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Estimation of the Major Source and Sink of Methylmercury in the Florida Everglades

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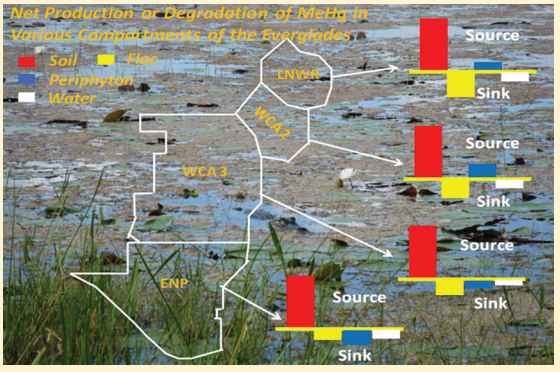
Supporting Information

ABSTRACT: Mercury methylation and/or demethylation have been observed in several compartments [soil (saturated soils covered by standing water), floc, periphyton, and water] of the Everglades, a wetland with mercury as one of the major water quality concerns. However, it is still unclear which compartment is the major source or sink due to the lack of estimation and comparison of the net methylmercury (MeHg) production or degradation in these compartments. The lack of this information has limited our understanding of Hg cycling in this ecosystem. This study adopted a double stable isotope ($^{199}Hg^{2+}$ and $Me^{201}Hg$) addition technique to determine the methylation/demethylation rate constants and the net MeHg production rates in each compartment. This study improved the previous models for estimating these parameters by (1) taking into account the difference between newly input and ambient mercury in methylation/demethylation efficiency and (2) correcting the contribution of photodemethylation to $Me^{199}Hg$ concentration when calculating methylation rates in water. The net MeHg production rate in each compartment was then estimated to identify the major sources and sinks of MeHg. The results indicate that these improvements in modeling are necessary, as a significant error would occur otherwise. Soil was identified to be the largest source of MeHg in the Everglades, while the floc and water column were identified as the major sinks. The role of periphyton varies, appearing to be a source in the northern Everglades and a sink in the southern Everglades. Soil could be the largest source for MeHg in the water column, while methylation in periphyton could also contribute significantly in the northern Everglades.



INTRODUCTION

Over the last several decades, methylmercury (MeHg) has emerged as one of the most widespread contaminants due to its prevalent existence, high toxicity, and bioaccumulation through the food chain. As a crucial part of understanding the cycling of mercury (Hg), great efforts have been made to identify the major production and degradation processes of MeHg in aquatic systems. Methylation of Hg in sediment by anaerobic bacteria (sulfate-reducing bacteria[1] or iron-reducing bacteria[2]) was deemed to be the major pathway for MeHg production[2−7] in most aquatic systems, while photodemethylation in water was widely proposed to be the major process of MeHg elimination.[8,9] Methylation of inorganic mercury in the water column was reported to be another significant pathway of MeHg formation in some ocean (e.g., the Arctic[10]) and freshwater[11] systems.

The double stable isotope addition technique is a useful tool for measuring Hg methylation/demethylation rates owing to its high accuracy, precision, and simultaneous determination of the methylation and demethylation rates. In recent years, this technique has been widely applied in estimating the net production of MeHg. However, two significant defects exist in previous models using this technique for identifying the major source and sink of MeHg. One is omission of the difference between the ambient and newly input Hg species in methylation/demethylation efficiency.[12] Their difference was often neglected in previous studies on estimating the net MeHg production rate.[13,14] A significant error could occur with this omission because a significant difference in methylation efficiency has been reported.[15] The other defect is related to the calculation of Hg methylation rate constant of the spiked mercury tracer (k_m) in water. This constant was usually calculated by the measured per-day increase in the amount of $Me^{n}Hg$ (with the assumption that $^{n}Hg^{2+}$ was spiked).[16,17] This calculation assumes that the degradation of ambient and newly

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produced Me^{n}Hg is negligible. However, this assumption often may not be valid for natural waters, where MeHg demethylation rate constant (k_d) can be 2−3 orders larger than k_m.[10] These two defects should be corrected for an accurate estimation of the production or degradation of MeHg by utilizing stable isotope tracer techniques.

The Everglades is a subtropical wetland ecosystem located in the south of Florida. Great efforts have been made to understand the cycling of mercury in this system[9,12,18−20] since the observation of elevated mercury levels in fish, wading birds, and other wildlife.[21] Hg methylation and/or MeHg demethylation were found to occur in various compartments of the Everglades, including soil,[12,20] floc (flocculent materials on top of soil),[19] periphyton,[18] and water.[9] Despite these findings, it is still unclear which compartment is the major source or sink of MeHg due to the lack of estimation and comparison of the net MeHg production in these compartments. A few previous studies have investigated the relationship of MeHg distribution in a certain compartment of the Everglades to its in situ methylation or demethylation. The spatial pattern of MeHg was previously found to be positively related to that of MeHg methylation rate in Everglades soil.[12] MeHg concentration in Everglades water was reported to be negatively related to photodemethylation in water.[9] Nevertheless, there is a lack of knowledge on the relationships of MeHg in a certain compartment to methylation/demethylation in other compartments. This information is crucial since MeHg present in one compartment (e.g., water) could be determined by the methylation/demethylation occurring both in that compartment and in other compartments (e.g., floc, periphyton, or soil).

The objectives of this study were to investigate Hg methylation/demethylation in various compartments of the Everglades, to assess the role of these processes in the spatial distribution of MeHg, and to identify the major source and major sink of MeHg in this ecosystem. To achieve these objectives, double stable isotope ($^{199}Hg^{2+}$ and $Me^{201}Hg$) addition experiments were conducted to study the methylation/demethylation of mercury in various compartments and areas of the Everglades. The net production or degradation rates of ambient and newly input Hg were calculated in soil, periphyton, floc, and water of the Everglades.

MATERIALS AND METHODS

Reagents. MeHgCl standard was purchased from Ultra Scientific (N. Kingstown, RI). Enriched ^{201}HgO (atomic percentage 96.17% ± 0.56%) and ^{199}HgO (atomic percentage, 91.09% ± 0.05%) were purchased from Oak Ridge National Laboratory (Oak Ridge, TN). $^{199}HgCl_2$ solution (measured atomic percentage 91.19% ± 0.46%) was prepared by dissolving ^{199}HgO in 10% HCl (v/v). $Me^{201}HgCl$ (measured atomic percentage 93.25% ± 0.42%) was synthesized from isotope-enriched ^{201}HgO by use of methylcobalamin.[22] Other reagents used were of reagent grade or higher.

