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Growing Rice Aerobically Markedly Decreases Mercury Accumulation by Reducing Both Hg Bioavailability and the Production of MeHg

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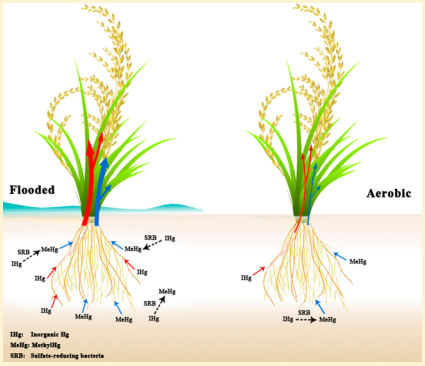
Supporting Information

ABSTRACT: Rice consumption represents a major route of mercury (Hg) and methylmercury (MeHg) exposure for those living in certain areas of inland China. In this study we investigated the effects of water management on bioavailable Hg, MeHg, and sulfate-reducing bacteria (SRB, abundance and community composition) in rhizosphere soil, and total Hg (THg) and MeHg in rice plants grown under glasshouse and paddy field conditions. Aerobic conditions greatly decreased the amount of THg and MeHg taken up by rice plants and affected their distribution in different plant tissues. There were positive correlations between bioavailable Hg and THg in brown rice and roots and between numbers of SRB and MeHg in brown rice, roots, and rhizosphere soil. Furthermore, the community composition of SRB was dramatically influenced by the water management regimes. Our results demonstrate that the greatly reduced bioavailability of Hg and production of MeHg are due to decreased SRB numbers and proportion of Hg methylators in the rhizosphere under aerobic conditions. These are the main reasons for the reduced Hg and MeHg accumulation in aerobically grown rice. Water management is indicated as an effective measure that can be used to reduce Hg and MeHg uptake by rice plants from Hg-contaminated paddy fields.



1. INTRODUCTION

Mercury (Hg) is a global and extremely toxic contaminant which has received considerable attention because of its accumulative and persistent nature. Methylmercury (MeHg), the most common form of organic Hg, is of greater concern due to its higher toxicity for humans and its ability to be more readily biomagnified in food chains.[1,2] As a result of the long history of Hg mining activities in some provinces of China (e.g., Guizhou), a large area of farmlands, including paddy fields, has been seriously contaminated by Hg.[3−5] Consumption of marine products such as fish and shellfish is usually considered as a major pathway for MeHg exposure to humans.[6] In recent years, however, rice (*Oryza sativa* L.) consumption has been recognized as another primary source of MeHg for humans in Hg mining areas and also in certain inland areas in Southwestern China.[7−9]

Rice can accumulate high levels of Hg and MeHg in its grains. Concentrations of MeHg and ratios of MeHg:total Hg (THg) in rice grains are usually much higher than in other local edible crop plants grown within the same mercury mining area.[3,8,10] Furthermore, it is notable that grains of rice accumulate the highest ratios of MeHg:Hg compared to other rice plant tissues (roots, leaves, and stems).[11,12] There is therefore an urgency to develop effective management practices to reduce THg and MeHg in rice grains and so ensure food safety for paddy fields contaminated by Hg.[5]

The Hg methylation process is primarily mediated by certain anaerobic microbes (e.g., sulfate-reducing bacteria (SRB), iron-reducing bacteria, and methanogens),[13−15] and SRB have been considered to play the most important role.[16,17] Studies have shown that SRB are abundant in paddy fields, because the flooded condition can favor the reoxidation process of reduced sulfur compounds.[18,19] Moreover, anaerobic conditions in paddy fields may increase Hg mobilization into the soil solution and favor the methylation of Hg by anaerobic microbes, leading to an enhancement of Hg and MeHg bioavailability to rice plants.[20,21] Soil redox potential (Eh) plays an important role in influencing THg/MeHg bioavailability. Controlling soil Eh through water management has been proven to effectively decrease the concentrations of arsenic and cadmium in rice.[22−24] It has also been reported that water-saving measures can reduce THg and MeHg concentrations in rice grain.[25] Peng et al.[26] observed that rice grown aerobically

