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Methanogenesis Is an Important Process in Controlling MeHg Concentration in Rice Paddy Soils Affected by Mining Activities

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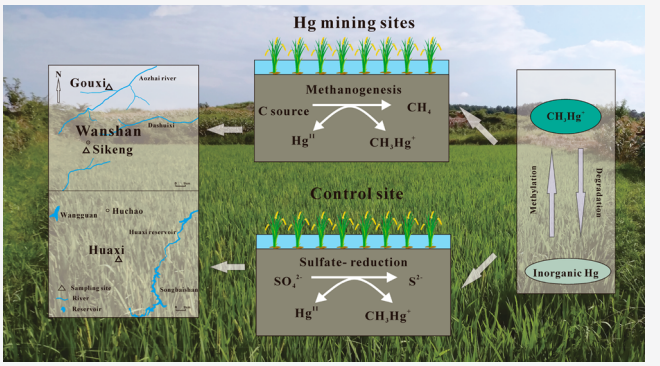
ABSTRACT: Rice paddies are agricultural sites of special concern because the potent toxin methylmercury (MeHg), produced in rice paddy soils, accumulates in rice grains. MeHg cycling is mostly controlled by microbes but their importance in MeHg production and degradation in paddy soils and across a Hg concentration gradient remains unclear. Here we used surface and rhizosphere soil samples in a series of incubation experiments in combination with stable isotope tracers to investigate the relative importance of different microbial groups on MeHg production and degradation across a Hg contamination gradient. We showed that sulfate reduction was the main driver of MeHg formation and concentration at control sites, and that methanogenesis had an important and complex role in MeHg cycling as Hg concentrations increased. The inhibition of methanogenesis at the mining sites led to an increase in MeHg production up to 16.6-fold and a decrease in MeHg degradation by up to 77%, suggesting that methanogenesis is associated with MeHg degradation as Hg concentrations increased. This study broadens our understanding of the roles of microbes in MeHg cycling and highlights methanogenesis as a key control of MeHg concentrations in rice paddies, offering the potential for mitigation of Hg contamination and for the safe production of rice in Hg-contaminated areas.



KEYWORDS: *rice paddy, microorganism, mercury, methylation, demethylation*

INTRODUCTION

Mercury (Hg) is a global pollutant that can be methylated to the potent neurotoxin, methylmercury (MeHg). This methylation occurs mostly through biological pathways in aquatic environments.[1] MeHg can be bioaccumulated in organisms and biomagnified in aquatic food webs.[1,2] Although fish consumption has been identified as one of the predominant Hg exposure pathways worldwide,[3,4] recent work has revealed rice consumption as another significant exposure route.[5] In some areas of China, such as the Wanshan Hg mine in Guizhou Province, MeHg in rice can reach levels ≥100 $\mu g \cdot kg^{-1}$,[6] far exceeding the national food safety limit in China (20 $\mu g \cdot kg^{-1}$, GB2762−2017). The positive relationship between hair MeHg levels and daily rice intake by local people in the Wanshan area is of special concern, as it highlights the risk of human exposure to MeHg via rice consumption.[5,7]

The risk of human exposure to MeHg is not limited to mining areas, but also to other rice growing areas around the world. Indeed, paddy soils represent an environment conducive to the production of MeHg. With varying redox potential, intermittent anoxia, high nutrient concentrations, and the presence of known methylators, paddy soils are hot spots for Hg methylation and subsequent MeHg accumulation in rice plants.[8] It is therefore critical to better understand MeHg production and degradation in rice paddies to better manage Hg pollution, assess exposure risk to MeHg, and develop remediation strategies.

Inorganic Hg in rice can be absorbed by roots (as Hg^{II}) or captured by plants from the atmosphere (as Hg^0).[8] In contrast, MeHg in rice grains mainly originates from in situ production in paddy soils.[8−12] Chemical speciation of Hg, pH, redox potential, organic matter molecular composition and availability, and microbial activity are all important variables that affect the amount of MeHg formed in aquatic systems.[13−15] In agricultural (rice growing) wetlands,[16] the spatiotemporal variation of Hg methylators activity had a larger effect on MeHg production variability than did the variation in Hg^{II} availability, highlighting the importance of microorganisms in Hg methylation.

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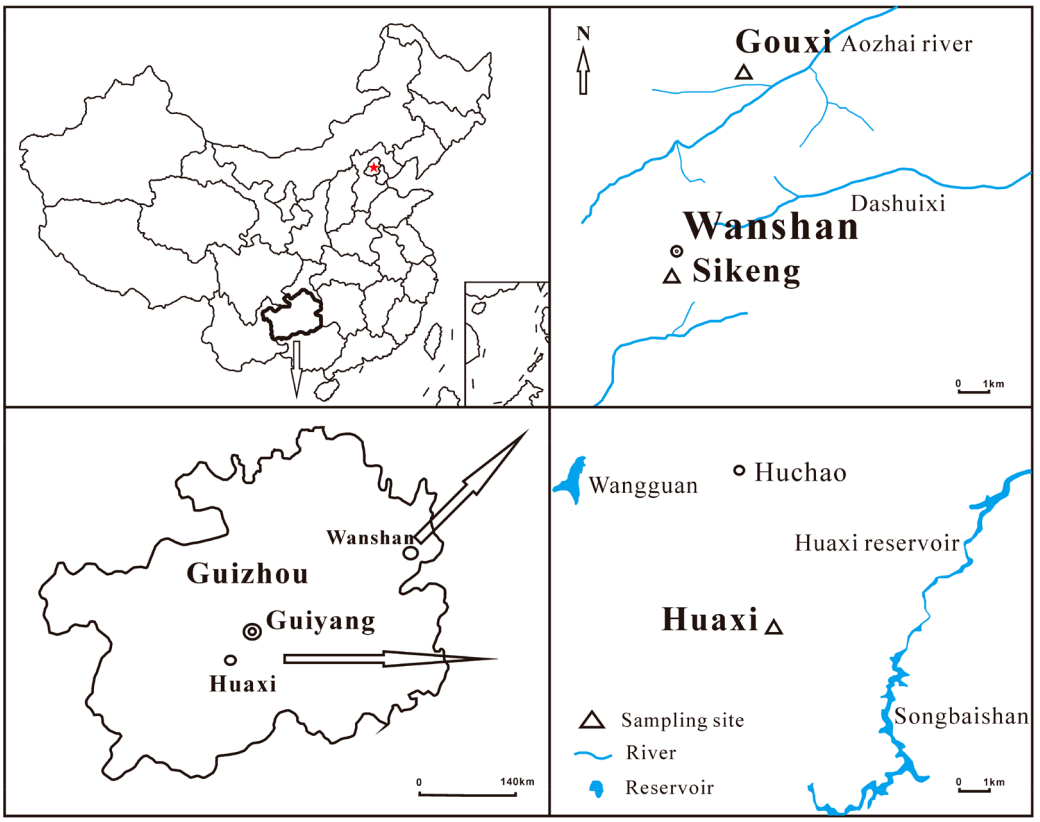

