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High methylmercury formation in ponds fueled by fresh humic and algal derived organic matter

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Abstract

Neurotoxic methylmercury causes adverse effects to ecosystem viability and human health. Previous studies have revealed that ponding alters natural organic matter (NOM) composition and increase methylmercury concentrations in rivers, especially in the first years after flooding. Here, we investigate the influence of NOM composition (i.e., sources and degradation status) on mercury methylation rate constants in nine boreal beaver ponds of different ages across Sweden. We show that increased methylmercury concentrations in surface waters is a consequence of enhanced mercury methylation in the pond sediments. Moreover, our results reveal that during the first years after the initial flooding, mercury methylation rates are fueled by the amount of fresh humic substances released from the flooded soils and by an increased production of algal-derived NOM triggered by enhanced nutrient availability. Our findings indicate that impoundment-induced changes in NOM composition control mercury methylation processes, causing the raise in MeHg levels in ponds.

Scientific Significance Statement

Ponds are the most abundant type of aquatic ecosystem at boreal, subarctic, and artic latitudes. Accelerated permafrost thawing caused by global warming and an increasing number of beaver populations now recovering from near extirpation are the main causes of ponding in the boreal region. Unfortunately, ponding is associated with increased concentrations of neurotoxic methylmercury, particularly during the first years after the initial flooding. An understanding of the processes controlling methylmercury formation is thus required to develop management strategies that aim to reduce mercury exposure. Our results show that ponding enhanced the released of fresh humic organic matter and nutrients triggered algae growth, both determining the final concentration of methylmercury in ponds.

Introduction

Ponds and impoundments are the most abundant type of aquatic ecosystems at boreal, subarctic, and artic latitudes (Pienitz 2008; Verpoorter et al. 2014). Created either by natural processes or anthropogenic activities, ponds have important socio-economical and environmental implications (Deemer et al. 2016; Yvon-Durocher et al. 2017). The recovery of beaver populations (Halley et al. 2012) and the accelerated permafrost thawing caused by global warming (Payette et al. 2004) is known to trigger the formation of ponds and the subsequent remobilization of carbon pools that have been accumulated over thousands of years (Breton et al. 2009; Catalán et al. 2017). Besides the implications of that enhanced carbon remobilization for the global carbon cycle (Schuur et al. 2008; Holgerson and Raymond 2016; Yvon-Durocher et al. 2017), pond formation has been associated with increased concentrations of methylmercury (MeHg) in aquatic networks (Hall et al. 2005; Roy et al. 2009; Levanoni et al. 2015; MacMillan et al. 2015) over the first years after the initial flooding (Roy et al. 2009). Indeed, MeHg concentrations in pioneer beaver systems can be up to

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3.5-fold higher downstream than upstream of the ponds (Levanoni et al. 2015). Such, increased MeHg levels in aquatic systems can be of critical importance since MeHg is bio-accumulated and biomagnified in food webs, eventually affecting wildlife and human health (Scheuhammer et al. 2007). Beaver populations (*Castor fiber* and *Castor canadensis*) inhabit freshwater streams and lakes of the Northern Hemisphere. While they surpassed 60 million individuals in the 18th century, overhunting, together with a reduction of their habitat, almost led to extirpation of beaver populations during the 19th century in many parts of Europe and North America. However, successful protection and reintroduction measures were undertaken during the 20th century, bringing the global beaver population to over 1 million individuals (Halley et al. 2012). In boreal freshwater networks, high densities of beaver impoundments have previously been associated with enhanced NOM bioavailability (Lapierre et al. 2013; Catalán et al. 2017) and high MeHg levels (Hall et al. 2005; Roy et al. 2009; Levanoni et al. 2015). The processes causing enhanced MeHg levels following the establishment of beaver impoundments are however not known.

MeHg is produced in oxygen-limited sediments, soils, and stratified water columns (Pak and Bartha 1998; Eckley and Hintelmann 2006; Gascon Diez et al. 2016). The content and composition of NOM and several additional environmental factors such as temperature, redox potential, pH, and sulfur chemistry modulate biological methylation rates of inorganic divalent mercury (Hg(II)) (Ullrich et al. 2001; Drott et al. 2007). Among these factors, NOM seems to play a key role for the process, and a recent study revealed that NOM molecular composition controls Hg(II) methylation rates in boreal lake sediments (Bravo et al. 2017). The molecular composition of NOM in inland waters is complex and dynamic since several interdependent processes (microbial processing, photo-degradation and flocculation) contribute to its transformation and degradation (Kothawala et al. 2014). In aquatic systems, NOM is a mixture of autochthonous and allochthonous compounds that might affect the formation of MeHg in contrasting ways, by controlling the overall activity of microbial communities (Bravo et al. 2017) or by affecting Hg(II) solubility and availability for bacterial uptake and methylation (Schaefer and Morel 2009; Jonsson et al. 2012, 2014). Recent studies have suggested that autochthonous algal-derived NOM enhances Hg(II) methylation by boosting the activity of microbial communities (Bravo et al. 2017) and by providing low molecular mass thiols forming bioavailable complexes with Hg(II) (Leclerc et al. 2015). However, the components of NOM controlling Hg(II) methylation processes in boreal streams, which are normally poor in autochthonous NOM but enriched in allochthonous terrigenous NOM (Kothawala et al. 2015), remain unknown.