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markedly reduced THg and MeHg concentrations as well the proportion of MeHg in the grain under glasshouse conditions, due to the decreased THg and MeHg concentrations in the soil solution. However, previous studies have mainly focused on the effect of water treatments on accumulation of Hg and MeHg in rice. The reasons why the aerobic treatment has such an effect and the possible factors involved in the process of Hg and MeHg uptake and accumulation in rice in different water treatments all remain unclear.

The bioavailability of Hg and MeHg in the rhizosphere has been recognized to be one of the most important factors affecting their accumulations in rice plants.[12,26] Changes in local conditions in the rhizosphere can impact on the bioavailability of Hg and MeHg[20,26] and the various microbially facilitated processes, including Hg methylation and demethylation that affect Hg speciation.[21] Importantly, as the dominant Hg methylators, SRB can show great variation in numbers due to changes in rhizosphere conditions.[27−29] However, the specific impacts of different water management regimes on the bioavailability of Hg and MeHg[25] and the SRB communities in the rhizosphere of rice plants grown in Hg-contaminated paddy fields are not fully understood.[26] The aims of the present study were therefore to investigate the impacts of different water management regimes under glasshouse and paddy field conditions on (1) the accumulation and distribution of THg and MeHg in rice plants, (2) the bioavailability of Hg, (3) the concentration of MeHg in soil, and (4) the SRB community composition in the rhizosphere, and their correlations with the aforementioned.

2. MATERIALS AND METHODS

2.1. Pot Experiment. A rhizobag pot experiment was performed in 2012 (Guangzhou, China) in a glasshouse at ambient temperatures (25−36 °C) under daylight conditions. Soil was collected from the plough layer (0−20 cm) of a paddy field which was located at Wanshan in Guizhou Province, the largest Hg producing area in Asia. Mining at Wanshan was initiated in 221 B.C. and lasted until 2001 when lower demand for Hg and increasing environmental concerns brought about mine closure.[30] The paddy soil studied contained 2.34% total C, 0.14% total N, 46 μg g^{-1} total Hg, and 3.7 ng g^{-1} MeHg and had a pH of 7.0. The concentration of THg in the paddy soil was much higher than the domestic environmental quality standard for an agricultural soil in China (0.5 μg g^{-1}) (GB 15618-2008). A previous study[4] has also shown that the THg concentrations in the soils collected from rice paddy fields in the Wanshan Hg mining area varied from 5.1 to 790 μg g^{-1}. Thus, the concentration of THg in the soil employed in this study was typical for paddy fields in the Wanshan Hg mining area. After air-drying, sieving to <8 mm, and homogenization, the soil was supplemented with basal fertilizers (125 mg N kg^{-1} soil as urea, 80 mg P kg^{-1} and 125 mg K kg^{-1} soil as KH_2PO_4 and K_2SO_4), mixed thoroughly, and equilibrated for 1 month. A compartmentalized rhizobag culture system was used in this experiment. A 0.8 kg amount of soil was placed in each rhizobag (made of 30 μm nylon mesh, 12 cm diam, 15 cm height). The rhizobag was then transferred to a lightproof PVC pot (20 cm diam, 18 cm height), and the gap between the bag and the PVC pot was filled with the same soil. Each pot contained 2 kg of the soil.

There were four water management regimes employed in this experiment: flooded (CF) or aerobic (CA) throughout the entire rice growth period, flooded followed by aerobic after heading (F-A), and aerobic followed by flooded after heading (A-F). Two rice cultivars, one *japonica,* cv. Zixiang (ZX) and one *indica,* cv. Nanfeng (NF), were selected for use, as they are widely grown in China. Disinfected seeds were germinated in moist sand and allowed to grow for 20 days, and then uniform seedlings were selected and transplanted into the rhizobags (two plants per bag) on April 2, 2012. The water management was changed at the beginning of the last ear emergence day (day 72) of each treatment. Deionized water was added daily to raise the water status to full saturation capacity with approximately 2.5 cm of standing water for flooded rice and to 70% of the soil water-holding capacity for aerobic rice. Soil Eh was measured at approximately 5 cm below the soil surface on days 2, 31, 50, 72, 99, and 122. Rice plants were harvested on August 5, 2012.