Figure 1. Sampling sites in Guizhou Province in Southwest of China. The control site (Huaxi) is located near Guiyang City while the Hg mining sites (Gouxi and Sikeng) are located in the Wanshan Hg mining area.

The water−sediment interface is important for Hg methylation as both active Hg methylation and high MeHg concentration are observed in this critical zone.[17,18] Rice paddy ecosystems are typically vertically stratified with a 5−10 cm layer of overlying surface water, followed by a ca. 2 cm top surface soil layer supporting a dynamic and active microbial community,[19,20] considered to be an important site for Hg methylation.[21] Deeper (>2 cm), the rhizosphere soil is chemically different from the surface soil, with rice plant roots exuding organic acids. This can promote the growth of anaerobic microbes and decreases pH.[22−24] This may enhance the bioavailability of Hg^{II} to microbes potentially favoring Hg methylation.[25]

In aquatic systems and soils, Hg methylation is mainly mediated by anaerobic microorganisms, especially sulfate- and iron-reducing bacteria, methanogenic Archaea, and to a lesser extent, Firmicutes and syntrophs.[26−28] So far, the key methylators described in paddy soils have been identified as sulfate-reducing bacteria, iron-reducing bacteria, and methanogens.[29,30] One study reported on a correlation between *hgc*A copy numbers and total Hg (THg) and MeHg levels in soils affected by mining activities.[31] *hgc*A genes identified from the mining area were from lineages identified as *Deltaproteobacteria*, *Firmicutes*, *Chloroflexi*, *Euryarchaeota*, and two unclassified groups of microbes.[32] Novel Hg methylators were identified as *Geobacter anodireducens*, *Desulfuromonas* sp. DDH964, and *Desulfovibrio* sp. J2[30] and Catenulisporaceae, Frankiaceae, Mycobacteriaceae, and Thermomonosporaceae were found to be the potential MeHg demethylators in paddy soils.[33] The relative importance of these microbial groups in MeHg production and degradation, however, remains unclear.

The net concentration of MeHg measured in paddy soils is the result of MeHg production (methylation) and degradation (demethylation).[21,34−37] Although methylation has received considerable attention over recent decades, we know very little of the mechanisms that contribute to MeHg degradation. This is a major gap in knowledge as demethylation can possibly represent a key process limiting MeHg accumulation in the environment. To fill these knowledge gaps, the objectives of our study were: (1) to determine the contributions of known microbial metabolisms to Hg methylation and demethylation in rice paddy soils along a Hg contamination gradient; and (2) to quantify the relative importance of methylation and demethylation over the spatially stratified rice paddy environment. We used a combination of incubation experiments with specific metabolic inhibitors and/or stimulants coupled with Hg isotope tracers to quantify the relative importance of microbial metabolisms involved in MeHg cycling in rice paddy soils.

MATERIALS AND METHODS

Site Description. Experiments were performed at three sites: an abandoned Hg mining site (Sikeng, E 109°12′18.36″, N 27°30′38.12″), an artisanal Hg mining site (Gouxi, E 109°13′30.86″, N 27°33′56.91″), and a control site (Huaxi, E 106°32′1.46″, N 26°24′40.46″). The abandoned Hg mining site and artisanal Hg mining site are located in the Wanshan Hg mining area, Guizhou Province, China (Figure 1). The control site of Huaxi is situated southwest of Guiyang City, Guizhou Province. These sites represent three different levels of Hg pollution.

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Table 1. Amounts of Specific Inhibitors/Stimulants Added for Each Incubation Experiment[a]

no.	inhibitors/stimulants	conc. (mM)	anticipated effects
A	Na_2MoO_4	1	inhibits sulfate-reduction
		5	
		10	
		20	
		50	
B	BES	5	inhibits methanogenesis
		10	
		20	
		50	
C	Na_2MoO_4 + BES	1 + 5	inhibits both sulfate-reduction and methanogenesis
D	Na_2SO_4	1	promotes sulfate-reduction
E	FeOOH	1	promotes iron-reduction
F	control	no addition	control
G	autoclaved	121 °C, 30 min	abiotic

[a]The Mo and BES concentrations were 1 mM and 5 mM, respectively, unless otherwise stated.

