reducers, Methanomicrobia are putative methanogens while Bacteroidia are known to contain some genera able to act as fermenters. To support **Fig. 4**, we performed linear regression models between *hgcA* total abundance and *hgcA* assigned to Methanomicrobia (r^2 = 0.54; p < 0.01; **Fig. 5A**), as well as total *hgcA* abundance and *hgcA* assigned to putative sulfate reducers (Syntrophia + BSN033 + Desulfobacteria) (r^2 = 0.53; p < 0.01; **Fig. 5B**). The random effect of site location was tested using a LMM to consider the dependency of the abundance between sites but this effect was not significant. Those regressions showed that among all sites, the proportions of sulfate reducers (31 ± 10%) and methanogens (15 ± 10%) were similar among sites even if the total abundance varied. Thus, it appears that sulfate-reducers > methanogens > fermenters are the most abundant members of the *hgcA*-harboring community. To investigate distribution patterns within the *hgcA*-containing community and to identify associations between particular methylators and depths/locations along the river section, we compared the microbial community composition between the sites and depths using a Non-Metric Multidimensional Scaling (NMDS). However, no clear pattern in distribution of samples was observed (**Fig. S6**).

3.4. Contribution of different metabolic guilds to the methylating prokaryotic community

To further assess the contribution of methanogens and sulfate reducers to Hg methylation in the sediments of the St. Maurice River, we first explored if methanogens and/or sulfate reducers were correlated to Hg variables (THg, MeHg or %MeHg) and then we estimated the fraction of methanogens and sulfate reducers harboring the *hgcA* gene. We found no significant correlation between Hg variables and abundance of methanogens (inferred by *mcrA* gene abundance) or sulfate reducers (inferred by *dsrB* abundance) (n=15, $p > 0.05$) except for a weak correlation between *dsrB* and HgT concentrations ($r^2 = 0.27$, n=15, $p = 0.027$).

We found a strong significant relationship between *mcrA* abundance and *hgcA* abundance assigned to the known class of methanogenic archaea, Methanomicrobia ($p < 0.0001$; r^2 marginal = 0.75; r^2 conditional = 0.87; **Fig. 6A**), suggesting that the whole community of methanogens in the St. Maurice sediments are represented by the class Methanomicrobia. An increase in the methanogens community will leads to an increase of the Methanomicrobia *hgcA*-containing community. The average proportion of Methanomicrobia-containing hgcA which is about 14% is likely slightly varying depending on the location. The class of Methanomicrobia includes, among others, the genera *Methanolinea*, *Methanoregula* and *Methanospirillum* and constitutes the majority of putative methanogens in our samples (95%). Interestingly, no correlation was observed between *dsrB* abundance and *hgcA* abundance assigned to known class of putative sulfate reducers ($p = 0.05$; **Fig. 6B**), suggesting thatputative sulfate reducers include in the *hgcA*-containing community represent only a small fraction of whole community of sulfate reducers in these sediments.

4. Discussion

4.1. Organic matter drives Hg accumulation in pondages

Total Hg concentrations in sediments ranged from 8 to 200 ng g^{-1} (with a mean of 57 ± 46 ng g^{-1}) and are comparable with those found in sediments from natural aquatic environments in North America (French et al., 1999; Kamman et al., 2005; Mosher et al., 2012). French et al. (1999) reported mean values of 39 ng g^{-1} THg in sediments of 34 lakes from Newfoundland (Canada) which is a similar average with the one measured in the present study. The highest concentrations (> 100 ng g^{-1}) measured from CAUP and RDC pondages are slightly higher than background concentrations found in aquatic systems from the western United States of America (Fleck et al., 2016) but comparable with reservoirs in Eastern Canada (Kamman et al. 2005) or those of the Three Gorge Reservoir in China (Meng et al., 2016). The similarity of the measured THg concentrations in pondages compared to other reservoirs in North America could be related to the retention of sediments upstream of the dams.

Indeed, the higher levels of THg and OM in the pondage sites of the CAUP and RDC sectors suggests that pondages acted as sediment traps accumulating THg and OM (**Fig. 1**). The positive correlations of THg as a function of OM proportions (**Fig. 2A**) underscore the role of OM as a driver for the presence of Hg in the sediments. The coupling between Hg and OM in freshwater ecosystems is well known (Meng et al., 2016; Ravichandran, 2004; Teisserenc et al., 2011). In addition, the higher OM levels observed in CAUP and RDC sectors are likely a result of the watershed disturbances, namely forest fire and logging, which favored the flux of Hg and OM from the drainage basin to the river system and may thus partially account for the Hg accumulation in the pondages. This result is supported by the C/N ratio (**Fig. 3**), used as an indicator of whether the OM resulted from autochthonous or

allochthonous processing (Bravo et al., 2017; Drott et al., 2007; Kaushal & Binford, 1999). To further support this assumption, the lower THg and OM levels found in RDC-REF (pondage without logging) also suggests that it is the interaction between pondages and the concomitant presence of the perturbations that is responsible for higher OM and Hg levels. RoRs located in undisturbed watersheds may cause less or no alteration of Hg cycling, as has been reported by Silverthorn et al. (2018). Further research on these interactions between watershed disturbances and RoRs on a number of rivers stretches may clarify this hypothesis.