2.2. Paddy Field Experiment. A paddy field experiment was conducted in the same field where the soil was collected for the pot experiment. Due to the difficulties and inconvenience in the switch between flooding and aerobic conditions in the paddy field, only two water management regimes were applied: CF and CA treatments. In the CA treatment, the 3 m × 3 m plots were piled up to be 10 cm above the normal surface using soil collected from the same paddy field. The plots were then boarded with surround planks to protect from any rain-wash. No changes were made in the CF treatment, with the soil surface at the normal height. When the paddy field was irrigated, the water was maintained ∼5 cm above the normal surface. Therefore, soils in the CF treatment were totally submerged throughout the entire rice growth season, whereas the surface soil in the CA treatment maintained aerobic conditions over the same period. No specific water irrigation was required in the plots with the CA treatment. There were four replicate plots for each treatment, with a 1 m wide buffer strip between each plots. All plots were randomized in the paddy field. The germination of seeds, transplantation of rice plants (June 6, 2012), and soil fertilization were all similar to those used in the pot experiment, and rhizobags of the same size were also employed. Plants were harvested on October 6, 2012.

2.3. Sampling. After the grain had matured, the plants grown in the glasshouse and the paddy field were harvested and separated into roots, straw, husk, and brown rice. All the plant samples were freeze-dried, milled, ground to a fine powder, and stored at 4 °C for further analysis. The soil within the rhizobags was regarded as the rhizosphere soil; it was collected at about 5 cm below the surface. The soil samples collected from the paddy field were sealed, double-bagged, and stored in an ice-cooled container before being shipped to the laboratory within 24 h. All rhizosphere soil samples were then divided into two subsamples, one for chemical analysis and the other for microbial analysis. The subsamples for chemical analysis were freeze-dried at −50 °C, crushed, and sieved to 150 mesh for THg and MeHg analysis.[12] The subsamples for microbial analysis were freeze-dried (−50 °C) and stored at −80 °C for subsequent DNA extraction and molecular analysis.

2.4. THg and MeHg Analysis. For THg analysis, the soil and plant samples were crushed and microwave-digested in concentrated HNO_3 (16 mol L^{-1}), and Hg in the digests was measured by atomic fluorescence spectrometry (AFS, Beijing Titan Instrument Co., Ltd.). For MeHg analysis, plant samples were prepared by KOH−methanol/solvent extraction. Soil samples were prepared using a KBr−$CuSO_4$/solvent extraction. For both, MeHg was determined with a MERX Automatic