In this study, we quantified Hg(II) methylation rates using enriched Hg(II) isotope tracers, and characterized NOM quality using optical spectroscopy methods across nine Swedish beaver impoundments of contrasting ages. Our results show that fresh humic and algal-derived NOM control Hg(II) methylation rates in pond sediments. These drivers of enhanced Hg(II) methylation rates, here elucidated for beaver impoundments, are expected to be the same in other types of natural and anthropogenic impoundments.

Material and methods

Study area and sampling

In late September and early October 2014 (two years after Levanoni et al. 2015), sediment and water samples were collected from nine beaver ponds distributed in a latitudinal gradient across Sweden (Table 1). The ponds where classified according to a previous study and based on the years since damming (New: < 10 yr, Old: > 10 yr). This age limit was selected considering the effects of age influencing MeHg concentration in downstream waters observed in previous studies (Levanoni et al. 2015). Surface waters (0.5 m depth) were collected with a 1 L Ruttner sampler for measurements of MeHg, total-phosphorus (TP), SO_4^{2-} and dissolved organic carbon (DOC) concentrations as well as for characterization of DOM (Supporting Information Table S1). Oxygen, conductivity, pH, and temperature were measured both at the surface and the water overlying the sediment at each sampling site (WTW Conductometer LF 191, WTW, Germany) (Table 1). From each pond, three replicate sediment cores of about 30 cm depth with 30 cm of overlying water were collected using a 6-cm diameter gravity corer (UWITEC, Austria). Cores were kept upright at 4°C until further processing within 12 h of sampling.

Chemical water and sediment characteristics

Surface water subsamples of 0.2 L were filtered through a 70 mm Whatman GF/C Whatman precombusted glass-fiber filter, which was then preserved frozen at −20°C for later chlorophyll *a* (Chl *a*) analysis (Jepersen and Christoffersen 1987). Filtered water was collected in duplicate precombusted glass vials that were kept at 4°C and analyzed for DOC concentration (acidified to pH 2) and optical DOM characterization within 10 d of sampling. DOC concentration was measured with high temperature catalytic oxidation (Shimadzu TOC analyzer) (Benner and Strom 1993). Unfiltered water was kept cold (4°C) in a polypropylene tube for total phosphorous (TP) measurements (Murphy and Riley 1962). Sulfate (SO_4^{2-}) was measured by ion chromatography (Metrohm 833 Basic IC plus). Between 7 mg and 10 mg of freeze-dried sediment were packed tightly in pressed tin capsules (Elemental Microanalysis, 6x4 mm) and analyzed for elemental content of C and N by high temperature catalytic oxidation with a COTECH ECS 4010 elemental analyzer calibrated with sulfanilamide standard (C 41.84%, N 16.27%, H 4.68%, O 18.58%, S 18.62%).

Dissolved organic matter characterization

Absorbance spectra (200–800 nm) were measured with a Lambda 40 spectrophotometer (Perkin-Elmer) following

Table 1. Location, morphometrics, and physico-chemical characteristics for the studied beaver impoundments. The age (N: New < 10 yr old and O: Old > 10 yr old) of the beaver system was previously described elsewhere (Levanoni et al. 2015), in parenthesis the nomenclature of the site in their paper.

	Coordinates		Morphometry		Land use (%)*			Watershed/ pond area	Physico-chemical properties				
Site	Latitude (N)	Longitude (E)	Area (Ha)	Depth (m)	Wetlands	Arable land	Forest		DO (%)	C (μS cm^{-1})	pH	T (°C)	Age*
1	59°42′31″	16°5′35″	2.9	0.9	0.4	4.4	95	90	31.4	84.4	5.66	-	7–10, N (23)
2	59°41′18″	16°1′53″	1.65	0.7	5	0	86	485	66.8	71.6	5.75	-	2–7, N (24)
3	59°14′52″	14°50′25″	0.93	0.6	3.7	5.3	83.2	2151	84.6	70.6	6.28	-	10–18, N (21)†
4	59°14′11″	14°52′6″	0.28	0.5	3.5	4.3	87	12143	90.9	82.5	6.36	8.7	2–7, N (22)
5	62°19′5″	16°49′45″	2	0.8	1.4	1	91.4	1100	95	37	6.46	5.7	>18, O (13)
6	62°13′12″	16°48′38″	1.71	0.4	5.8	0.7	91.5	643	98	33.2	6.51	7.3	>18, O (14)
7	66°3′18″	22°5′18″	1.6	0.8	6.8	0.4	76.7	781	87.8	21.11	6.02	5.7	2–8, N (1)
8	66°12′54″	21°53′9″	3.93	0.7	12.8	0	80.2	573	68.4	20.2	6.01	5.5	>18, O (2)†
9	66°13′37″	22°1′37″	0.3	0.4	26.1	0	65.9	1674	47.8	29.9	5.51	4.2	2–8, N (3)

* Data from Levanoni et al. (2015).
† Sites with intermediate age estimated were classified as New and Old based on the observations.