4.2. Pondage sediments have a high methylation potential

We found that pondages are likely accumulation sites for OM-bound Hg that could promote the Hg methylation leading to MeHg production and subsequent trophic chain contamination. The %MeHg which is generally accepted as an indicator for potential net Hg methylation (Drott et al., 2008), was higher or comparable to the values reported in the literature for reservoir in North America (Kamman et al. 2005). On average, MeHg accounted for less than 2% of THg in our sediments, which is consistent with ranges of values for stream sediments (Fleck et al., 2016; Mikac et al., 1999). However, the higher MeHg proportions above 3%, encountered in the flooded sites from CAUP and RDC sectors, are values usually linked to elevated methylation potential (Fleck et al., 2016) (**Fig. 1**).

In addition, the coupling between %MeHg and OM proportions was relatively weak in our data (**Fig. 2C**) suggesting that only part of the OM was available for bacterial processing. It is likely that variables that integrate OM sources and quality could be better correlated to Hg methylation potential. The C/N ratio is also a proxy for organic matter lability which directly influences Hg methylation (Kim et al., 2011). Thus, the strong correlation between the percentage of MeHg and the C/N ratio, both in surface sediments (**Fig. 3**) and along core

profiles (**Fig. S3**), implies that higher methylation potential was found in the pondages where sediments were more influenced by allochthonous inputs (Drott et al., 2007; Tjerngren et al., 2012). Specifically, the surface sediment in CAUP-06, CAUP-03 and RDC-14 harbored the highest C/N ratio and %MeHg. It is likely that in this river system, the main source of labile OM available for Hg methylation in sediments comes from the watershed inputs rather than from autochthonous production. This contrasts with a recent study conducted in lakes where autochthonous OM was shown to be the main driver of Hg methylation (Bravo et al., 2017). Indeed, it is traditionally accepted that terrestrial/allochthonous OM is generally recalcitrant to biological degradation and therefore not significantly contributing to biogeochemistry cycles. However, recent studies shows that biologically degradable OM is strongly coupled to terrestrial inputs (for instance inferred from colored DOM) in aquatic environments when considering the gradient landscape (Lapierre et al., 2013; Lapierre & del Giorgio, 2014). Indeed, the continental aquatic environments such as rivers, lakes and wetlands are highly connected to terrestrial watersheds, and thus to allochthonous inputs representing freshly imported OM which could be readily degraded by microbial degradation as it gets more labile through its circulation along the watershed. More studies are clearly needed to understand this shift in the coupling of OM of different quality and origins, and Hg methylation in lakes, streams and RoR-impacted systems.

With respect to the constructed wetlands, these systems are often hotspots for Hg methylation (St. Louis et al., 1994; Tjerngren et al., 2012). This was not the case in our study, since the two sites located in the recently constructed wetlands (CC-3 and CC-4) harboured the lowest OM content and THg concentrations (**Fig. 1A, B, C**). These wetlands were built on former terrestrial land. The excavation process led to a removal of the top organic layer, presumably richer in Hg. This likely explains the unexpectedly low MeHg concentrations and %MeHg found in the sediments of these constructed wetland. However, these wetlands are

also characterized by a large periphytic community that could potentially methylate Hg (Desrosiers et al., 2006; Hamelin et al., 2015). Thus, further research is needed to assess the overall role of these wetlands on MeHg production and transfer to biota.

In summary, pondages that underwent additional landscape alterations (forest fire and logging) likely promote net Hg methylation. It would be important to better understand these interactions between watershed disturbances and in-river Hg and carbon processing in harnessed rivers. This could be achieved by selecting a large number of sites differently affected by combinations of watershed perturbations.

4.3. A diverse Hg-methylating community in the St. Maurice River

In this study, we described the abundance and diversity of the *hgcA*-carrying community along the studied river stretch and highlighted the contribution of major metabolic guilds to Hg methylation. The *hgcA* abundances could not be clearly linked to any of the analyzed environmental and mercury parameters (**Fig. S4**). This absence of correlation is concordant with previous studies focusing on a broad range of compartments including water, sediments and soils (Bae et al., 2014; Bravo et al., 2016; Christensen et al., 2019; Goñi-Urriza et al., 2015; Schaefer et al., 2014), although a strong correlation between *hgcA* abundance and MeHg content was observed previously in paddy soils historically contaminated with Hg in China (Liu et al., 2014). Predicting the extent of MeHg production from *hgcA* gene abundance is challenging because (i) the presence of a gene does not ensure the presence of a functional protein but rather the genomic potential for Hg methylation (Christensen et al., 2019) and (ii) the presence of *hgcAB* may not be Hg-driven but regulated by yet unknown environmental parameters, as the functional diversity of *hgcAB* is not yet fully understood. In addition, OM characteristics (e.g. terrestrial source vs. algal source) may influence the bioavailability of Hg and thus the ability for methylating microbial communities to perform Hg methylation (Bravo

et al., 2016, 2017). Therefore, only a lesser and unknown fraction of measured Hg may be available to be methylated, impeding a reliable correlation.

The *hgcA* sequences identified in the sediments were taxonomically diverse (**Table S2**). We did not observe a significant change in the relative abundance of *hgcA*-harboring classes across sites and depths along the river section despite changing conditions (**Fig. 4B** and **Fig. S6**). Our results showed that *hgcA* sequences associated with Syntrophia, Bacteroidia and Methanomicrobia were dominant in the metagenomes (**Fig. 4A**). This is concordant with recent studies where most of the *hgcA* sequences identified are from the known Hg methylators within Deltaproteobacteria, Bacteroidetes, and Methanomicrobia (Bae et al., 2014; Gilmour et al., 2013; McDaniel et al., 2020; Podar et al., 2015).