The first sampling site, Sikeng, is located in the abandoned Hg mining area. The activities of Hg mining in the Wanshan district can be traced back to the Qin Dynasty (221 B.C.), and intensified in the last century, making it historically important for the local residents' economy. Although these activities were phased out since 2002, Hg from the large number of smelting tailings and waste rocks continues to be discharged into the nearby rivers and streams, resulting in pollution of the irrigation water and soils of the rice paddy fields. The second sampling site, Gouxi, is located in area with small-scale artisanal smelting activities resulting in high levels of gaseous Hg in the air.[12] The soil and water were polluted by dry/wet deposition of atmospheric Hg, which was associated with an increased Hg methylation in rice paddies at this site.[21] The third sampling site, Huaxi, is located ca. 30 km away from Guiyang City. It was chosen as the control site because the Huaxi and Wansahn mining areas had a similar climate.[8] There is no distinct Hg pollution source near this site as indicated by the low concentrations of gaseous Hg in the ambient air and THg in the paddy soils.[8]

Sampling. The sampling campaign was conducted from August 11th to 13th 2017 at the heading stage of rice growing period. This corresponded to day 50 to 60 after the rice seedings were transplanted into the paddies. The timing of sampling was chosen to reflect conditions optimal for Hg methylation as supported by high concentrations of THg, MeHg, Fe^{2+}, and HS^- found in pore water of rice paddies in previous studies during this period.[12,21] Both the surface soil layer (0−2 cm deep below the soil−water interface) and the rhizosphere soil (10−15 cm, soil attached to the rice root) were collected for incubation experiments.

Three surface soil samples were carefully collected using a plastic shovel for each of the sampling sites, without disturbing the deeper soils or losing the interface layers. Similarly, three rhizosphere soil samples were obtained by pulling up the plants and grabbing the soil from the rice roots by hand with sterile gloves. Soil samples and overlying water samples for incubation were stored in 500 mL polypropylene bottles (Nalgene, U.S.A.). After sampling, the bottles for soil samples were filled up with a 2 cm of corresponding overlying water, sealed, double-bagged, and transported to the lab under 4 °C condition, within 24 h. Samples were stored at 4 °C in a refrigerator in the dark until incubation. Three additional surface/rhizosphere soil samples were collected in 50 mL plastic centrifuge tubes (Jet Bio-Filtration Co., Ltd., China) and stored at −20 °C in the laboratory for THg and MeHg analyses.

Incubation Experiment Design. We used Na_2MoO_4 and BES to inhibit sulfate-reduction and methanogenesis, respectively.[38,39] A wide range concentration of inhibitors (molybdate and BES) are generally reported in published studies to possibly inhibit sulfate-reduction and methanogenesis depending on the soil/sediment types. For example, 0.2−20 mM molybdate and 10−30 mM BES were employed for estuarine/coastal Marine/peat sediment or swamps soil.[26,35,36,39−41] To obtain a proper and reasonable concentration, a gradient of inhibitor concentrations (1−50 mM molybdate and 5−50 mM BES) were tested in this study. Our results showed that the sulfate-reduction and methanogenesis were effectively inhibited when treated with 1 mM molybdate and 5 mM BES (Figure S1 of the Supporting Information, SI). Therefore, 1 mM molybdate and 5 mM BES are selected in the following experiments. The concentration of inhibitors and stimulants are showed in Table 1. Na_2SO_4 and FeOOH were used to stimulate sulfate-reduction and iron-reduction, respectively. These two metabolic pathways are recognized as potentially involved in Hg methylation in paddy soil.[41] Autoclaved samples were used as abiotic controls for microbial Hg methylation and MeHg degradation. Although the autoclaving step can alter the physical properties of the soil sample, it was shown to be appropriate for short time incubation (1 day) of soil samples.[42] Stock solutions were prepared in sterile water. The methylation and demethylation rate constants were determined using a multi-isotope technique including $^{202}Hg(NO_3)_2$ and $CH_3{}^{198}HgNO_3$.[43] $CH_3{}^{198}HgNO_3$ was prepared by the methylcobalamin method.[44] $^{202}Hg(NO_3)_2$ (ISOFLEX, San Francisco, CA, U.S.A.) solution was prepared before incubation. The addition of Hg isotopes increased THg and MeHg in soils by 10% and 100% of the ambient concentrations, respectively.[21]

Incubation experiments were conducted in an anaerobic chamber (25 °C, 90% Ar + 10% H_2), including sample preparation, Hg isotope tracers spiking, inhibitor/stimulant additions, and sub sampling. The independently collected three surface/rhizosphere soil samples and corresponding overlying water samples were combined to prepare the slurry (water contents were 50−60%) in a 2-L beaker for each of the sampling sites. Plant roots and pebbles were removed before