Kothawala et al. (2013). Specific UV absorption coefficients at wavelength λ a_λ (m^{-1}) were calculated according to Beer Lambert law: $a_\lambda = A_\lambda \ln 10/L$, where A_λ is absorbance and L is the path length (m) (Stedmon et al. 2000). The $SUVA_{254}$ (Supporting Information Table S2) was calculated as the ratio a_{254} and DOC (mg C L^{-1}).

Fluorescence emission excitation matrices (EEM) were obtained with a fluorescence spectrophotometer (Fluoromax-4, Horiba Jobin Yvon) using a 1 cm quartz cuvette and Milli-Q water as a blank. The area underneath the water Raman scan was used to normalize sample intensities. Corrections for instrument specific biases, Raman scattering and inner filter effect were applied using the FDOMcorr toolbox for MATLAB (Mathworks, Natick, Massachusetts, U.S.A.) following Kothawala et al. (2013). We obtained the main fluorescent peaks associated with DOM (Peaks A, T and C, and the total fluorescence (FT)) together with several optical indices that provide information about the composition and origin of DOM (Biological index (BIX), Humidification index (HIX), and $SUVA_{254}$). A summary of the descriptors used is provided in Table 2 and the results are provided in Supporting Information Table S2.

Hg(II) methylation assays

The upper 2 cm of the sediment cores were sectioned in a glove box under an inert N_2-atmosphere. Sediment slurries were prepared following Bravo et al. (2017). Briefly, a Hg(II) isotope tracer ($^{198}HgCl_2$) was added to sediment slurries at concentrations similar to ambient Hg(II) values. One replicate was immediately frozen (t_0) and another three replicates (t_f) were incubated for 24 h in the glove box at 18°C and subsequently frozen. Hg(II) and MeHg were extracted from sediment with focused microwave assisted acid extraction for 4 min at 80 W and subsequently analyzed by species specific isotope dilution using capillary gas chromatography hyphenated to inductively coupled plasma mass spectrometry (GC-ICPMS) (Rodriguez-Gonzalez et al. 2013). Hg(II) methylation rate constants (k_m) were calculated from the initial and final concentrations of the $Me^{198}Hg$ species. The isotopes used in this research were supplied by the United States Department of Energy Office of Science by the Isotope Program in the Office of Nuclear Physics.

MeHg concentrations in the water column

Unfiltered water samples for MeHg concentration determination were collected in factory-fresh brown glass bottles. The concentration of MeHg in water was determined by cold vapor atomic fluorescence spectrometry following EPA 1630 method (USEPA 1998) at the Swedish Environmental Institute (IVL). The detection limit of the analytical method was 0.02 ng L^{-1}.

Quality assessment and quality control (QA/QC)

All materials in contact with samples were washed in an Extran® (phosphate free) bath for 1 h, rinsed with MilliQ

Table 2. Summary and description of the optical properties used in this study. *Modified from literature* (Catalán et al. 2013).

Parameter	Description	Interpretation
$SUVA_{254}$ ($L\ mg^{-1}\ m^{-1}$)	Ratio of the specific UV absorbance at 254 nm and the DOC concentration in mg L^{-1}(Weishaar et al. 2003)	Informs on the aromaticity of DOM, with values generally ranging between 1 $L\ mg^{-1}\ m^{-1}$ and 6 $L\ mg^{-1}\ m^{-1}$ (Weishaar et al. 2003)
a_{440} (m^{-1})	Absorption coefficient at 440 nm (Cuthbert and Del Giorgio 1992)	Proxy for colored DOM concentration (Cuthbert and Del Giorgio 1992; Ask et al. 2009)
Biological Index (BIX)	Ratio of the emission intensities at 380/430 nm for an excitation of 310 nm (Huguet et al. 2009)	Indicator of recent biological activity (Huguet et al. 2009) or recently produced DOM (Wilson and Xenopoulos 2009)
A/C ratio (α'/α)	Ratio of intensities of Peaks A and C (Kothawala et al. 2012)	Indicator of biological (Kothawala et al. 2012) or photochemical (Moran et al. 2000) degradation of the humic fraction of DOM
Peak A (or α')*	250 Ex – 450 Em (Coble 1996; Huguet et al. 2009)	Humic, aromatic substances of terrestrial origin (UVA-humic like; Fellman et al. 2010)
Peak C (or α)*	350 Ex – 450 Em (Coble 1996; Huguet et al. 2009)	Humic substances from terrestrial sources (Fellman et al. 2010) UVC-humic like (Stedmon and Markager 2005)
Peak T (or δ)*	280 Ex – 330 Em (Coble 1996; Parlanti et al. 2000)	Protein-like material from microbial sources (Moran et al. 2000) (resembling the amino acid Tryptophan signal; Fellman et al. 2010)

* The peaks were reported normalized by the total intensity of the sample.

water and then acid-washed during 1 h in two different ultrasonic baths of 10% HNO_3 v/v and one of HCl 10% v/v. Between the baths and before use, all materials were rinsed with Milli-Q. Glass fiber filters GF/F (0.7 μm) were muffled for 1 h at 550°C.