With respect to *hgcA*-harboring methanogens, almost all have been identified in the Methanomicrobia class to date (Bae et al., 2014; Gilmour et al., 2018) and the importance of methanogens as Hg methylators in natural environments is increasingly recognized (Gilmour et al., 2018). A recent study on the widespread distribution of *hgcA* among the tree of life in various environments revealed that Syntrophobacterales were the dominant order among Deltaproteobacteria (McDaniel et al., 2020). The order of Syntrophobacterales is known to contain sulfate-reducing Hg methylators and interestingly, they have also been described as major contributors for the beneficial interaction between non-Hg-methylating syntrophs and Hg-methylating SRB and methanogens (Bravo et al., 2018a). Thus, the dominance of methanogens and sulfate reducers in the *hgcA*-containing community in the St. Maurice River seems to be concordant with the common literature on freshwater sediments, while Bacteroidia have only recently been implicated as methylators and is prevalent in freshwater sediments as fermenters (Gionfriddo et al., 2020).

The positive relationships between the *hgcA*-containing total community and *hgcA*-containing Methanomicrobia (**Fig. 5A**) and between the abundance of the *mcrA* marker gene

for methanogenesis and *hgcA*-containing Methanomicrobia (**Fig. 6A**) indicate methanogens significantly contribute to Hg methylation in the studied sediments and are mainly represented by the class Methanomicrobia. Indeed, methanogens, and more particularly, Methanomicrobia were previously shown to be important Hg methylators in some systems such as paddy soils and boreal lake sediments (Bravo, Peura, et al., 2018; Vishnivetskaya et al., 2018)

Interestingly, despite the high abundance of *hgcA*-containing putative sulfate reducers among the identified Hg methylators (mostly associated with Syntrophia) and a total SRB population (based on the abundance of the marker gene *dsrB*) relatively high in our data, we found no correlation between *dsrB* gene abundance and *hgcA*-containing putative sulfate reducers **(Fig. 6B**). This lack of correlation is an indication that *hgcA*-harboring Syntrophia, which were the most abundant microbial taxa assigned to putative sulfate reducers, may be rare among the total community of SRB. The majority of SRB may not contain the *hgcA* gene and are thus not able to methylate Hg. However, the correlation between total *hgcA* abundance and *hgcA* assigned to sulfate reducers (**Fig. 5A**) is an indication that sulfate reducers may be as important as methanogens in Hg methylation in surface sediments.

In summary, archaeal methanogens, sulfate reducers (Syntrophia) and Bacteroidia appear to be the primary Hg methylators in the sediments of our studied sites. It is known that SRB and methanogens can act in syntrophy and that representative groups of each of these guilds can methylate Hg (McInerney & Bryant, 1981; Pak & Bartha, 1998). This syntrophy in the sediments may be due to their metabolic interdependence on the interspecies electron transfer via formate, H_2 or acetate providing carbon and electrons for both species. Interestingly, these intermediate products can be provided through the fermentative activity of Bacteroidia, the third major Hg-methylating group identified in our metagenomes. Indeed, species able to degrade OM such as the Bacteroidia play a key role in the hydrolysis and decomposition of cellulosic organic matter and produce hydrogen, a major end-product of fermentation released

into the environment and used by methanogenic archaea (Briée et al., 2007; Hatamoto et al., 2014). Such an anaerobic syntrophy would allow the complete mineralization of OM while being crucial to promote Hg methylation in the sediments of the St. Maurice River.

5. Towards a better understanding of the impact of RoRs on Hg cycling

Overall, this study highlights for the first time that the construction of RoRs can alter the Hg cycle in sediments and that this phenomenon is likely exacerbated by the co-occurrence of local watershed disturbances. Indeed, at the watershed scale, pondages developed during RoR construction likely led to the disruption of sediment transport and the subsequent accumulation of THg bound to OM as highlighted by the higher THg concentration and OM content, observed in the pondages upstream of the RoRs. In addition, the concomitant presence of landscape perturbations such as wildfire and deforestation impact the riverscape and promote the transport of terrigenous OM. This input of fresh terrestrial OM is potentially leading to higher Hg methylation as indicated by the higher MeHg and %MeHg. In this river stretch, the Hg methylating community was ubiquitous and diverse with methanogens, sulfate reducers and fermenters being dominant. To minimize the risk of increased MeHg production, the biogeochemical interactions between RoR construction and local watershed disturbances must be considered, particularly since RoR are usually built on medium-size rivers where allochthonous inputs of OM may be significant.

In the current context of growing energy demands worldwide and climate change, the hydroelectric sector is in expansion. Issues relative to small hydropower, such as RoRs, must be seriously considered. We recommend monitoring Hg concentrations in biota, sediments and water before and after the construction of small hydropower in order to better address this potential environmental footprint.

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Figures captions

FIGURE 1: *Biogeographic gradient of [THg], [MeHg], %MeHg and %OM in surface bulk sediments.* Histogram representation of (A) [THg], (B) [MeHg], (C) %MeHg and (D) % organic matter in the 15 sites sampled along the St. Maurice River. Sites are ordered from upstream to downstream. The error bars stand for standard deviation (n=2 -5).

FIGURE 2: *Relationship between Hg variables and organic matter content in surface bulk sediments*. Relationship between (A) [THg] and %OM, (B) [MeHg] and %OM, (C) %MeHg (ratio MeHg/THg) and %OM. Error bars are mean standard deviations.