Collection of Samples. Unfiltered surface water was collected at five sites in September−October 2009 to study the photodemethylation of MeHg (results were previously published)[9] and the methylation of inorganic mercury in water. Soil, floc, and periphyton samples were collected at four sites in September−October 2009 and at 12 sites in July 2010 (Figure S1, Supporting Information) to study the methylation and demethylation of mercury in these compartments. Although covered by water, the term "soil", rather than sediment, is generally used in the literature for most areas of the Everglades except the canals. Detailed sampling procedures can be found in the Supporting Information. Upon arrival of samples at the laboratory, Hg methylation/demethylation experiments were conducted within 3 h. Trace-metal clean techniques were followed during sample collection, shipment, and analysis.[23]

Incubation Experiments. *Methylation and Demethylation of Mercury in Soil, Floc, and Periphyton.* Double stable isotope addition method ($^{199}HgCl_2$ and $Me^{201}Hg$) was employed to simultaneously measure the methylation and demethylation rate constants. Soil (0−10 cm), floc, and periphyton were homogenized with a blender. Predetermined quantity of isotope-enriched $^{199}HgCl_2$ and $Me^{201}Hg$ were added to approximately 30 g of soil (2.03 ng of $^{199}Hg{\cdot}g^{-1}$ and 0.22 ng of $^{201}Hg{\cdot}g^{-1}$ wet sample), floc (0.44 ng of $^{199}Hg{\cdot}g^{-1}$ and 0.17 ng of $^{201}Hg{\cdot}g^{-1}$ wet sample), or periphyton (0.44 ng of $^{199}Hg{\cdot}g^{-1}$ and 0.17 ng of $^{201}Hg{\cdot}g^{-1}$ wet sample). Concentrations of the spiked $^{199}HgCl_2$ and $Me^{201}Hg$ were at the ambient total Hg and MeHg levels (0.1−1.7 times that of ambient total Hg and 0.3−9.6 times that of ambient MeHg). All these procedures were conducted under a N_2 saturated atmosphere. Triplicates were employed for each trial. Spiked samples were divided into two portions, of which one was immediately frozen to −20 °C, representing t = 0 days (t_0). The other portion was incubated in darkness at 29 ± 1 °C (in situ water temperature ranged from 26 to 31 °C during the sampling period) under N_2 saturated atmosphere for 2 days, representing t = 2 days (t_2). Samples were collected and preserved at −20 °C and then analyzed for $Me^{199}Hg$, $Me^{201}Hg$, and $Me^{202}Hg$ via aqueous phenylation followed by gas chromatography and inductively coupled plasma mass spectrometry (GC-ICP-MS).[24]

Methylation/Demethylation of Mercury in Everglades Water. Unfiltered water samples (200 mL) were transferred to 0.5-L FEP (fluorinated ethylene−propylene) Teflon bottles and then spiked with $^{199}HgCl_2$ and $Me^{201}Hg$ to form final concentrations of approximately 50 and 0.6 ng·L^{-1} as Hg, respectively. Spiked samples were divided into two groups and were incubated for 6 days. One group was incubated under ambient temperature and light conditions, while the other was incubated under dark condition by wrapping the bottles with aluminum foil. Triplicates were employed for each trial. After incubation, water samples were preserved by adding concentrated HCl to form a final concentration of 1% (v/v) and were stored at 4 °C until analysis. Concentrations of $Me^{199}Hg$, $Me^{201}Hg$, and $Me^{202}Hg$ in the incubated samples were determined after 0, 2, 4, and 6 days of incubation.

Determination of Total Hg, MeHg, and Other Ancillary Parameters. Detailed analytical procedures for MeHg, total Hg (THg), and other ancillary parameters can be found in the Supporting Information.

Data Analysis. *Calculation of Specific Hg^{2+} Methylation/MeHg Demethylation Rate Constants in Soil, Floc, and Periphyton.* The specific methylation and demethylation rate constants of newly spiked $^{199}Hg^{2+}$ and $Me^{201}Hg$ (k_m and k_d) and measured net ambient MeHg production (or degradation) rate (R) in soil, floc, and periphyton were calculated from the increased amount of $Me^{199}Hg$ derived from the spiked $^{199}Hg^{2+}$ ($\Delta[Me^{199}Hg]_{sp}$), the decreased amount of spiked $Me^{201}Hg$ ($\Delta[Me^{201}Hg]_{sp}$), and the net change in the amount of ambient $Me^{202}Hg$ ($\Delta[Me^{202}Hg]_n$), respectively (eqs 1−3). In many previous studies,[16] the change in concentrations of measured $Me^{199}Hg$ and $Me^{201}Hg$ were used to substitute for

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$\Delta[Me^{199}Hg]_{sp}$ and $\Delta[Me^{201}Hg]_{sp}$ to simplify the calculation. However, this simplification could cause a significant error if the methylation or demethylation of ambient mercury is not negligible. In this study, this defect was overcome by directly calculating the values of$\Delta[Me^{199}Hg]_{sp}$, $\Delta[Me^{201}Hg]_{sp}$, and $\Delta[Me^{202}Hg]_{n}$ to determine k_m, k_d, and R. They were calculated from equations similar to previously proposed functions for detecting transformations of Hg species.[25] A detailed derivation of these equations is provided in the Supporting Information. A t-test was then conducted on measured k_m, k_d, and R triplicate values to assess whether the measured methylation/demethylation rates of the spiked and ambient Hg were statistically significant ($p < 0.05$ level).

$$k_m = \frac{\Delta[Me^{199}Hg]_{sp}}{[^{199}Hg^{2+}]_{sp}t} \quad (1)$$

$$k_d = \frac{\ln \dfrac{[Me^{201}Hg]_{sp}}{[Me^{201}Hg]_{sp} - \Delta[Me^{201}Hg]_{sp}}}{t} \quad (2)$$

$$R = \frac{\Delta[Me^{202}Hg]_{n}}{P_{202}t} \quad (3)$$

where k_m is the specific methylation rate constant of spiked $^{199}Hg^{2+}$ (per day); k_d is the specific demethylation rate constant of spiked $Me^{201}Hg$ (per day); R is the measured production ($R > 0$) or degradation ($R < 0$) rate of ambient MeHg (nanograms per gram per day); t is the incubation time (days); $[^{199}Hg^{2+}]_{sp}$ and $[Me^{201}Hg]_{sp}$ are the concentrations of spiked $^{199}Hg^{2+}$ and $Me^{201}Hg$ (nanograms per gram), respectively; and P_{202} is the natural abundance of ^{202}Hg in ambient mercury (29.86%).[26]