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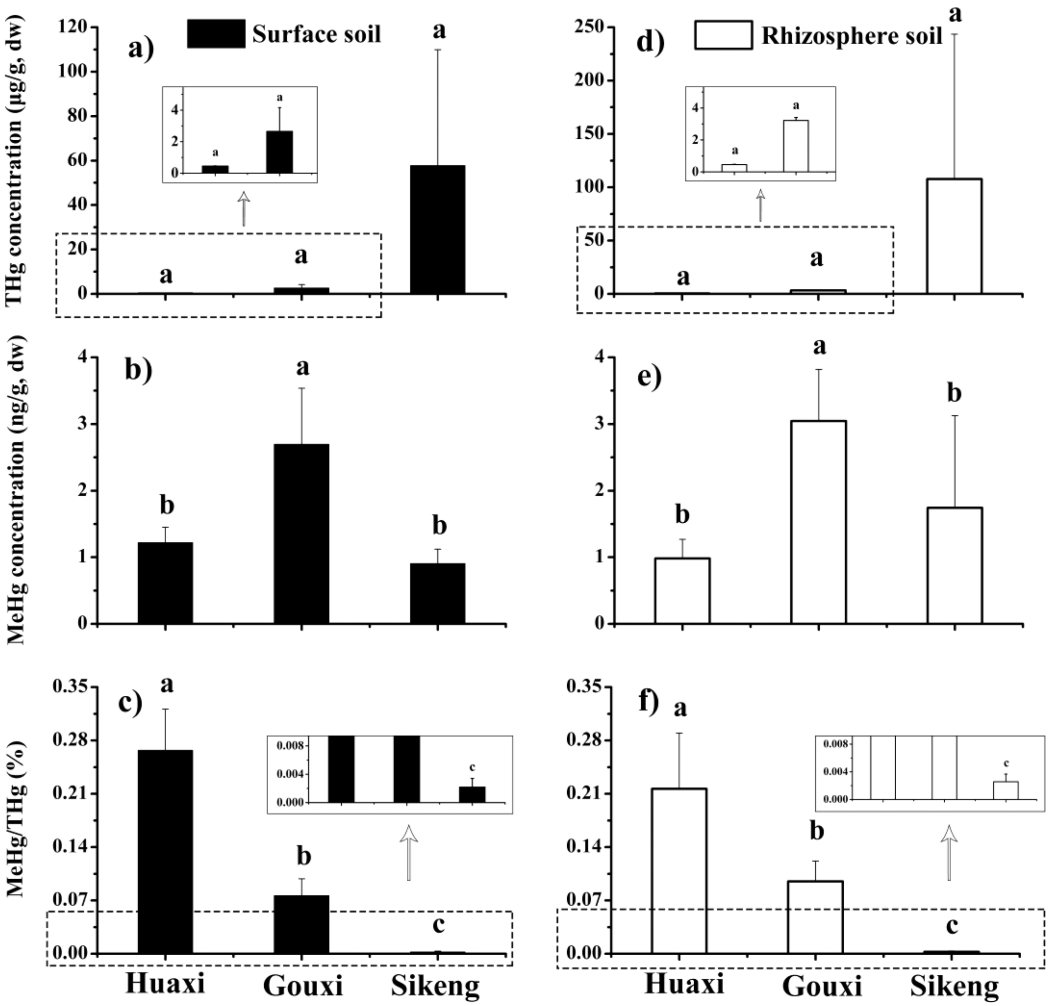

Figure 2. THg and MeHg concentrations and ratio of MeHg/THg in surface soils and rhizosphere soils of rice paddies at the different sites: Huaxi (a control site), Gouxi (the artisanal Hg mining site), and Sikeng (the abandoned Hg mining site); THg concentration in surface soils (a) and rhizosphere soils (d); MeHg concentration in surface soils (b) and rhizosphere soils (e); the ratio of MeHg in THg in surface soils (c) and rhizosphere soils (f). Data shown is based on three replicate samples; the error bar represents the standard deviation of the triplicate samples (n = 3). Markings "a", "b", and "c" above the bars indicate a significant difference (Tukey post-hoc test, $p < 0.05$).

homogenization. Approximately 15 mL of slurries were dispensed into 100 mL serum bottles with three replicate samples for each treatment (triplicate reactions), followed by the addition of inhibitors or stimulants and methylation/demethylation tracers (7 treatments, Table 1). The serum bottles were sealed with butyl rubber septa and aluminum crimp caps. After incubations for 24 h in the dark,[21,28,45] samples were frozen at −20 °C immediately and then freeze-dried for isotopic Hg species analysis.

Sample Preparation and Analysis of THg and MeHg. Paddy soil samples were freeze-dried and then homogenized with a mortar and sifted through a 200-mesh sieve. The THg concentration of soil samples were determined by cold vapor atomic fluorescence spectrometry (CVAFS, Tekran 2500, Tekran Instruments). For this analysis, ∼0.2 g of soil samples (accurate to 0.0001g) were weighed and put into a clean colorimetric tube. Then 5 mL of DDW and 5 mL of freshly prepared aqua regia were added to the mix. Samples were heated in a water bath at 95 °C for 5 min and digested for an additional 30 min after addition of 1 mL of bromine chloride. A 25 mL portion of DDW was added to the samples. After 24 h, the supernatant was taken and THg was analyzed by CVAFS. MeHg concentrations in samples were digested following the established methodology:[46] ∼0.1 g of dry soil samples were digested with 7.5 mL nitric acid (25%, v/v) and 1.5 mL of saturated $CuSO_4$. After extraction and reverse extraction, MeHg concentrations of samples were determined by CVAFS (Brooks Rand Model III, Brooks Rand Laboratories, U.S.A.).

Calculation of Specific Methylation and Demethylation Rate Constants. The concentrations of MeHg isotopes were measured using a gas chromatography inductively coupled plasma mass spectrometry (GC−ICP−MS, Agilent 7700x, Agilent Technologies, Inc.) system following the ethylation−purge−trap method,[47,48] including $CH_3{}^{198}Hg^+$ (demethylation tracer), $CH_3{}^{199}Hg^+$ (ambient), $CH_3{}^{200}Hg^+$ (internal standard), $CH_3{}^{201}Hg^+$ (ambient), and $CH_3{}^{202}Hg^+$ (methylation tracer). The methylation rate constants (k_m) and the demethylation rate constants (k_d) were calculated from the increase of $CH_3{}^{202}Hg^+$ and the decrease of $CH_3{}^{198}Hg^+$ through eqs 1 and 2:

$$k_m = \frac{[Me^{202}Hg]_{t2} - [Me^{202}Hg]_{t0}}{[^{202}Hg^{2+}] \times t} \quad (1)$$