FIGURE 3: *Relationship between %MeHg and C/N ratio.* Each point represents an average of the first five centimeter for the replicates of each site. The error bars stand for standard deviation

FIGURE 4: *Prokaryotic diversity of the Hg methylating community in the sediments of the St-Maurice river along a gradient of perturbations.* (A) The relative abundances of the dominant classes in all pooled samples. (B) The relative abundance of dominant classes at each site of the river section.

FIGURE 5: *Relationship between total hgcA abundance and abundance of hgcA assigned to sulfate reducers and methanogens.* Linear regression between (A) the total *hgcA* gene abundance and *hgcA* assigned to Methanomicrobia abundance (p-value <0.01, r^2=0.54), (B) the total *hgcA* gene abundance and *hgcA* assigned to putative sulfate reducers (Syntrophia, BSN_033 and Desulfobacteria) (p-value <0.01, r^2=0.53).

FIGURE 6: *Relationship between marker genes for the main metabolic guilds involved in Hg methylation and the hgcA gene involved in mercury methylation*. Linear regression between (A) the *mcrA* gene involved in methanogenesis, and the abundance of *hgcA* assigned to methanogens (Methanomicrobia) (p-value < 0.0001; r^2=0.75), (B) the *dsrB* gene involved in sulfate reduction and *hgcA* assigned to putative sulfate reducers (Syntrophia, BSN_033 and Desulfobacteria)(p-value=0.05).