Calculation of Specific Hg^{2+} Methylation/MeHg Demethylation Rate Constants in Water. A model based on first-order chemical kinetics was used to describe the degradation of MeHg in water (eq 4).[8,27] The rate constant of MeHg degradation, k_d, was then obtained by linear regression of ln $([Me^{201}Hg])_t$ against t, using Origin (version 6.0 for Windows; OriginLab Corp., Northampton, MA). A new model was developed to calculate the methylation rate constant of the spiked $^{199}Hg^{2+}$ (k_m) in water (eq 5a,5b). In this model, contributions of both $^{199}Hg^{2+}$ methylation and $Me^{199}Hg$ demethylation are taken into account in the function describing the variation of $Me^{199}Hg$ concentration (eq 5a,5b). According to this equation, k_m could be calculated by nonlinear regression of $[Me^{199}Hg]_t$ against t. However, $[Me^{199}Hg]_t$ is not sensitive to the value of k_m in the case of $k_d \gg k_m$, and an error will occur during the regression process under this condition. To correct for this error, a variable with higher sensitivity to the k_m value, $[Me^{199}Hg]_t$ /$[Me^{202}Hg]_t$ ratio $[R_{202}^{199}(t)]$, was employed to calculate k_m in water (eq 6a,6b). This ratio is expected to increase through the incubation period if methylation occurs in water. A detailed derivation of these equations is provided in the Supporting Information.

$$\ln([Me^{201}Hg]_t) = \ln([Me^{201}Hg]_0) - k_d t \quad (4)$$

When $k_d > 0$, $[Me^{199}Hg]_t$ is given by eq 5a

$$[Me^{199}Hg]_t = \frac{k_m[^{199}Hg^{2+}]_0 - (k_m[^{199}Hg^{2+}]_0 - k_d[Me^{199}Hg]_0)e^{-k_d t}}{k_d} \quad (5a)$$

and when $k_d = 0$, $[Me^{199}Hg]_t$ is given by eq 5b:

$$[Me^{199}Hg]_t = k_m[^{199}Hg^{2+}]_0 t + [Me^{199}Hg]_0 \quad (5b)$$

When $k_d > 0$, $R_{202}^{199}(t)$ is given by eq 6a

$$R_{202}^{199}(t) = \frac{[Me^{199}Hg]_t}{[Me^{202}Hg]_t} = \frac{k_m[^{199}Hg^{2+}]_0 - (k_m[^{199}Hg^{2+}]_0 - k_d[Me^{199}Hg]_0)e^{-k_d}}{k_m[^{202}Hg^{2+}]_0 - (k_m[^{202}Hg^{2+}]_0 - k_d[Me^{202}Hg]_0)e^{-k_}} \quad (6a)$$

and when $k_d = 0$, $R_{202}^{199}(t)$ is given by eq 6b:

$$R_{202}^{199}(t) = \frac{[Me^{199}Hg]_t}{[Me^{202}Hg]_t} = \frac{k_m[^{199}Hg^{2+}]_0 t + [Me^{199}Hg]_0}{k_m[^{202}Hg^{2+}]_0 t + [Me^{202}Hg]_0} \quad (6b)$$

where $[Me^{m}Hg]_0$ (m = 199, 201, or 202) is the concentration of m isotope MeHg at day 0 (nanograms per liter); $[Me^{m}Hg]_t$ is the concentration of m isotope MeHg at time t (nanograms per liter); and $[^{199}Hg^{2+}]_0$ and $[^{202}Hg^{2+}]_0$ are the concentrations of $^{199}Hg^{2+}$ and $^{202}Hg^{2+}$ at day 0 (nanograms per liter).

Estimation of Net Production or Degradation Rate of MeHg in Everglades Soil, Floc, Periphyton, and Water. Net production or degradation rates of MeHg in soil, floc, periphyton, and water were estimated at the four management units of the Everglades [Loxahatchee National Wildlife Refuge (LNWR), Water Conservation Areas 2 and 3 (WCA 2 and WCA 3), and Everglades National Park (ENP)] by use of the measured methylation and demethylation rate constants (k_m and k_d) and net production or degradation rates (R) (eqs 7a–8e). Net MeHg production rates of ambient [G_X^P(ambient)] and newly input Hg^{2+} [G_X^P(newly)] were calculated separately due to their difference in methylation/demethylation efficiency. The details in calculating the net MeHg production rate in each compartment can be found in Supporting Information.

$$G_X^P(\text{ambient}) = G_X^M(\text{ambient}) - G_X^D(\text{ambient}) = \frac{\bar{R}_X M_X}{A} \quad (7a)$$

$$R_X = k_m(X)\alpha_X[Hg^{2+}]_X - k_d(X)\beta_X[MeHg]_X \quad (7b)$$

$$G_X^P(\text{newly}) = \frac{\overline{k_m(X)}\Delta[Hg^{2+}]_X M_X}{A} \quad (7c)$$

$$G_W^P(\text{ambient}) = G_W^M(\text{ambient}) - G_W^D(\text{ambient}) \quad (8a)$$

$$G_W^M(\text{ambient}) = \int_0^D \frac{dC_{MeHg}(Z)}{dt} dZ = k_M \Delta[Hg^{2+}]_W PAR(0)\frac{1 - e^{-k_{PAR}D}}{k_{PAR}} \times 10^3 \quad (8b)$$