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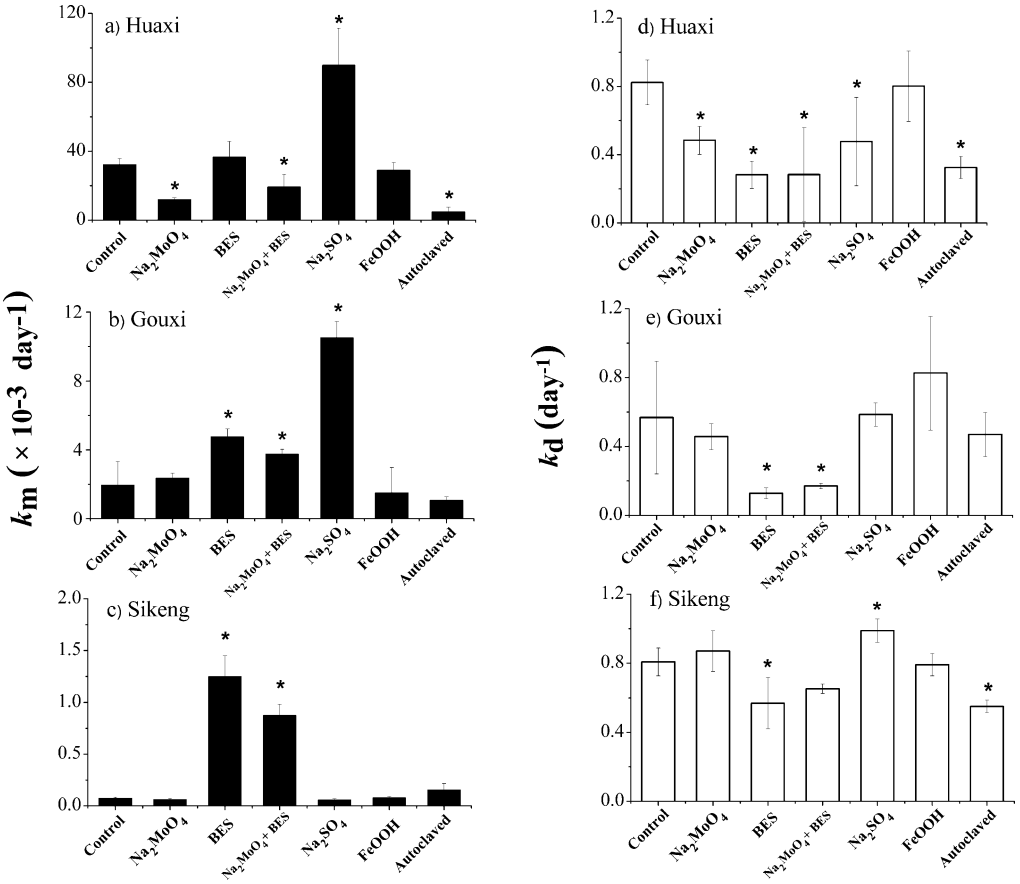

Figure 3. Potential methylation/demethylation rate constants ($k_m \pm$ STD, $k_d \pm$ STD, $n = 3$) in surface soils for the rice paddies under different treatments with inhibitors/stimulants. Huaxi (a and d, the control site); Gouxi (b and e, the artisanal Hg mining site); Sikeng (c and f, the abandoned Hg mining site). An asterisk denotes significant difference from the control treatment (Dunnett's test, $p < 0.05$); Data shown is based on three replicate samples for each of the treatments; the error bar represents the standard deviation of the triplicate reactions ($n = 3$).

$$k_d = \frac{\ln([Me^{198}Hg]_{t0}) - \ln([Me^{198}Hg]_{t2})}{t} \quad (2)$$

where $[Me^{202}Hg]_{t0}$ and $[Me^{198}Hg]_{t0}$ represent $Me^{202}Hg$ and $Me^{198}Hg$ concentrations at 0 h (initial concentration); $[Me^{202}Hg]_{t2}$ and $[Me^{198}Hg]_{t2}$ represent $Me^{202}Hg$ and $Me^{198}Hg$ concentrations in soils after incubation for 24 h; $[^{202}Hg^{2+}]$ was the $^{202}Hg^{2+}$ concentration in the spike added to the soils; and $[t]$ was the incubation time after spiking with isotopes.

QA/QC. Quality control and assurance measurements for all analytes were performed using method blanks, triplicates, and certified reference material. GBW07405 (GSS-5) and ERM-CC580, obtained from National Standard Material Center, were used as the standard materials for THg and MeHg analyses. Recovery rates of reference materials ranged from 75% to 114% and 116% to 118% for MeHg and THg in soil samples, respectively. Quantitative determination of MeHg isotopes was based on standard calibration curves, $r^2 \geq 0.99$. The method detection limit (3σ) for MeHg isotope analysis was 0.013 ng·L^{-1}. The variability between the triplicate samples was less than 8.8% and 0.73% for MeHg and THg for soil samples. Quality analysis was performed with SPSS 22.0 software. A one-way ANOVA (Dunnett's test, $p < 0.05$) was used to test for significant differences between controls and other treatments. The significant differences of THg and MeHg concentrations between different sampling sites were determined using a Tukey post-hoc test ($p < 0.05$).

RESULTS AND DISCUSSION

THg and MeHg Concentrations Across a Hg Contamination Gradient. We studied three sites corresponding to different geographical areas, over a Hg contamination gradient associated with Hg mining activities: Huaxi (control site), Gouxi (an artisanal Hg mining site), and Sikeng (an abandoned Hg mining site). Concentrations of THg and MeHg ([THg] and [MeHg]) and the ratios of MeHg to THg (MeHg/THg) in both the surface soil layer (0−2 cm) and the rhizosphere layer (10−15 cm) of rice paddy soils are shown in Figure 2.