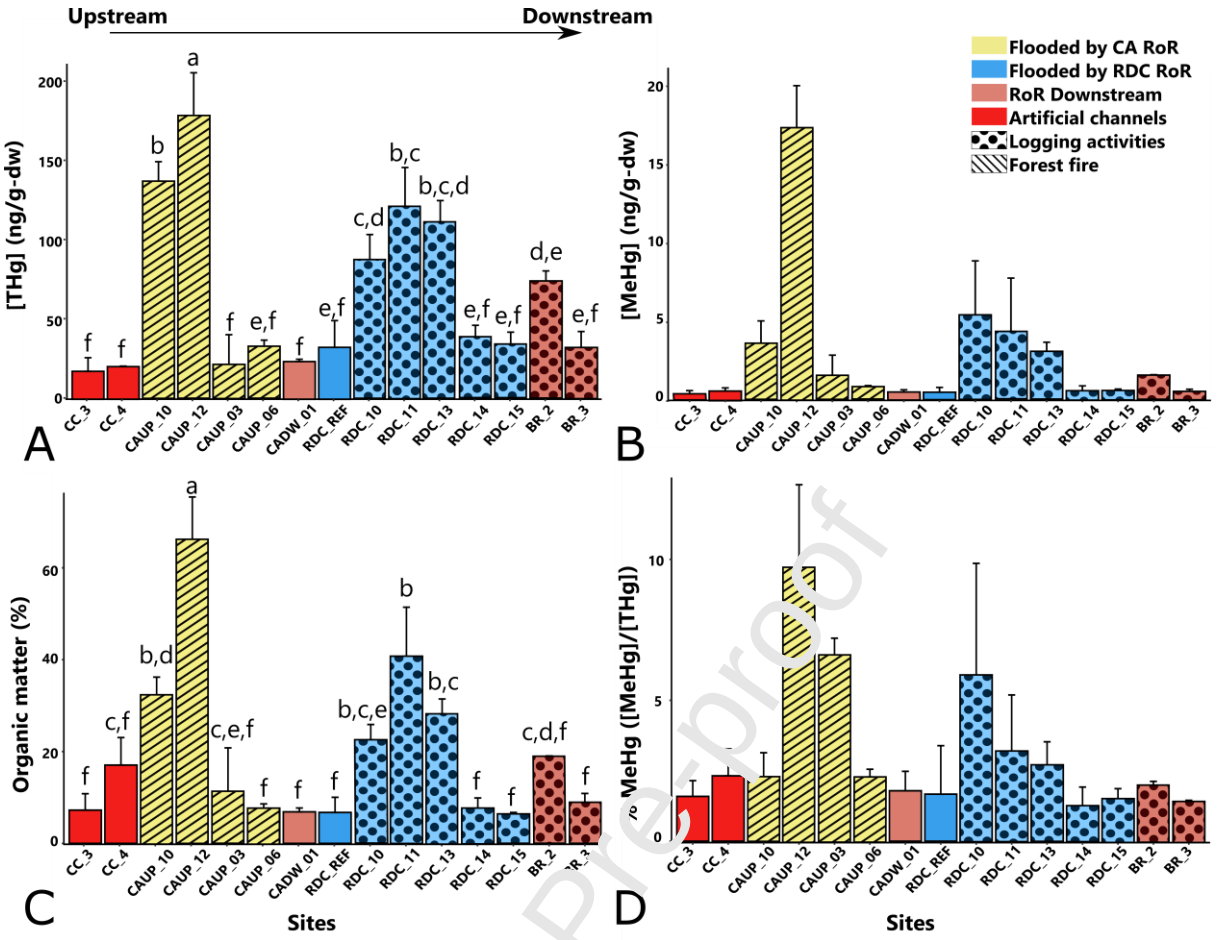


Figure 1; Millera Ferriz et al., 2020

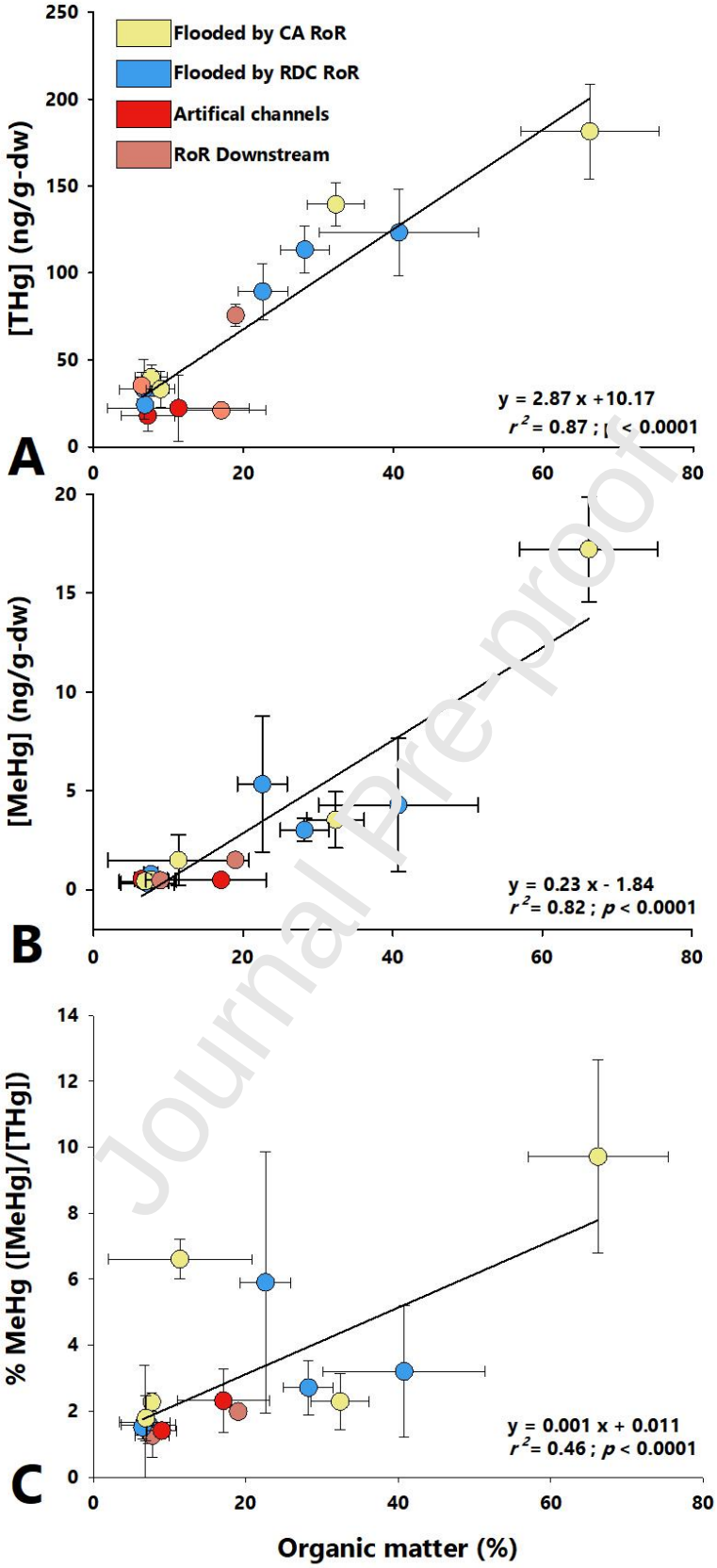


Figure 2; Millera Ferriz et al. 2020

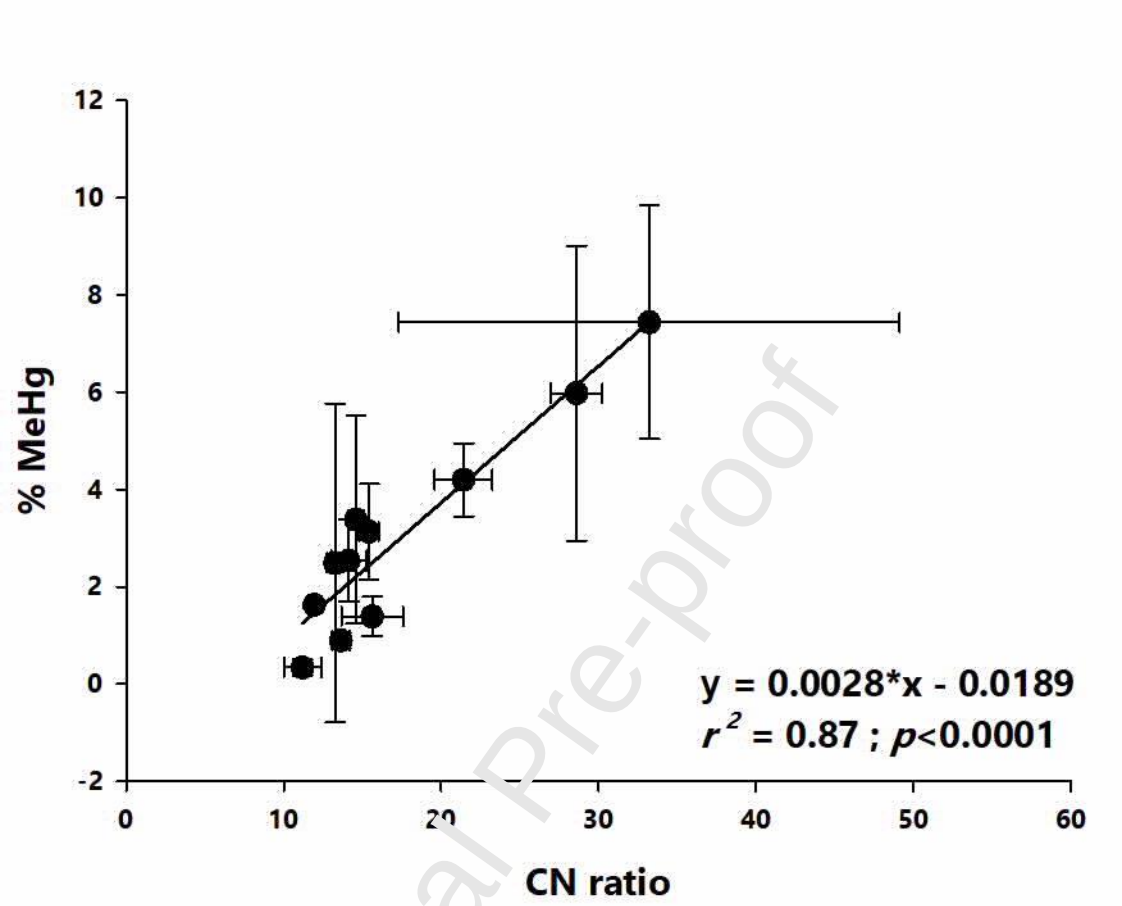


Figure 3; Millera Ferriz et al. 2020

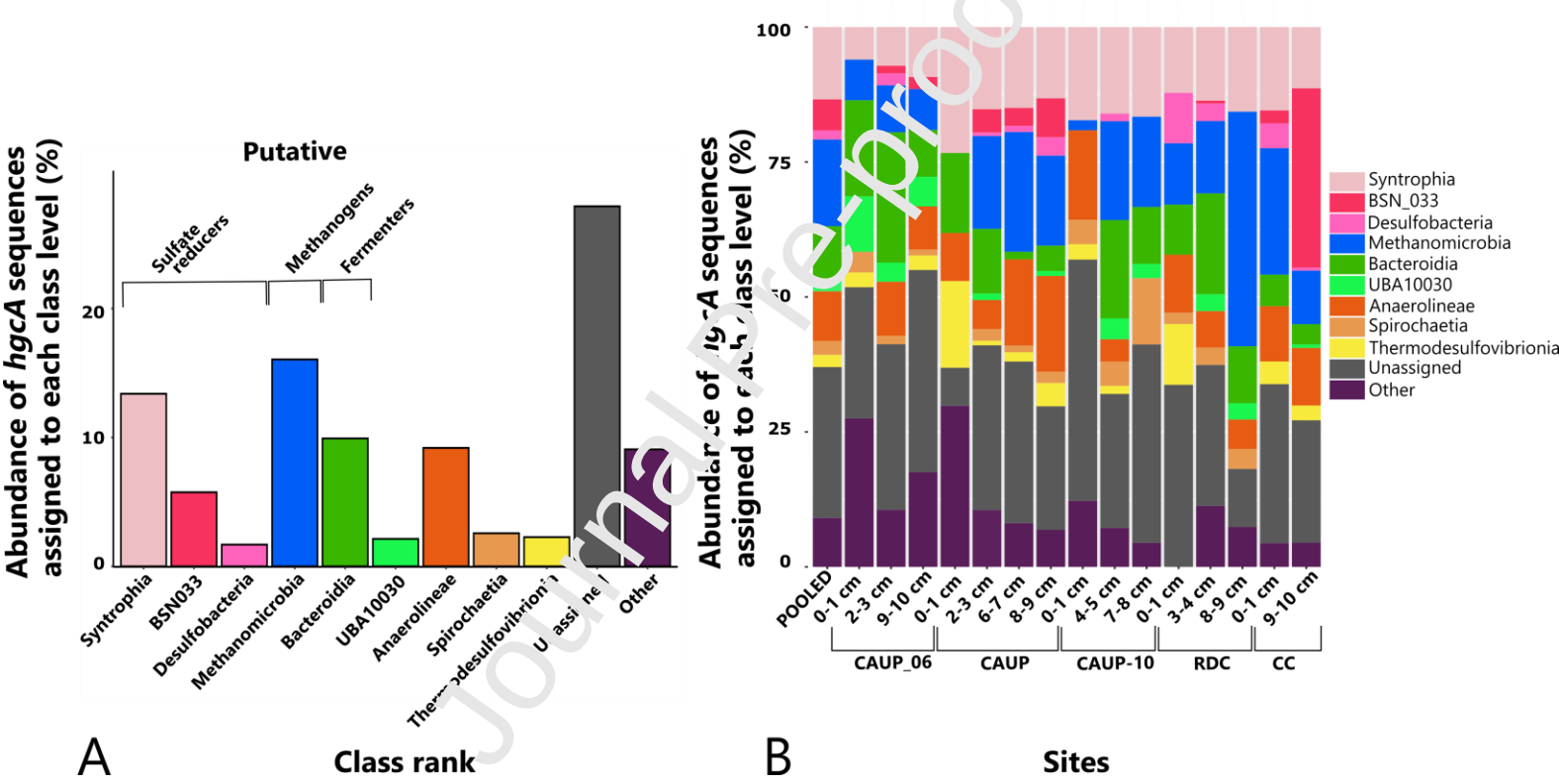


Figure 4; Millera Ferriz et al. 2020

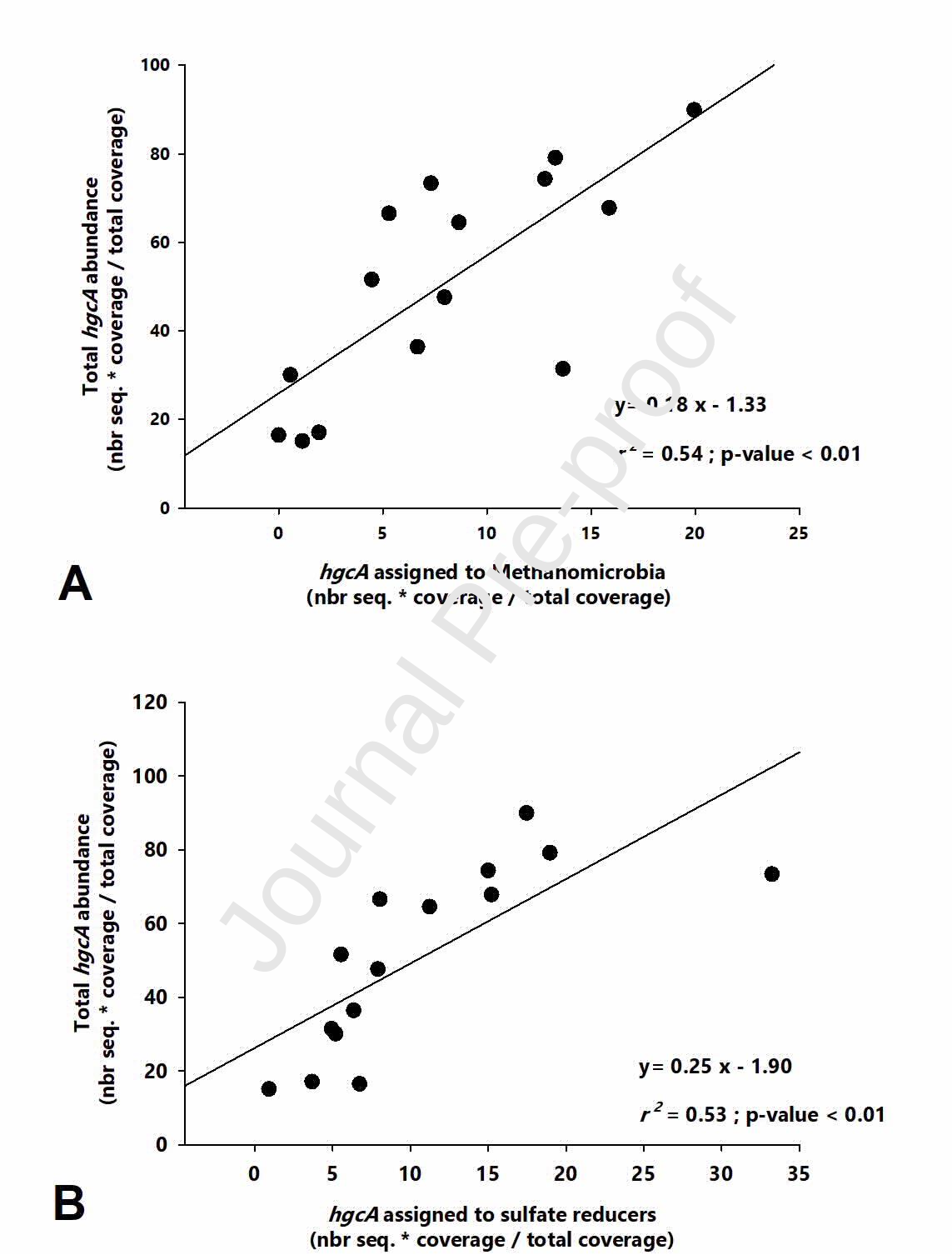


Figure 5; Millera Ferriz et al., 2020

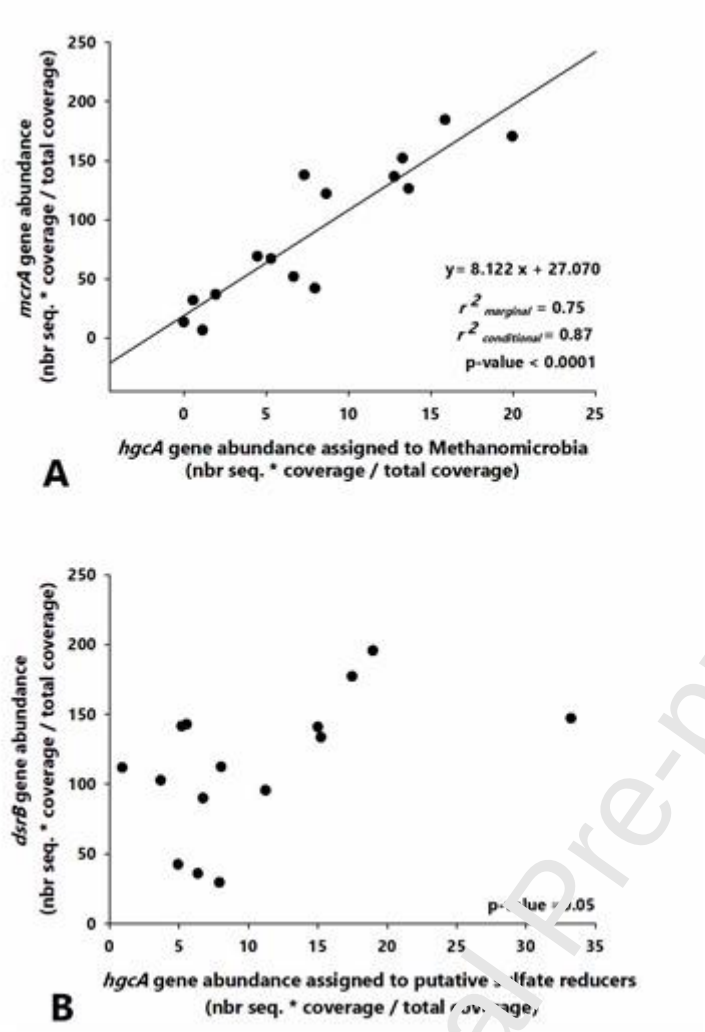