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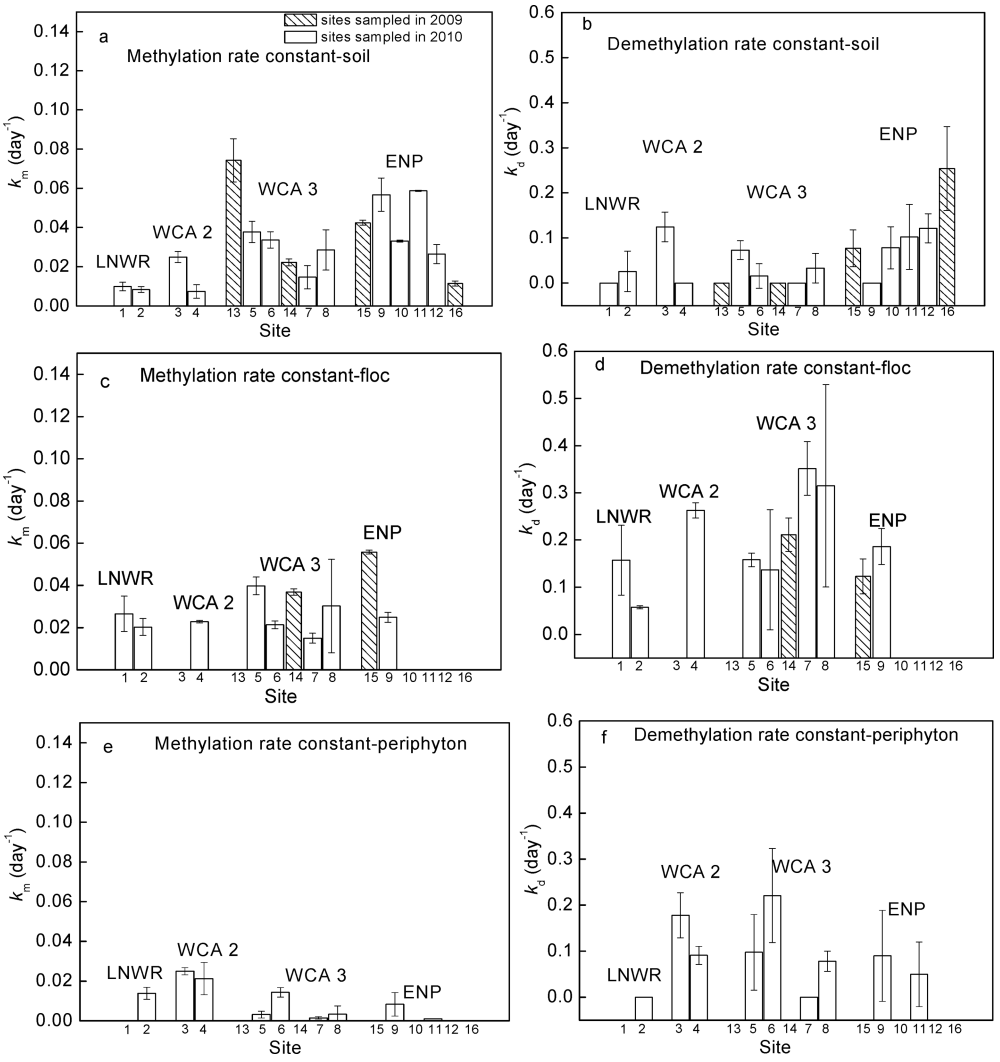

Figure 1. $^{199}Hg^{2+}$ methylation rate constant (per day) and $Me^{201}Hg$ demethylation rate constant (per day) in Everglades soil (a, b), floc (c, d), and periphyton (e, f). The *x*-axis represents the sampling sites. Sites 1−12 were sampled in 2010, while sites 13−16 were sampled in 2009. These sites were grouped according to their locations in the Everglades, from north to south. As samples of floc and periphyton could not be collected at some sites, k_m and k_d of these two compartments were not available at all sampling sites. Error bars represent the difference between triplicate samples.

$$k_M = \frac{k_m}{PAR} \tag{8c}$$

$$G_W^D(\text{ambient}) = \sum_I \left(k_D(I)[MeHg]_W PAR(0) \frac{1 - e^{-k_I D}}{k_I} \times 10^3 \right) \tag{8d}$$

$$G_W^P(\text{newly}) = \int_0^D \frac{dC_{MeHg}(Z)}{dt} dZ = k_M \Delta[Hg^{2+}]_W PAR(0) \frac{1 - e^{-k_{PAR} D}}{k_{PAR}} \times 10^3 \tag{8e}$$

G_X^P(ambient) and G_X^P(newly) are the net MeHg production or degradation rates of ambient and newly input Hg^{2+} in compartment X (nanograms per square meter per day), where X represents a specific compartment (soil, S; floc, F; periphyton, P; or water, W); G_X^M(ambient) and G_X^D(ambient) are the specific production or degradation rates of ambient MeHg in compartment X (nanograms per square meter per day); Superscripts M and D in G_X^M and G_X^D mean methylation and demethylation of Hg, respectively; $\overline{R}_X$ is the average net production or degradation rate of ambient MeHg (nanograms per gram per day); α_X is the ratio of methylation rate constant of ambient to newly spiked Hg for compartment X; β_X is the ratio of demethylation rate constant of ambient to newly spiked MeHg; $M_X$is the mass of compartment X in a specific management unit of the Everglades (grams); *A* is the area of a specific management unit in the Everglades (square meters); $[Hg^{2+}]_X$ and $[MeHg]_X$ are the concentrations of Hg^{2+} and MeHg in compartment X (nanograms per gram or nanograms per liter); $\Delta[Hg^{2+}]_X$ is the per-day increased concentration of Hg^{2+} in compartment X by newly input Hg (nanograms per gram or nanograms per liter); PAR is the photosynthetically active radiation (Einstein per square meter per day); PAR(0) is the photosynthetically active radiation above the surface of the water (Einstein per square meter per day); *Z* is a specific depth of water (meters); $k_D(I)$ is the photodegradation constant of I type of sunlight with respect to PAR(0) (square meter per Einstein), where I represents UV-A (I = UV-A), UV-B (I = UV-

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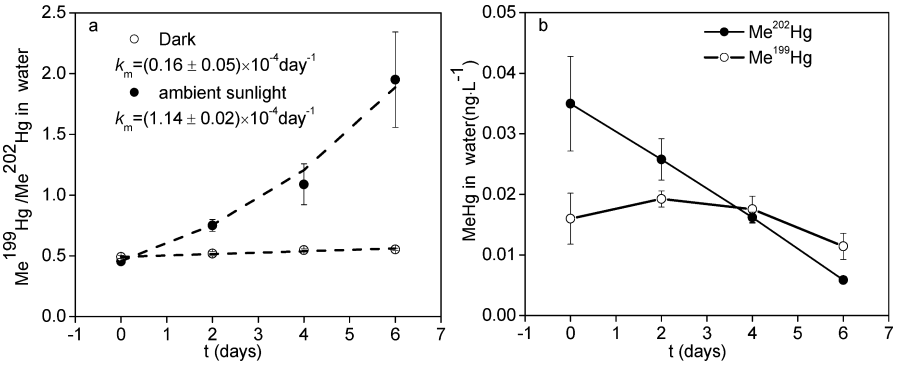