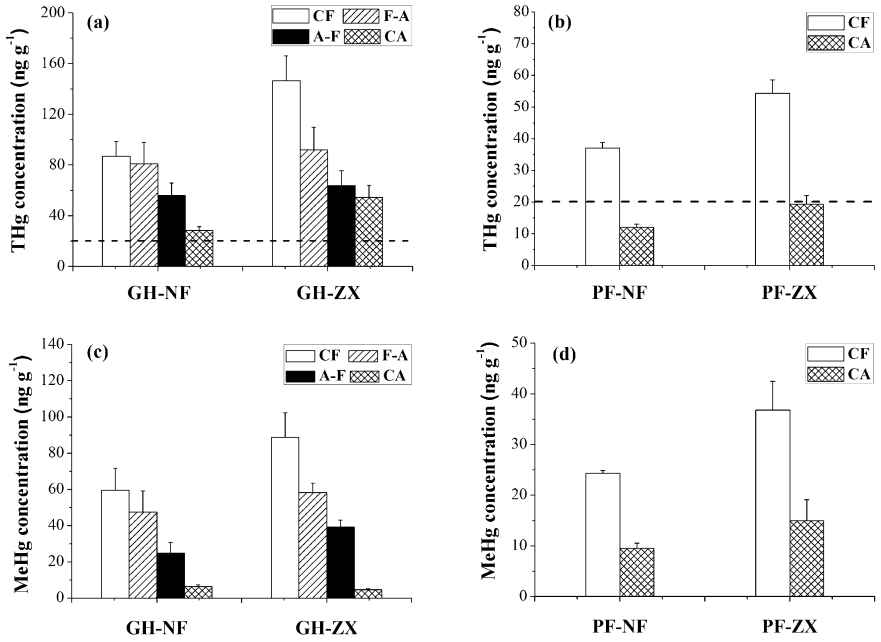

Figure 1. Concentrations of THg (a, b) and MeHg (c, d) in brown rice of two rice cultivars (NF: Nanfeng; ZX: Zixiang) grown under glasshouse (GH) and paddy field (PF) conditions. CF = continuously flooded, F-A = flooded−aerobic, A-F = aerobic−flooded, CA = continuously aerobic. Data are means plus SE (n = 4). The dashed line in a and b refers to the National Guidance Limit for crops recommended by the Chinese National Standard Agency.

Methylmercury System (Brooks Rand Laboratories, Seattle, WA), following method 1630.[31] For Hg bioavailability analysis, a sequential extraction technique for soil and sediment[32] was employed. Within the five fractions, only two (water-soluble and "human stomach acid"-soluble) represented the "bioavailable inorganic Hg".[33] The detection limits of this method were 0.001 mg L^{-1} for THg and Hg bioavailability, and 0.002 ng L^{-1} for MeHg in the samples. Information on quality assurance/quality control (QA/QC) for our analytical data is available in the Supporting Information.

2.5. Microbial Community DNA Extraction, qPCR, and Construction of dsrAB Gene Libraries and Sequence Analyses. Microbial community DNA was extracted from 0.25 g of rhizosphere soil using a "Powersoil" DNA extraction kit (MO BIO Laboratory, Carlsbad, CA). The extracted DNA was evaluated on a 1% agarose gel, and the concentration and purity of the extracts were estimated by spectrophotometry (NanoDrop, Wilmington, DE).

The final stage of the anaerobic sulfate reduction (reduction of sulfite to sulfide) is catalyzed by dissimilatory (bi)sulfite reductase, encoded by the *dsrAB* gene.[34] This gene is found in all known sulfate reducers and is therefore a key functional marker for molecular analysis and detection of SRB.[35] Furthermore, the *dsrAB* gene has been applied to characterize the abundance and composition of SRB communities involved in Hg methylation.[36] In our study, abundances of SRB were determined by quantitative real-time PCR analysis of *dsrAB* gene β-subunit (350 bp) using the primer set DSRp2060F (5′-CAACATCGTYCAYACCCAGGG-3′) and DSR4R (5′-GTGTAGCAGTTACCGCA-3′) described by Geets et al.[37] Additionally, clone libraries were constructed using *dsrAB*-PCR products amplified by the specific primer set DSR1F (5′-AC[C/G]CACTGGAAGCACG-3′) and DSR4R (5′-GTGTAGCA GTTACCGCA-3′) as previously described.[35] Positive clones were randomly selected from each library and the full-length inserts (1.9 kb) were sequenced on an ABI 3730*xl* DNA Sequencer (Applied Biosystems). The neighbor-joining tree was constructed based on the amino acid sequences deduced from the clone sequences and their closest relatives in the GeneBank database. Additional details on qPCR, clone library construction, and sequence analysis for *dsrAB* gene are provided in the Supporting Information.