For Hg(II) and MeHg measurements, the analytical quality was assured by analyzing every sample at least three times. The measurement variability was usually about 10% and always below 15%. Blanks were checked for contamination. The accuracy of the MeHg measurements was verified by analyzing the certified reference material (CRM) IAEA-405. Recoveries of MeHg to the CRMs were found to be 91% ± 3%.

Data analysis

Distributional properties of the data were checked prior to the analysis of the data. Normality assumptions were tested in all cases and when normality assumptions were not met, data were log transformed. Principal component analysis (PCA) was applied to nutrients (Chl *a*, sulfate, TP), DOC concentration and NOM optical properties. Data were centered and standardized before performing the PCA. PCA analysis was carried out in SIMCA, version 13.0.2 (Umetrics AB, Umeå, Sweden). Non-linear regression analyses between k_m and DOC and MeHg were carried out with SigmaPlot 13 (Systat Software). A two parameters exponential growth function was fitted to k_m and MeHg concentration relationship. A two parameters exponential rise to a maximum function was fitted to the k_m and DOC relationship.

Results and discussion

Transient increase of water column MeHg concentration is caused by enhanced Hg(II) methylation in the pond

The Hg(II) methylation rate constant (k_m, d^{-1}), determined in sediments using enriched isotope tracers, varied between 0.004 and 0.035 d^{-1} (Table 3), with a median value of 0.026 d^{-1} (interquartile range = 0.01–0.031). We observed a decrease in Hg(II) methylation rate constant with the age of the pond (Table 3). The k_m was a factor of 2.2 higher in new ponds (< 10 yr old; *see* "Methods") than in older ones (> 10 yr old) ($p < 0.05$). The concentrations of MeHg in the water column of the impoundments ranged between 0.14 ng L^{-1} and 1.0 ng L^{-1} and were, in agreement with previous studies, higher in new impoundments (Hall et al. 2005; Roy et al. 2009; Levanoni et al. 2015). The increase of MeHg concentrations in rivers affected by beaver colonization is however transient and decrease with the pond age (Roy et al. 2009).

The increase in sediment Hg(II) methylation rates in new ponds was associated with an exponential increase in pond-water MeHg concentrations (two-parameter exponential function; Fig. 1, $R^2 = 0.77$, $p = 0.0012$). The model indicates that about 80% of the increase in pond-water MeHg concentration can be explained by MeHg production in pond sediments (Fig. 1). We did not find any significant correlation between MeHg degradation rate constants (k_d) or $k_m\ k_d^{-1}$ ratio and MeHg levels in ponds (Supporting Information Fig. S1). Indeed, MeHg degradation rate constants in pond sediments varied independently of pond age (Supporting Information Fig. S1). Our results thus showed that the increase in

Table 3. Mercury in ponds. Mean and standard deviation values for Hg(II) and MeHg concentrations (ng g^{-1}), MeHg percentage of total Hg and Hg(II) methylation rate constant in sediments (k_m, d^{-1}), and MeHg concentration (ng L^{-1}) in the water column, of nine beaver impoundments.

Site	Hg(II) ng g^{-1}	MeHg ng g^{-1}	MeHg %	k_m day^{-1}	MeHg ng L^{-1}
*New**					
1	87.2 ± 10.4	2.7 ± 0.3	3.1	0.035 ± 0.002	1.0
2	176.6 ± 17.4	3.7 ± 0.3	2.1	0.031 ± 0.003	0.47
3	125.1 ± 13.9	10.1 ± 1.8	8.1	0.026 ± 0.001	0.24
4	42.9 ± 19.5	1.8 ± 0.8	4.2	0.031 ± 0.001	1.0
7	47.4 ± 10.1	5.6 ± 1.8	11.8	0.01 ± 0.002	0.16
9	47.4 ± 16.6	3.6 ± 0.7	7.6	0.032 ± 0.009	0.71
*Old**					
5	66.1 ± 14.8	2.6 ± 0.6	3.9	0.004 ± 0.002	0.12
6	135.5 ± 23.5	6.6 ± 0.8	4.9	0.009 ± 0.001	0.17
8	114 ± 20	6.4 ± 3.7	5.6	0.014 ± 0.002	0.14

* "*New*" refers to ponds < 10 yr old, and "*Old*" refers to ponds > 10 yr old.