Figure 2. Variation of $Me^{199}Hg/Me^{202}Hg$ ratio (a) and concentrations of $Me^{199}Hg$ and $Me^{202}Hg$ (b) during the incubation of Everglades water. $Me^{199}Hg/Me^{202}Hg$ ratio was used to calculate the methylation rate constant of spiked $^{199}Hg^{2+}$(eq 6a,6b). Points in panel (a) represent the measured values, while the dashed line shows the simulated results.

B), or visible sunlight (I = PAR); k_I is the light attenuation coefficient of I type of sunlight (per meter); D is the water depth (meters); and k_M is the PAR normalized rate constant of MeHg photomethylation (square meter per Einstein).

Statistical Analysis. Linear regression of MeHg concentrations in Everglades soil, floc, periphyton, and surface water on methylation and demethylation related variables in these compartments were conducted with Origin 6.0. Outliers were detected by Cook's distance measurements with SPSS (version 17 for Windows, SPSS Inc., Chicago, IL).

Pearson correlation analyses between MeHg concentration in water ($MeHg_{water}$) and 15 biogeochemical parameters were performed by using SPSS (version 17 for Windows, SPSS Inc., Chicago, IL). The 15 parameters included MeHg degradation potential (P_{PD}, a previously defined photodemethylation-related parameter),[9] dissolved organic carbon (DOC), MeHg concentration in floc layer ($MeHg_{floc}$), MeHg concentration in soil ($MeHg_{soil}$), MeHg concentration in periphyton ($MeHg_{peri}$), nitrate (NO_3-N), chlorophyll *a* (Chl-a), sulfate (SO_4-S), H_2S, total Hg concentration in water (THg_{water}), temperature (T), dissolved oxygen (DO), pH, Turbidity (Turb), and soluble reductive phosphate (SRP). Multiple linear regression analyses of MeHg against the parameters significantly correlated with MeHg were conducted by using SPSS.

RESULTS AND DISCUSSION

Hg^{2+} Methylation and MeHg Demethylation Potential in Everglades Soil, Floc, Periphyton, and Surface Water. Figure 1 illustrates the methylation rate constants of spiked $^{199}Hg^{2+}$ (k_m) and the demethylation rate constants of spiked $Me^{201}Hg$ (k_d) in Everglades soil, floc, and periphyton. Significant methylation ($p < 0.05$) of the spiked $^{199}Hg^{2+}$ (0.01−0.07 day^{-1}, average 0.03 ± 0.02 day^{-1}) was observed in all incubated soil samples (Figure 1a). Soil k_m values in the two northern areas (LNWR and WCA 2) were lower than that in the two southern areas (WCA 3 and ENP). Soil k_d was in the range of 0−0.25 day^{-1} (average 0.05 ± 0.05 day^{-1}) and generally illustrated an increasing trend from north to south (Figure 1b). Both k_m and k_d were significantly higher than 0 in all floc samples (Figure 1c,d). Values of floc k_m (0.02−0.06 day^{-1}, average 0.03 ± 0.01 day^{-1}) were similar ($p > 0.1$) to that of soil, while floc k_d (0.06−0.35 day^{-1}, average 0.20 ± 0.09 day^{-1}) was much higher (about 3.5 times) than that of soil. No significant spatial distribution trend was shown for floc methylation and demethylation. Significant methylation of Hg^{2+} (0.001−0.02 day^{-1}, average 0.01 ± 0.01 day^{-1}) and demethylation of MeHg (0−0.22 day^{-1}, average 0.09 ± 0.07 day^{-1}) were also found in periphyton (Figure 1e,f). The potential Hg methylation ($k_m[Hg^{2+}]$) and demethylation (k_d[MeHg]) rates were further calculated and are shown in Figure S2 and Table S1 in Supporting Information. Potential methylation rates were 4.52 ± 0.67 ng·g (dry)$^{-1}$·day^{-1} in soil, 3.07 ± 0.50 ng·g (dry)$^{-1}$·day^{-1} in floc, and 0.54 ± 0.13 ng·g(dry)$^{-1}$·day^{-1} in periphyton. Potential demethylation rates were estimated to be 0.04 ± 0.02 ng·g (dry)$^{-1}$·day^{-1} (soil), 0.51 ± 0.15 ng·g (dry)$^{-1}$·day^{-1} (floc), and 0.44 ± 0.18 ng·g (dry)$^{-1}$·day^{-1} (periphyton). Values of k_m and k_d obtained in this study are comparable to the values reported in previous studies conducted at WCA 2 and WCA 3 of the Everglades.[12,18,19,28] Compared to other ecosystems (Table S1, Supporting Information), methylation rate constants in Everglades soil, floc, and periphyton were at the high end of the range of this parameter, while moderate values of demethylation rate constants were observed in the Everglades. In comparison to the potential methylation rate of spiked $^{199}Hg^{2+}$, the net methylation rate of the ambient Hg was much smaller (<5% in soil; Table S1, Supporting Information). In addition, a large difference was observed between the estimated overall MeHg production rate ($k_m[Hg^{2+}] - k_d$[MeHg]) and the measured net production rate of ambient MeHg production rates (determined by $Me^{202}Hg$) (Table S1, Supporting Information), indicating that there are significant differences between ambient and newly input Hg species in methylation or demethylation efficiency. As periphyton is an important food source for externally feeding macroinvertebrates and fishes, methylation of mercury in periphyton may have a significant effect on the bioaccumulation of MeHg in the food chain.