2.6. Statistical Analyses. Data were analyzed using the SPSS 17.0 statistical software package and summarized by means ± standard errors (SE). Treatment means were compared by the least significant difference (LSD) at the 5% level. Coefficients of determination (R^2) and significance probabilities (P) were computed for linear regression fits.

3. RESULTS

3.1. Total Hg and MeHg in Plants. Flooding markedly decreased soil redox potential (Eh) compared with the aerobic treatment, whereas draining water at the ear emergence stage (day 72) led to a rapid increase in Eh (Figure S1, Supporting Information). Yields of brown rice showed no significant difference between the continuous flooding (CF) and the continuous aerobic (CA) treatments under paddy field conditions (Table S1, Supporting Information).

The concentration of total plant Hg followed the order: roots > straw > husk > brown rice, whereas MeHg followed the order: brown rice > roots > straw > husk. Whether under glasshouse or paddy field conditions, different water treatments produced a dramatic effect on THg and MeHg accumulation in rice plants (Figure 1 and Table S2, Supporting Information). Plants accumulated the highest THg and MeHg concentrations in the CF treatment and the lowest in the CA treatment. Overall, by comparison with the CF treatment, the CA treatment decreased THg concentrations by 65%, 66% and MeHg concentrations by 92%, 60% for brown rice under the glasshouse and paddy field conditions, respectively. Furthermore, it should be noted that in the CA treatment, the THg concentration in brown rice grown in the paddy field was below

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the National Guidance Limit for crops (<20 ng g^{-1}) as recommended by the Chinese National Standard Agency (Figure 1b). Under glasshouse conditions, THg and MeHg concentrations in brown rice in the aerobic-flooded (A-F) treatment were 46% and 57% lower than those in the CF treatment. However, the decreasing trend in THg and MeHg concentrations was not as obvious in the flooded-aerobic (F-A) treatment. Furthermore, cv. Zixiang (ZX) accumulated significantly higher concentrations of THg and MeHg in brown rice than cv. Nanfeng (NF) ($P < 0.05$) (Figure 1).

The distribution of MeHg content (calculated as biomass multiplied by MeHg concentrations in specific tissues) in rice plants was influenced by the water treatments (Figure 2).

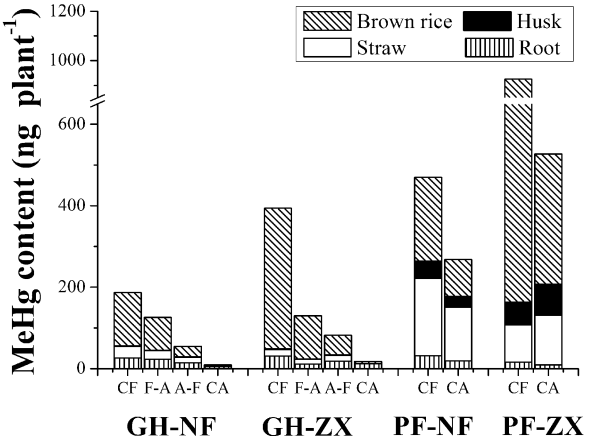

Figure 2. MeHg content in tissues of two rice cultivars (NF: Nanfeng; ZX: Zixiang) grown under glasshouse (GH) and paddy field (PF) conditions. CF = continuously flooded, F-A = flooded−aerobic, A-F = aerobic−flooded, CA = continuously aerobic.

MeHg content distribution ratios of brown rice in the CA treatment were 2.7- and 1.3-fold lower than those in the CF treatment, under the glasshouse and paddy field conditions, respectively.

3.2. Hg, MeHg Concentration and Hg Bioavailability in Rhizosphere Soil. The THg concentration was 46 ± 2.4 μg g^{-1} in rhizosphere soil and showed no significant difference between the water treatments either under glasshouse or paddy field conditions (data not shown). However, the MeHg concentrations were greatly affected by the water treatments (Table 1). By comparison with CF, the CA treatment decreased MeHg concentrations in rhizosphere soil by 38% and 44% under both glasshouse and paddy field conditions, respectively.