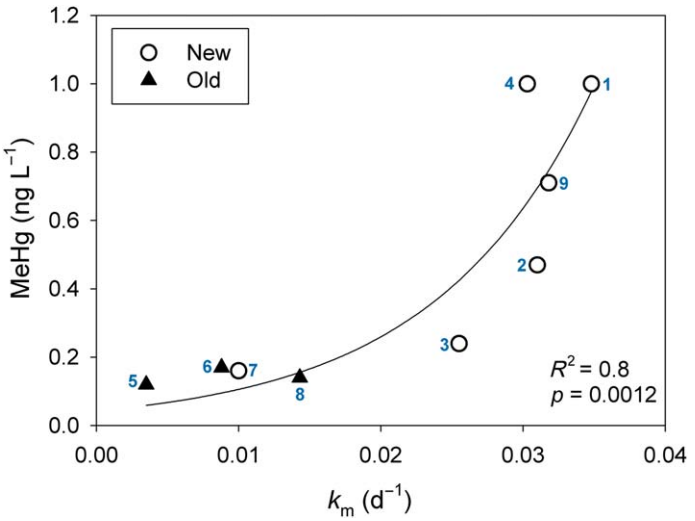

Fig. 1. High Hg(II) methylation rate constants in pioneer impoundments. Relationship between mercury methylation rate constant (k_m, d^{-1}) in sediments vs. unfiltered methylmercury (MeHg) concentration in pond water column (ng L^{-1}). Site numbers are marked in blue.

MeHg concentrations in recently established beaver impoundments is primarily caused by enhanced in situ Hg(II) methylation. Therefore, although recently flooded forest soils and wetlands rich in humic substances are likely to be important sources of MeHg for aquatic ecosystems by mobilizing MeHg accumulated in soils (Hall et al. 2005), our model indicates that this process barely contributed to the 20% of the increase in MeHg concentrations in pond-water (Fig. 1). The recently established pond number 7 deserves special attention because it features both a low Hg(II) methylation rate and a low MeHg concentration in the water column (Table 3). Thus it was an exception to the general pattern of high MeHg production and concentration in new ponds. Actually the new pond 7 was re-established on a previously impounded site. This shows that factors other than the age of the beaver impoundment *per se*, that is for example the pond geochemistry, history and/or hydrology, affect Hg(II) methylation rates.

In older ponds where the vegetation disappeared and the pond is characterized by open water, lower MeHg concentrations could be explained by (1) low Hg(II) methylation rates (Fig. 1), (2) high MeHg photodegradation rates (Fernández-Gómez et al. 2013) in the water column and/or (3) changes in MeHg solubility.

Increased inputs of fresh humic and algal-derived NOM control Hg(II) methylation in new ponds

Previous studies showed that impoundment establishment modifies the sources and the biodegradability patterns of riverine NOM (Catalán et al. 2017). Moreover, the role of molecular composition of NOM on Hg(II) methylation processes in boreal lake sediments has been recently highlighted (Bravo et al. 2017). In this study, we employed a set of optical spectroscopy parameters widely applied to assess the source (i.e., produced within the aquatic system vs. imported from the terrestrial environment) and degradation status (e.g., due to photochemical and/or biological processing) of dissolved NOM (Fellman et al. 2010) to reveal that increased inputs of fresh humic and algal-derived NOM control Hg(II) methylation rate constants. Moreover, the lack of correlation between Hg(II) and MeHg in pond sediments ($p = 0.159$) suggests that MeHg concentrations are controlled by in situ MeHg formation, which is limited by OM composition, not by Hg(II), especially in pioneer ponds (that accounted for six of the nine studied sites).

The NOM composition in recently established ponds was enriched in algal-derived NOM (e.g., Chl *a*, Supporting Information Table S1) and was more humic and less processed (denoted from now onwards "fresh humic") compared to older ponds (Supporting Information Table S2). The differences in NOM composition among the nine beaver impoundments were further described using principal component analysis (PCA, Fig. 2). The first principal component (PC1) explained 52.7% of the total variance (Fig. 2). Positive loadings on PC1 indicated that the presence of nutrients (i.e., TP and sulfate) and internally produced algal-derived NOM (i.e., Chl *a*) were associated with the presence of terrigenous humic acids (FT) (Jaffé et al. 2008) and aromatic compounds ($SUVA_{254}$) (Weishaar et al. 2003) (Fig. 2A). These groups of compounds were indeed particularly abundant in the recently constructed ponds 1 and 2 (positive scores on PC1 Fig. 2B, Supporting Information Table S5), both featuring high k_m. The results in Fig. 2A therefore indicate that