Figure 2a shows the methylation of spiked $^{199}Hg^{2+}$ in surface water. The 199/202 ratio of MeHg increased gradually from 0.5 to ∼2 after 6 days of exposure to sunlight, while negligible change occurred in the dark. To further validate the effect of sunlight on methylation, k_m values at trials with and without sunlight were calculated according to eq 6a,6b. The $^{199}Hg^{2+}$ in water had a k_m of (1.14 ± 0.02) × 10^{-4} day^{-1} under ambient sunlight, while it was (0.16 ± 0.05) × 10^{-4} day^{-1} in the dark. These results suggest that methylation, which is dependent upon sunlight, occurs in Everglades water. However, its rate was much slower than that of MeHg photodemethylation (k_d = 0.26 ± 0.04 day^{-1}),[9] indicating that methylation in water plays a minor role in the cycling of MeHg in the Everglades. The changes in $Me^{202}Hg$ concentration were taken into account in

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order to correct for the effect of MeHg demethylation during the incubation. No significant increase in $Me^{199}Hg$ concentration was observed, but a substantial decrease in $Me^{202}Hg$ did occur (Figure 2b), due to the faster rate of photodemethylation compared to methylation. This indicates that contributions of the photodemethylation of ambient and newly produced $Me^{199}Hg$ were not negligible for the variation of $Me^{199}Hg$. These results suggest that photodegradation of ambient and newly produced $Me^{199}Hg$ should be considered when determining k_m in water, especially for systems with $k_m \ll k_d$.

Effects of MeHg Production and Degradation on the Spatial Distribution of MeHg in Everglades Soil, Floc, Periphyton and Surface Water. The importance of MeHg production and degradation in the spatial distribution of MeHg in various compartments of the Everglades was estimated by use of k_m and k_d values obtained in this study. The few previous studies on this topic focused only on the relationship between MeHg distribution in a compartment and its in situ methylation or demethylation.[9,12] However, MeHg present in a particular compartment (e.g., water) could be determined by the methylation/demethylation that occurred both in that compartment and in other compartments (e.g., floc, periphyton, or soil). Thus, the relationships of MeHg in one compartment to the methylation/demethylation in that compartment and other compartments were evaluated in this study. Four parameters (k_m, $k_m[Hg^{2+}]$, k_d (or P_{PD} in water), and $k_d/k_m[Hg^{2+}]$) associated with mercury methylation or demethylation were selected to study the effects of MeHg production and degradation on the spatial distribution of MeHg. k_m and $k_m[Hg^{2+}]$ represent the potential and rate of methylation, while k_d (or P_{PD} in water) can reflect the demethylation potential of MeHg. If spatial distribution of MeHg is significantly affected by methylation or demethylation, it is expected to be positively related to that of k_m and $k_m[Hg^{2+}]$ or negatively related to that of k_d. k_m and k_d were the two common parameters used to evaluate the influence of methylation and demethylation on MeHg distribution. However, neither of them can reflect the combined effect of Hg methylation and demethylation. This could be a problem if neither of these two processes can overrule the other. Under such conditions, the results could be inconclusive. Thus, a new parameter ($k_d/k_m[Hg^{2+}]$, the reciprocal of steady-state MeHg concentration) was derived (eqs 9 and 10) and used to represent the combined effect of methylation and demethylation. A negative relationship is expected to be observed between MeHg concentrations and $k_d/k_m[Hg^{2+}]$ if both methylation and demethylation significantly affect MeHg distribution. Regression analysis was then conducted to study the relationship between the MeHg present in soil, floc, or water and the values of the four parameters in these compartments (Figure S3, Supporting Information).

$$\frac{d[MeHg]}{dt} = k_m[Hg^{2+}] - k_d[MeHg] \quad (9)$$

$$\frac{1}{[MeHg]} = \frac{k_d}{k_m[Hg^{2+}]} \quad (10)$$

Except for site 1 (identified as an outlier), MeHg concentration in soil was closely correlated to k_m ($p < 0.05$; Figure S3a, Supporting Information) and $k_m[Hg^{2+}]$ ($p < 0.01$; Figure S3b, Supporting Information) of soil. Other tested parameters did not show a significant effect on soil MeHg concentration ($p > 0.1$). Similar results were observed for periphyton (Figure S3d,e, Supporting Information, except for site 8, which was identified as an outlier). These results suggest that MeHg concentrations in soil and periphyton are mainly related to the in situ methylation. Floc MeHg concentration was found to be inversely proportional to $k_d/k_m[Hg^{2+}]$ of floc ($0.05 < p < 0.1$; Figure S3c, Supporting Information). Other tested parameters (including floc k_m and k_d) did not show a significant effect on floc MeHg concentration ($p > 0.1$), indicating that both methylation and demethylation of MeHg in this compartment are important in controlling its MeHg concentration. The lack of a significant correlation between MeHg concentration and methylation or demethylation alone in floc suggests that neither of them dominates the other. For surface water, MeHg concentrations were strongly correlated to k_m ($p < 0.05$; Figure S3f, Supporting Information) and $k_m[Hg^{2+}]$ ($p < 0.05$; Figure S3g, Supporting Information) of periphyton and P_{PD} of water column ($p < 0.05$; Figure S3h, Supporting Information). MeHg in water was not positively affected by the methylation in floc and not significantly related to the other three parameters in floc ($p > 0.1$). In addition, MeHg in water showed no significant relationships to soil methylation/demethylation related parameters ($p > 0.1$). These results suggest that the methylation of mercury in periphyton and photodemethylation could significantly affect the levels of MeHg in Everglades water.

Cycling of MeHg in the Everglades water column is very complex as multiple processes could have a significant influence, for example, methylation in soil, floc, and periphyton and photodemethylation in the water column. Data obtained in this study and the monitoring investigation[21] in 2005 were employed to further investigate factors influencing MeHg concentrations in water. Ten (P_{PD}, DOC, $MeHg_{floc}$, $MeHg_{soil}$, $MeHg_{peri}$, SO_4-S, H_2S, THg_{water}, DO, and Turb) of the 15 environmental parameters were found to significantly correlate with MeHg concentrations in Everglades water ($p < 0.01$ level). Multiple linear regression analyses indicated that THg_{water}, $MeHg_{peri}$, and P_{PD} were the three most important parameters influencing the distribution of $MeHg_{water}$ (Table S2, Supporting Information), as implicated by their higher standardized coefficients (β). With respect to the positive relationship of $MeHg_{water}$ to $MeHg_{peri}$, there are two possible explanations. One is that MeHg in periphyton is taken up or adsorbed from surface water and thus controlled by the concentration of surface water MeHg. The other is that MeHg in water could be significantly affected by the methylation of Hg in periphyton. The latter explanation may be more reasonable in the Everglades, as a positive relationship of MeHg in periphyton to periphyton k_m was observed (Figure S3d,f, Supporting Information), meaning that $MeHg_{peri}$ may reflect the Hg methylation in periphyton. These results suggest that methylation in periphyton and photodemethylation in water may influence the MeHg levels in Everglades water.