Figure 6; Millera Ferriz et al., 2020

Credit author statement

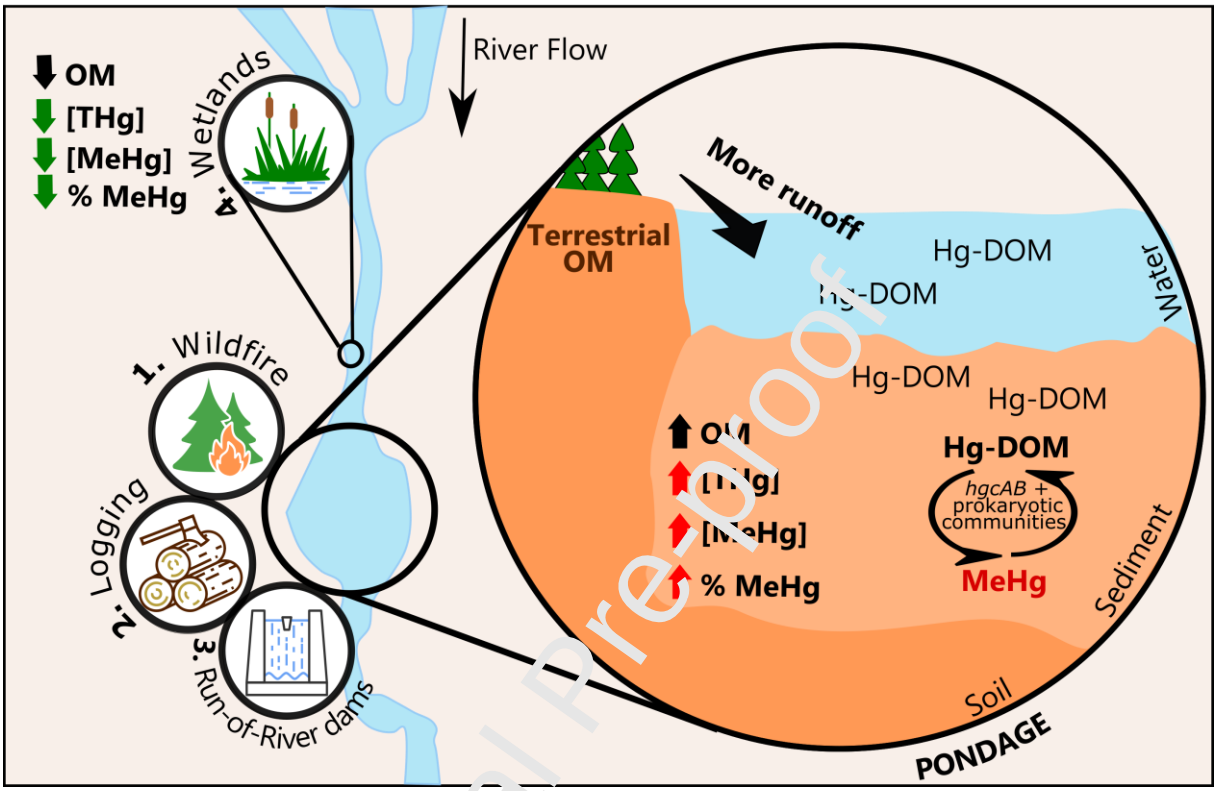
Lise Millera Ferriz : Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Writing – Original Draft, Writing – Review & Editing; **Dominic Ponton:** Conceptualization, Methodology, Investigation, Writing – Review & Editing; **Veronika Storck:** Methodology, Investigation, Supervision, Writing – Review & Editing; **Maxime Leclerc:** Conceptualization, Methodology, Investigation, Writing – Review & Editing; **François Bilodeau:** Conceptualization, Writing – Review & Editing, Funding acquisition; **David Walsh:** Conceptualization, Methodology, Investigation, Writing – Review & Editing, Supervision; **Marc Amyot:** Conceptualization, Methodology, Review & Editing, Supervision, Project Administration, Funding acquisition

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical abstract



Highlights

- The St. Maurice watershed was impacted by logging, wildfire and hydropower dams
- Sediment Hg levels were higher in the pondages created by run-of-river dams
- Sediment Hg and methylHg levels were strongly correlated to organic matter
- Hg methylation potential was related to the C/N ratio in sediments
- Hg-methylating community included sulfate reducers, methanogens and fermenters