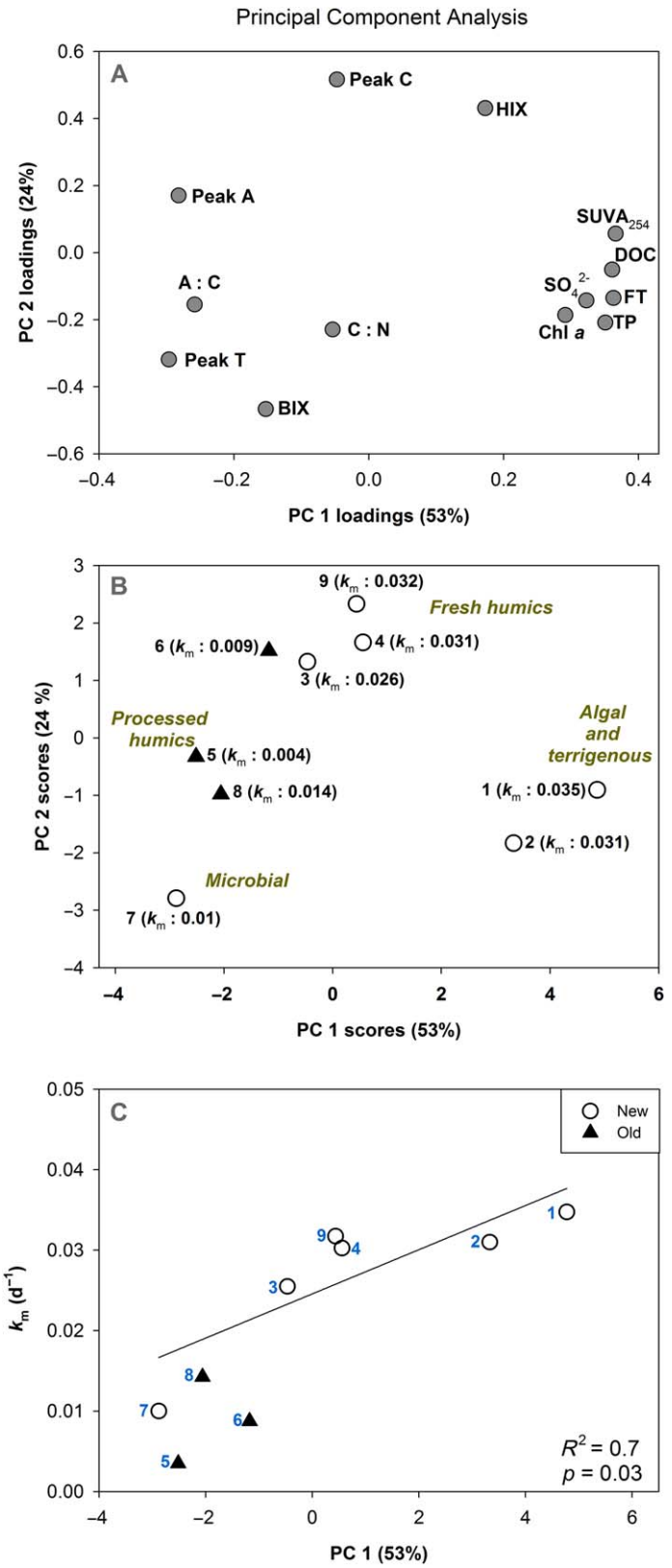

Fig. 2.

the initial flooding of soils entails a resuspension and transport of soil humic NOM and nutrients (Supporting Information Tables S1, S2) into the water as previously reported in other studies (Hall and Louis 2004; Roy et al. 2009). Moreover, an increase in nutrients led to an enhanced primary production and hence to an increase in the abundance of algal-derived NOM in pioneer beaver impoundments 1 and 2 (Fig. 2B), as also observed elsewhere (Hall et al. 2005).

Negative loadings on PC1 were found for the fluorescence peak A, the ratio of the peaks A to C (A : C) and peak T (Fig. 2A). Although both peaks A and C are linked to the presence of humic substances (Kothawala et al. 2012), increases in A : C values have been related to extensive NOM processing, as the peak C is lost preferentially to peak A under both photo- and biodegradation processes (Kothawala et al. 2012). Therefore, negative loadings of PC1 indicated processed NOM, in particular humic NOM. Based on this, we conclude that highly processed humics dominated the NOM composition of the old ponds 5, 6 and 8, all three characterized by low k_m (negative scores of PC1, Fig. 2B). High peak T fluorescence intensity and biological index (BIX) have been linked with the presence of microbial-derived organic compounds and NOM derived from biological processes (Moran et al. 2000; Kothawala et al. 2014). Negative loadings on PC2 (24.3%) were therefore associated to microbial and processed NOM (Fig. 2A). Accordingly, pond 7 that featured low k_m rates despite a young age, presented evidence of strongly processed NOM (positive scores on PC2, Fig. 2B) and low Chl *a* concentrations (Supporting Information Table S1). In contrast, positive loadings on PC2 were observed for peak C and HIX, linked to fresh humic substances (Kothawala et al. 2012) and the humification degree (Ohno 2002), respectively. Positive loadings on PC2 were thus associated with fresh humic materials, highly humified (i.e., high HIX, Fig. 2A) and yet not strongly degraded by photo-degradation (i.e., high peak C, Fig. 2A), most likely recently leached from the inundated soils. Hence new ponds 3, 4 and 9, all characterized by high k_m, were dominated by fresh humic NOM (positive scores on PC2, Fig. 2B). New ponds were thus characterized by higher algal-derived (ponds 1 and 2), fresh humic (ponds 3, 4, and 9) or by degraded NOM (pond 7) (Fig. 2A,B; Supporting Information Table S2). Differences on the NOM composition among new ponds might be