Production or Degradation of MeHg in Various Compartments of the Everglades. The net daily production (or degradation) rates of MeHg in soil, floc, periphyton, and water of the Everglades were estimated from the results of isotope addition experiments. Estimation of these rates is crucial in order to identify the major source and sink of MeHg. As a difference in the efficiency of methylation or demethylation was expected to exist between ambient and newly input Hg species, the net production (or degradation) rates of newly input and ambient Hg species were calculated separately. The production rate of MeHg from the newly input

Table 1. Net Production and Degradation Rates of MeHg in Various Compartments of Everglades[a]

	net per-area production (or degradation) rate of MeHg ($ng \cdot m^{-2} \cdot day^{-1}$)			
	LNWR	WCA 2	WCA 3	ENP
		Soil		
G_S^p(ambient)	418 ± 289	2105 ± 1466	3980 ± 2621	6236 ± 8317
G_S^p(newly)	$(5 \pm 0.6) \times 10^{-1}$	$(9 \pm 7) \times 10^{-1}$	$(18 \pm 11) \times 10^{-1}$	$(21 \pm 10) \times 10^{-1}$
total	418	2105	3982	6238
$[G_S^p$(newly)$]$/total	0.1%	0.4%	0.4%	0.3%
		Floc		
G_F^p(ambient)	−126 ± 54	−91 ± 4	−145 ± 83	−9 ± 9
G_F^p(newly)	$(8 \pm 2) \times 10^{-2}$	$(4 \pm 0.2) \times 10^{-2}$	$(6 \pm 2) \times 10^{-2}$	$(1 \pm 0.5) \times 10^{-2}$
total	−126	−91	−145	−9
$[G_F^p$(newly)$]$/total	0.06%	0.04%	0.04%	0.1%
		Periphyton		
G_P^p(ambient)	3 ± 0.15	52 ± 22	−9 ± 10	−27 ± 23
G_P^p(newly)	$<1 \times 10^{-2}$	$<1 \times 10^{-2}$	$<1 \times 10^{-2}$	$<1 \times 10^{-2}$
total	3	52	−9	−27
$[G_P^p$(newly)$]$/total	0.004%	0.005%	0.002%	0.002%
		Water		
G_W^p(ambient)	−5 ± 6	−4 ± 2	−4 ± 3	−4 ± 3
G_W^p(newly)	$<1 \times 10^{-6}$	$<1 \times 10^{-6}$	$<1 \times 10^{-6}$	$<1 \times 10^{-6}$
total	−5	−4	−4	−4
$[G_W^p$(newly)$]$/total	<0.0001%	<0.0001%	<0.0001%	<0.0001%

[a]LNWR (Loxahatchee National Wildlife Refuge), WCA 2 and WCA 3 (Water Conservation Areas 2 and 3), and ENP (Everglades National Park) are the four management units of the Everglades. G_X^p(ambient) and G_X^p(newly) (X = S, soil; F, floc; P, periphyton; or W, water) are the production rates of MeHg from ambient and newly input Hg, respectively. G_X^p(newly) was calculated according to the average measured k_m in compartment X $[k_m(X)]$ and the per-day increased concentration of Hg^{2+} by newly input Hg^{2+} ($\Delta[Hg^{2+}]_X$) (eq 7c). Values of $\Delta[Hg^{2+}]_X$ in each compartment were cited from ref 29.

Hg^{2+} can be estimated by using the k_m obtained from the spiked $^{199}Hg^{2+}$ (eq 7c). As for the production of MeHg from ambient Hg, it would be ideal to calculate the net methylation rate from the measurement of changes in ambient MeHg if such measurement is feasible. However, significant changes in ambient MeHg could be detected only at limited sampling sites (14 of 16 for soil, 5 of 10 for floc, and 6 of 9 for periphyton) (Figure S4, Supporting Information). This is due to the fact that the variation in ambient MeHg ($Me^{202}Hg$ in this study) during the incubation period is often too small to be detected. Previous studies usually calculated the overall MeHg production rate by the difference of potential methylation rate and potential demethylation rate ($k_m[Hg^{2+}] - k_d[MeHg]$).[13,14] However, such practice does not take consideration of the differences in bioavailability of the ambient and newly spiked Hg species. In this study, α (ratio of methylation rate constant of ambient to newly spiked Hg) and β (ratio of demethylation rate constant of ambient to newly spiked MeHg) in soil, floc, and periphyton were calculated by fitting the data of measured net MeHg production (or degradation) rate against the potential methylation rate ($k_m[Hg^{2+}]$) and potential demethylation rate ($k_d[MeHg]$) (eq 7b) of the limited sites where significant changes in ambient MeHg were observed (Figure S3, Supporting Information). α and β were estimated to be 0.06 and 0.93 in soil, 0.02 and 0.71 in floc, and 0.53 and 0.50 in periphyton. The differences of ambient and newly spiked Hg species in methylation and demethylation were ignored in water, as the MeHg produced in water was negligible and no difference was observed in the photodemethylation of the ambient and newly spiked MeHg.[27]