THg concentrations increased over the contamination gradient, from Huaxi to Gouxi and the Sikeng (Figure 2a,d). [THg] were 0.46 ± 0.01, 2.7 ± 1.5, and 57 ± 52 $\mu g \cdot g^{-1}$ in the surface soil layer and 0.46 ± 0.02, 3.2 ± 0.18, and 1.1 × 10^2 ± 1.4 × 10^2 $\mu g \cdot g^{-1}$ in the corresponding rhizosphere soil, at Huaxi, Gouxi, and Sikeng, respectively (Figure 2a,d). There was no statistically significant difference of THg concentration between the three sites ($p > 0.05$) due to the large variablility of concentrations at Sikeng. In line with previous studies, THg concentrations in paddy soil from the Hg mining area, Gouxi and Sikeng, were high, due to the long-term Hg smelting activities.[12] Although the human Hg smelting activities recently decreased at Gouxi, the impact has been sustained for many

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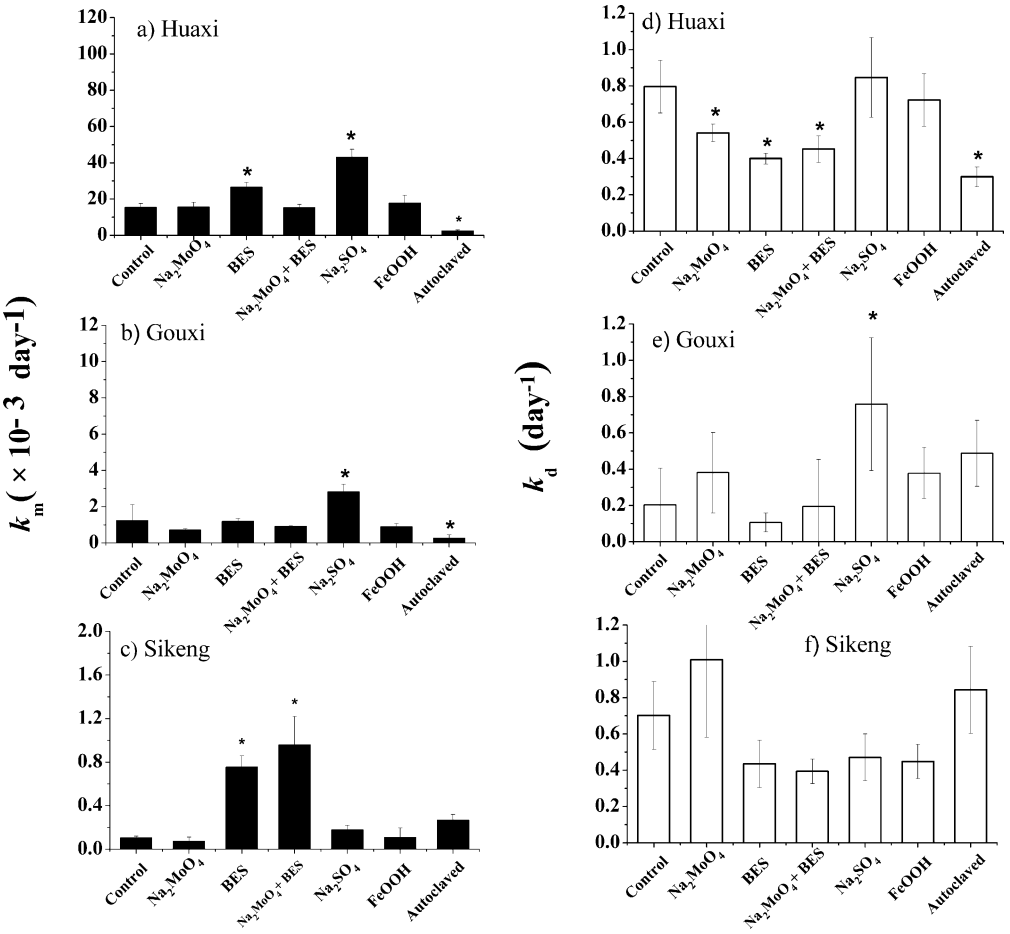

Figure 4. Potential methylation/demethylation rate constants (k_m ± STD, k_d ± STD, n = 3) in rhizosphere soils for the rice paddies treated with the inhibitors/stimulants: Huaxi (a and d, the control site); Gouxi (b and e, the artisanal Hg mining site); and Sikeng (c and f, the abandoned Hg mining site). An asterisk denotes significant difference from the control treatment (Dunnett's test, $p < 0.05$). Data shown is based on three replicate samples for each of the treatments; and the error bar represents the standard deviation of the triplicate reactions (n = 3).

years. Affected by slag, THg concentrations at Sikeng were strikingly high and consistent with, or higher than, another previously studied abandoned Hg mining site, namely Wukeng.[12]

MeHg concentrations did not linearly increase with the contamination gradient (Figure 2b,e). [MeHg] were 1.2 ± 0.23, 2.7 ± 0.84, and 0.9 ± 0.22 ng·g^{-1} in surface soils and 0.98 ± 0.29, 3.0 ± 0.78, and 1.7 ± 1.4 ng·g^{-1}, in corresponding rhizosphere soils at Huaxi, Gouxi, and Sikeng, respectively (Figure 2b,e). In fact, although THg concentration in paddy soil at Gouxi was much lower than that of Sikeng (Figure 2a,d), MeHg concentrations were significantly higher (p < 0.05, Figure 2b,e). The higher [MeHg] at Gouxi than at Sikeng reflects (i) greater methylation at Gouxi than at Sikeng, or (ii) a greater demethylation at Sikeng than at Gouxi. It is possible that Gouxi exhibited greater methylation than at Sikeng because of the elevated Hg^0 concentration in the atmosphere. Elevated Hg^0 at Gouxi likely provided a continuous source of newly deposited and bioavailable Hg at this site, stimulating MeHg production.[21] Hg methylation is directly associated with microbial activity and high Hg concentrations have previously been negatively associated with bacterial abundance.[49] Therefore, it is possible that the very high Hg concentrations at Sikeng were toxic to the microbial community, limiting Hg methylation. Finally, previous studies have shown a general decrease in [MeHg] over an increasing Hg contamination gradient.[50] Such patterns have been attributed to greater reductive demethylation at high [THg].[50] Therefore, our data possibly reflect greater methylation at Gouxi due to increased bioavailable Hg associated with enhanced Hg deposition at this site and greater demethylation at Sikeng.