Fig. 2. Statistical relations between NOM composition and the Hg(II) methylation rate constant. Principal Component Analysis (PCA) of nine beaver impoundments based on dissolved NOM descriptors: (**A**) loadings of principal component 1 (PC1, 52.7%) and 2 (PC2, 24.3%), (**B**) scores of PC1 and PC2 and (**C**) Principal Component Regression analysis (PCR) of PC1 and Hg(II) methylation rate constant (k_m, d^{-1}) in sediments of old (closed triangles) and new (open circles) beaver impoundments. Site numbers are marked in blue. See PCA details in Supporting Information Tables S3, S4, and S5.

explained by different land uses in the catchment of the river (Catalán et al. 2017). For example, a high surface of wetlands in the catchment of ponds 3, 4, and 9 (Table 1) might explain a higher content of humic terrigenous NOM (Peak C, Fig. 2A). Also, a higher area of ponds in the catchment of ponds 1 and 2 (Table 1) could explain a higher concentration of nutrients, released from the recently flooded soil to the pond water. Pond 7, new but built on a previously impounded site, with a flooded area similar to that seen for ponds 1 and 2, is located farther north, where catchments are typically characterized by low nutrient concentrations (Bergström et al. 2005). Therefore, even if pond formation enhances the release and accumulation of nutrients in ponds compared to upstream parts of the river (Catalán et al. 2017), our results indicate that final nutrient concentrations in pond 7 were still too low to promote algal growth (Supporting Information Table S1).

NOM composition in aquatic systems reflects the source, processing pathways and biological reactivity (Kothawala et al. 2014) but is intricate. Therefore, statistical approaches integrating the complexity and diversity of NOM are undoubtedly necessary for a high predictive power and fundamental understanding of Hg(II) methylation processes. We used a multivariate statistical approach (Principal Components Regression analysis, PCR) to assess the effect of several distinct NOM constituents on k_m. We observed that the first component of the PCA (Fig. 2C, $R^2 = 0.7$, $p < 0.05$), representing a gradient of both fresh algal-derived NOM and fresh terrigenous humic NOM, was positively related to k_m. The key role of algal-derived NOM on Hg(II) methylation has been recently highlighted in boreal lake sediments (Bravo et al. 2017). We propose that, similarly to boreal lake sediments (Bravo et al. 2017), the production of algal-derived NOM in pioneer beaver impoundments enhanced Hg(II) methylation by enhancing the activity of microbial communities. Algal exudates might also have raised the concentration of low molecular mass thiols and augment Hg(II) availability for Hg(II) methylating bacteria as previously suggested (Leclerc et al. 2015).

Besides algal-derived NOM, our results indicate an important role of terrigenous NOM in Hg(II) methylation processes in ponds. Indeed, k_m correlated positively with DOC (Fig. 3), $SUVA_{254}$ and total fluorescence FT (FT, Fig. 2A) and negatively with the A : C ratio and peaks A and T (Fig. 2C), indicating that an increase in available fresh terrigenous NOM was also associated with high Hg(II) methylation rates in pioneer beaver impoundments. In contrast, highly processed humic NOM, found in old ponds 5, 6, and 8, correlated negatively with k_m (negative loadings on PC1 and PCR analyses, Fig. 2A,C), indicating that the availability of fresh NOM responsible for enhanced Hg(II) methylation decreased over time in impoundments. Therefore, we conclude that positive correlations between k_m and DOC (Fig. 3) or $SUVA_{254}$ (Fig. 2A,C) likely occur when terrigenous NOM is mainly