By utilizing the obtained α, β, and k_m and k_d values, the net production (or degradation) rates of ambient MeHg were estimated by use of eq 7b at each site (Figure S5, Supporting Information). Values of the other parameters for estimating the net production (or degradation) rates are listed in Table S3 (Supporting Information). Finally, the production (or degradation) rates of MeHg (from ambient or newly input Hg) in soil, floc, periphyton, and water of the Everglades were estimated (see Table 1). Although the newly input Hg^{2+} has a much higher methylation/demethylation efficiency, the net daily produced MeHg from this source was found to account for a very small fraction of MeHg produced (<0.4%). This could be explained by the low fraction of newly input Hg to the ambient Hg in the Everglades. Soil was estimated to be the largest source of MeHg in all four management units of the Everglades, accounting for 98−100% of total produced MeHg. The net MeHg production rate in soil ranged from 418 to 6238 $ng \cdot m^{-2} \cdot day^{-1}$, in the order LNWR < WCA2 < WCA3 < ENP. Floc is a major sink of MeHg, with a degradation rate of −9 to −145 $ng \cdot m^{-2} \cdot day^{-1}$. Water is another sink for MeHg, accounting for approximately 2−10% of the total MeHg degradation. Periphyton was found to be a source for MeHg in the northern Everglades (LNWR and WCA 2) and a sink in the south (WCA 3 and ENP). The multiple role of periphyton in the Everglades is attributed to the great variety in periphyton k_m and k_d at different locations (Figure 1). It should be noted that each of the four areas of the Everglades (LNWR, WCA2, WCA3, and ENP) exhibits substantial spatial and temporal variation in factors (e.g., sulfide, sulfate, periphyton community), which could affect the production and degradation of MeHg. Given the limited sampling sites in this study, a relatively high standard deviation was observed for some estimated rates (Table 1). In this study, soil (0−10 cm), floc, and periphyton samples were collected separately in order to compare the production of MeHg in these compartments, and then the samples were homogenized and incubated. By

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adopting this approach, the vertical distribution of sulfate-reduction bacteria (SRB) activities in soil cores could not be reflected, which may cause some errors on the estimated production rates of MeHg. However, these errors should be acceptable due to the fact that the obtained methylation rate constants of surface sediment in this study are comparable to the reported results of a previous study using the intact cores.[12]

There is a significant difference between the ambient and newly input Hg species in methylation/demethylation efficiency. However, this was often neglected in the previous studies of estimating the net MeHg production rate via isotope addition technique.[13,14] If α and β were not included in the estimation model, the estimated net production (or degradation) rate of MeHg in soil $[G_S^p(\text{ambient})]$ could be overestimated by a factor of 20 (Table S4, Supporting Information). The average of the estimated net production (or degradation) rate of MeHg in floc $[G_F^p(\text{ambient})]$ would increase from −70 to 700 $ng{\cdot}m^{-2}{\cdot}day^{-1}$ (Table S4, Supporting Information). In that case, the net per-day increase in MeHg concentration in soil would account for 400% of ambient MeHg concentration (Table S4, Supporting Information). This fails to account for the mass balance of ambient MeHg. This ratio is decreased to 20% if the estimated α and β are included in the calculation. These results indicate that the difference in methylation/demethylation efficiency of the ambient and newly input Hg species must be taken into account when net MeHg production (or degradation) rates are estimated. In the Everglades, values of α were much lower than that of β in the soil and floc, suggesting the dissimilarities of ambient MeHg and inorganic Hg in bioavailability.[15] A much higher value of α was observed in periphyton (51%) in comparison to soil and floc (2−6%). This may be caused by the differences in absorbing divalent inorganic Hg and/or speciation of divalent inorganic Hg in these compartments, which could significantly affect the bioavailability of inorganic Hg.

In order to identify the major source of MeHg in the water column, the distribution rate of daily produced MeHg in the benthic layer to the water column was estimated. The rate in each management unit of the Everglades was calculated from the previously reported percentage of produced MeHg distributed to the compartments in water column[29] and the net MeHg production rates measured in this study (see details in Supporting Information). By using a mass balance model of MeHg, it was estimated that approximately 5.9% (LNWR), 20.8% (WCA 2), 15.2% (WCA 3), and 9.4% (ENP) of produced MeHg was transported into the compartments in the water column.[29] The rate of totally produced MeHg distributed to the compartments in the water column were estimated to be 17 $ng{\cdot}m^{-2}{\cdot}day^{-1}$ (LNWR), 429 $ng{\cdot}m^{-2}{\cdot}day^{-1}$ (WCA 2), 581 $ng{\cdot}m^{-2}{\cdot}day^{-1}$ (WCA 3), and 582 $ng{\cdot}m^{-2}{\cdot}day^{-1}$ (ENP). Methylation in periphyton was then estimated to account for 18% (LNWR) and 12% (WCA 2) of total produced MeHg imported into the water column, with the rest coming from the soil. These results suggest that soil could be the largest source for MeHg in the water column, while methylation in periphyton could also contribute significantly in the northern Everglades. From the estimated net methylation rate in periphyton (Table 1), the daily production of MeHg by periphyton was calculated to be 0.02 and 0.30 kg in the LNWR and WCA 2, respectively. These amounts accounted for approximately 10% and 140% of ambient MeHg in the water of these two management units (0.19 and 0.22 kg).[23] This estimation also supports the opinion that methylation in periphyton can significantly influence the levels of MeHg in Everglades surface water. Transport of mercury at the sediment−water interface is a complicated process and could be affected by many factors, such as methylation, redox condition, water depth, and physical properties of the sediments.[30,31] In consideration of the complexity of this process, results obtained in this study could be further refined by measuring and comparing the transport rates of MeHg from benthic layer and periphyton to water column.

Sulfate-reducing bacteria were deemed to be the methylators in Everglades soil, floc, and periphyton.[12,18] Sulfate, sulfide, nutrients, and dissolved organic matter (DOM) concentrations generally bear a decreasing gradient from north to south.[21,32] Sulfate can stimulate the activity of SRB and thus favor the Hg methylation process, while high sulfide would inhibit this process by reducing the bioavailability of Hg^{2+}.[33] DOM can either enhance the methylation process by stimulating SRB activity[34] or inhibit it via reducing Hg bioavailability.[35] The spatial distribution of these parameters may result in the variation of MeHg production rate in the Everglades. As the addition of sulfide was observed to inhibit the methylation of Hg^{2+} in the Everglades,[12] the north-to-south decreasing trend of sulfide may explain the larger methylation rate constants of Hg in the two southern areas of the Everglades. In addition, the composition of Everglades periphyton varies across the Everglades, ranging from filamentous green mats in the eutrophic areas to calcareous mats in less impacted areas.[18,36] It was found from a previous study that the filamentous green algal communities showed a more rapid rate of sulfate reduction compared to the calcareous periphyton,[18] indicating the high activity of SRB in the filamentous green algal communities. It could explain the decrease of periphyton methylation rate from north to south following a decreasing trend of nutrients.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional text and equations, five figures, and four tables as described in the text. This material is available free of charge via the Internet at http://pubs.acs.org/ .

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Notes

The authors declare no competing financial interest.

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