Microbial Hg^{II} Methylation across a Hg Contamination Gradient in Paddy Soils. In a subsequent series of experiments using metabolic inhibitors and stable isotope tracers, we aimed at determining the relative importance of methylation vs demethylation on [MeHg] at the three sites.

Although variations within sites were observed based on individual treatments, overall the data showed a marked decrease in methylation rates (k_m) over the contamination gradient (10×10^{-3} day^{-1} < k_m < 80×10^{-3} day^{-1} at Huaxi; 2×10^{-3} day^{-1} < k_m < 10×10^{-3} day^{-1} at Gouxi; and n/d < k_m < 1×10^{-3} day^{-1} at Sikeng, Figures 3 and 4, p < 0.05). This decrease in methylation rates over the Hg gradient may reflect (i) a toxic effect of increasing Hg concentrations on methylating microbes[49,51] or (ii) a limitation in the abundance of substrates required for methylation, such as nutrients or bioavailable Hg. The effects of autoclaving on methylation rates varied for the different sites (Figures 3 and 4). k_m in autoclaved treatments decreased by 45%∼78% in the surface (Figure 3) and rhizosphere soils (Figure 4) of Huaxi and Gouxi. However, k_m increased by 100% and 150% (p < 0.05) after autoclaving when comparing control and Sikeng surface

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soil and rhizosphere soil, respectively. The variable effects of autoclaving on methylation rates have previously been reported and could be associated with changes in soil physicochemical properties due to the high temperature and high pressure during autoclaving process.[42]

In surface soil samples from the control site (Huaxi), Hg methylation (k_m) was significantly inhibited by the presence of molybdate and enhanced by the addition of sulfate ($p < 0.05$, Figure 3a). In rhizosphere soil samples, molybdate had no significant effect on k_m, but sulfate addition increased k_m by 179% ($p < 0.05$, Figure 4a). These data suggest that sulfate-reduction dominated Hg methylation at Huaxi. The addition of molybdate had no effect on Hg methylation at the mining sites, whether it was at Gouxi or Sikeng. Typically, increased sulfate-reduction rates have been associated with an increase in MeHg production in lacustrine sediments.[34,52] Previous work showed that sulfate concentrations of pore water in paddy soil at the Hg mining sites (Gouxi and Wukeng) were 100−200 μM,[21] values higher than that typically recognized as an optimal SO_4^{2-} level for Hg methylation.[52] Surprisingly, the addition of sulfate increased Hg methylation significantly at Gouxi ($p < 0.05$, Figures 3b and Figure 4b) but not at Sikeng ($p > 0.05$, Figures 3c and Figure 4c). This suggests that sulfate might be a limiting factor for sulfate-reduction mediated Hg methylation at Gouxi site but not at Sikeng site. The lack of a response of Hg methylation to SO_4^{2-} addition at Sikeng suggests either that (i) Hg methylating sulfate reducers are not abundant at Sikeng or (ii) that the activity of methylating microbes is hampered by the high Hg levels (i.e., 264 mg·kg^{-1} at Sikeng in this study). Note that previous work showed that the abundance of one of the genes involved in Hg methylation gene, *hgc*A, associated with sulfate-reducing bacteria, was low in paddy soil at high Hg levels,[30] further suggesting a sensitivity of Hg methylating sulfate reducers to very high Hg levels.

Inhibiting methanogenesis using BES significantly promoted Hg methylation by 2.4- and 16.6-fold compared to the control in surface ($p < 0.05$, Figure 3) soil samples at Gouxi and Sikeng, and 1.7- and 7.2-fold compared to the control in rhizosphere soil samples at Huaxi and Sikeng, respectively. Interestingly, the stimulations were proportionally increased with the contamination gradient. A similar trend was observed for the rhizosphere soils, although less pronounced. These results suggest that there was strong competition, e.g., for substrates, between methanogenesis and other Hg methylating groups in paddy soils. Our data shows that methanogenesis was occurring at all sites and was hampered when BES was added or in autoclaved samples (Figure S2). This suggests that methanogenesis limited Hg methylation as Hg contamination increased. Compeau et al.[39] showed that the addition of BES increased Hg methylation in anoxic estuarine sediment, because sulfate reducers and methanogens might compete for carbon sources and electron donors in sediment.[53] The sulfate amendment treatment suggests that sulfate-reduction may be limiting Hg methylation at Gouxi, but not at Sikeng. FeOOH addition had no significant effects on k_m either for surface soil or rhizosphere soil samples at all studied sites ($p > 0.05$). This suggests that iron provided as FeOOH was not available, or not limiting, or that iron reducers are not important contributors to Hg methylation at these sites. The nature of the microbes that compete with methanogens for resources, (e.g., H_2, acetate), and involved in Hg methylation at high Hg concentration (Sikeng) remains unknown.