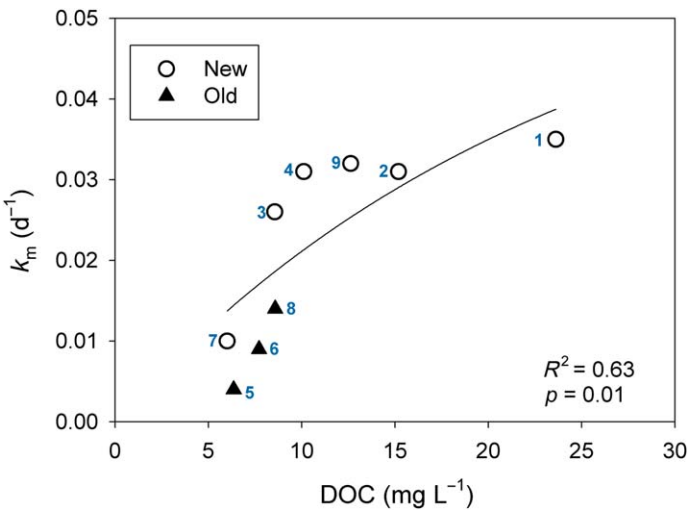

Fig. 3. Dissolved organic carbon in beaver impoundments. Relationship between the mercury methylation rate constant (k_m, d^{-1}) in sediments and dissolved organic carbon (DOC) concentration (mg L^{-1}). Site numbers are marked in blue.

composed of fresh humic compounds (positive loadings on PC1 and PC2). Although algal-derived NOM is preferentially used by bacteria (Kritzberg et al. 2004), fresh terrigenous NOM can also boost the activity of certain groups of microorganisms able to metabolize specific components of terrigenous NOM (Fasching et al. 2014) and therefore enhance (directly or indirectly) the activity of Hg(II) methylating microbial communities. In pioneer ponds, we observed a plateau effect for k_m relative DOC concentration in water column stabilizing at around $\sim$ 10 mg L^{-1} (Fig. 3). A similar threshold has been reported in laboratory studies carried out with pure cultures of bacteria and NOM isolates (Graham et al. 2013; Chiasson-Gould et al. 2014). The effect of DOC concentration on Hg(II) methylation potential may at least partly be because of its capacity to bind Hg(II) (Ravichandran 2004). Thus, depending on the strength of the binding (Ravichandran 2004), the amount of DOC could either increase or decrease bacterial Hg(II) methylation independently of its molecular composition.

In a previous study, a detailed characterization of the molecular composition of sedimentary NOM performed by pyrolysis–gas chromatography–mass spectrometry (Py-GC-MS) revealed the role of algal-derived NOM on Hg(II) methylation (Bravo et al. 2017). Py-GC-MS is a powerful technique for determining NOM deriving from algae (e.g., phytol), vascular plants (e.g., lignin oligomers), or non-vascular plants (e.g., some phenolic compounds) (Tolu et al. 2015). Additionally, the Py-GC-MS method is very useful for identifying pyrolytic compounds indicative of degradation products of high-molecular mass carbohydrates (e.g., furans) and proteins and chlorophylls (e.g., pyrrole and pyridine) in

sediments. Nevertheless, even if the origin and degradation status of the sedimentary NOM inferred by Py-GC-MS could be used to predict Hg(II) methylation rate constants in Bravo et al. (2017), in this study, the fluorescence properties could resolve differences in the composition of NOM and provide valuable insights about the diagenetic stage of the humic NOM. By using NOM fluorescence optical properties, the results of this study extend previous findings on the compounds controlling Hg(II) methylation processes (Bravo et al. 2017) and show that besides algal-derived NOM, fresh humic acids are also a key driver for k_m. The mechanisms controlling Hg(II) methylation and MeHg concentrations found here for beaver impoundments are most likely similar to those responsible for the increase in MeHg levels in thaw ponds (MacMillan et al. 2015), or any pond formed from flooded soils.

Relevant role of ponds in Hg(II) cycling in boreal, subarctic, and arctic regions

An increasing number of beaver populations (Halley et al. 2012) and an accelerated permafrost thawing (Payette et al. 2004) in boreal, subarctic, and arctic regions (Payette et al. 2004; Taylor et al. 2016), raise concerns about a possible transient increase in MeHg concentrations in aquatic networks at a global scale. Our findings represent an important step towards mechanistic understanding of MeHg dynamics following the establishment of impoundments. They show that the effects of beaver ponds on stream MeHg concentrations is caused by enhanced Hg(II) methylation rates driven by the release of nutrients, stimulating production of algal NOM, and fresh terrigenous humic NOM released from flooded soils. The increase in MeHg concentrations is transient because the NOM components promoting high Hg(II) methylation rates decrease with pond aging. While previous studies have associated the age of the pond with MeHg levels, our results show that NOM composition determined by the beaver colonization history, catchment properties and hydrology rather than pond ageing per se will control Hg(II) methylation rates in ponds and subsequently MeHg concentrations downstream beaver impoundments. This was indeed manifested by the low content of algal-derived and fresh humic NOM and the low MeHg concentration and k_m encountered in the pioneer beaver pond 7, which was re-established on a previously impounded site. Therefore, without the boost of terrigenous organic matter from the inundated surrounding soils and an enhanced algal-derived NOM production, beaver colonization does not lead to an increase in MeHg levels downstream in re-colonized ponds. We therefore conclude that both aging (Roy et al. 2009) and colonization history of the ponds (Levanoni et al. 2015) are critical aspects to include in the ongoing beaver relocation and reintroduction strategies.

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Conflict of Interest

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