The k_m of control groups was 32 ± 3.3 × 10^{-3}, 2.0 ± 1.37 × 10^{-3}, and 0.075 ± 0.0072 × 10^{-3} day^{-1} in surface soil and 15 ± 2.2 × 10^{-3}, 1.2 ± 0.87 × 10^{-3}, and 0.11 ± 0.014 × 10^{-3} day^{-1} in rhizosphere soil at Huaxi, Gouxi, and Sikeng, respectively. Methylation in the surface soils was slightly greater than that in corresponding rhizosphere soil samples at Huaxi and Gouxi. Additionally, the rhizosphere soil samples were less responsive to specific inhibitors/stimulants than surface soil (Figures 3 and 4). These results suggest that conditions in surface soils are more favorable to Hg methylation than in rhizosphere soils, likely associated with more active microbes and suitable physicochemical conditions. We noted that the k_m in surface soil at Sikeng was lower than corresponding rhizosphere soil, however.

Microbial MeHg Degradation across a Hg Gradient in Paddy Soils. MeHg degradation can be an important sink for MeHg in environmental samples.[41] MeHg degradation significantly decreased in autoclaved treatments ($p < 0.05$) for both surface (inhibited 60%) and rhizosphere (inhibited 60%) soil samples at Huaxi (Figures 3d and 4d), suggesting that microorganisms play an important role in the degradation of MeHg at the control site. Demethylation was not significantly affected by autoclaving at the Hg mining sites, (Gouxi and Sikeng, $p > 0.05$, Figures 3e and Figure 4e,f), except for the surface soil sample at Sikeng (32% decrease in k_d upon autoclaving), suggesting a role for abiotic demethylation that we discuss below.

Contrary to what was observed for methylation, MeHg degradation rates (as k_d) were more variable and were not affected by the Hg contamination gradient (Figures 3 and 4), with k_d values ranging from 0.2 to 1.2 day^{-1} at all three sites. However, for both surface and rhizosphere soil samples, Gouxi site (0.57 ± 0.33 and 0.21 ± 0.2 day^{-1}) exhibited lower demethylation rates than Huaxi (0.82 ± 0.13 and 0.80 ± 0.14 day^{-1}) and Sikeng (0.81 ± 0.08 and 0.70 ± 0.19 day^{-1}), possibly explaining the significantly higher [MeHg] at Gouxi ($p < 0.05$, Figure 2b,e).

MeHg degradation can occur via oxidative or reductive demethylation. Oxidative MeHg degradation is an anaerobic process that is poorly understood and for which the mechanisms remain unclear. Inhibiting anaerobic metabolisms such as methanogenesis, and to a lesser extent sulfate-reduction (using BES and molybdate, respectively), hampered MeHg degradation at all three sites, especially in surface paddy soil samples (Figure 3). At the control site (Huaxi), both molybdate and BES significantly inhibited MeHg degradation (40% and 70% at the surface and 30% and 50% in the rhizosphere, respectively, $p < 0.05$), whereas only the addition of BES inhibited MeHg degradation at Gouxi and Sikeng (77% and 29%, at the surface and 47% and 38% in the rhizosphere, respectively). MeHg degradation by methanogenesis has previously been reported in peat soil and fresh sediment,[36,41,54] but this study is the first to report on its potential importance in rice paddy soils and across a Hg contamination gradient. Our data do not support the "MeHg accumulation paradox" observed elsewhere, and for which [MeHg] is inversely correlated to [THg] because of a greater role of reductive demethylation (coded by the *mer* operon) as [THg] increases.[50] It is possible, although we did not test for it, that the absolute contribution of reductive demethylation, involving the *mer* operon, becomes more important as Hg concentrations increased. Indeed, microbial communities in Hg-impacted environment have been shown to adaptat to Hg,

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via the presence of *mer* genes and mRNA transcripts, indicating that reductive demethylation is occurring at mining sites.[50,54]

The effects of sulfate addition on MeHg degradation were variable and hence hard to interpret, but the results may point to a role for abiotic demethylation via the production of sulfides.[55−57] Indeed, both biotic and abiotic processes can be involved in MeHg degradation, and recent studies have identified sulfides as possibly being involved in MeHg degradation leading to dimethylmercury production.[55] We did not measure dimethylmercury production in our samples but the fact that we observed demethylation despite an autoclaving step supports an abiotic pathway that deserves further exploration. Although this study did not specifically test for the mechanisms involved in abiotic MeHg degradation, it is possible that abiotic demethylation at Hg mining sites could also be caused by the presence of high levels of Se (36.6[58]). Indeed, the Wanshan Hg mine is a Se-rich area,[58,59] and MeHg degradation can be mediated by selenoamino acids via a bis(methylmercuric)selenide intermediate.[60] Further investigation into the role of Se on MeHg cycling in a mining area is warranted.

Environmental Implications. In this study we investigated the microbial controls on Hg methylation and MeHg degradation in rice paddy soils over a gradient of Hg contamination. We show that sulfate-reduction contributes to MeHg formation and degradation at noncontaminated sites. In contrast, at Hg mining sites, methanogenesis exhibited a complex and important role in controlling MeHg cycling. Methanogenesis directly affected MeHg degradation via oxidative demethylation and indirectly affected MeHg production by out-competing other microbial guilds. We found that MeHg cycling was more active in the surface soil layer than in rhizosphere soil samples. We propose that management of methanogenesis at Hg mining sites may represent a lever which could be utilized to mitigate the production of MeHg and reduce the risk of human exposure to MeHg caused by rice consumption.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c00268.

Figure S1, potential methylation rate constants in surface soils for the rice paddies treated with the gradient inhibitors and Figure S2, methane production in surface soils treated with inhibitors/stimulants after incubation for 14 days (PDF)

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Notes

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