The "bioavailable inorganic Hg" was defined as the sum of water-soluble Hg and "human stomach acid" soluble Hg:[33] these two fractions accounted for 0.02−0.18% of the THg content of the soil. The CA treatment greatly decreased the "bioavailable inorganic Hg" concentration by 45−56%, compared to the CF treatment (Table S3, Supporting Information). Furthermore, there were significant positive correlations between the "bioavailable inorganic Hg" concentrations and the THg concentrations in brown rice ($R^2 = 0.38$, $P < 0.05$) and root ($R^2 = 0.83$, $P < 0.01$) tissues of rice plants (Figure 3a,b).

3.3. Abundance of Sulfate-Reducing Bacteria in the Paddy Soils. Significant differences ($P < 0.05$) in SRB abundance were detected between the water treatments (Table 2). Under glasshouse conditions, the *dsrAB* gene copy number

Table 1. MeHg Concentrations in Rhizosphere Soils of Two Rice Cultivars (NF: Nanfeng; ZX: Zixiang) Grown under Glasshouse (GH) and Paddy Field (PF) Conditions[a]

	MeHg concentration (ng g^{-1})			
water treatment	GH-NF	GH-ZX	PF-NF	PF-ZX
CF	3.7 ± 0.24 a	6.9 ± 1.6 a	7.0 ± 1.7 a	9.7 ± 3.5 a
F-A	2.9 ± 0.54 b	6.2 ± 1.9 a	–	–
A-F	3.6 ± 1.4 a	6.0 ± 1.4 a	–	–
CA	2.4 ± 1.1 b	4.1 ± 0.85 b	4.1 ± 0.98 b	5.2 ± 1.6 b

[a]Data are means ± SE ($n = 4$). Different letters within the same column indicate significant difference between the water treatments at the level of $P < 0.05$. CF = continuously flooding, F-A = flooding−aerobic, A-F = aerobic−flooding, CA = continuously aerobic.

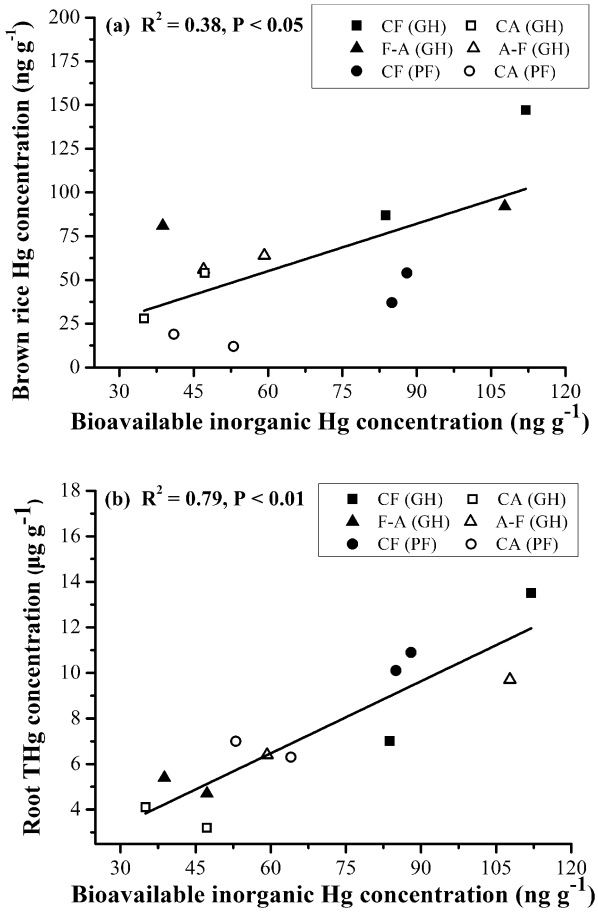

Figure 3. Relationship between bioavailable inorganic Hg concentrations in soils and THg concentrations in brown rice (a) and root (b) tissues of rice plant grown under glasshouse (GH) and paddy field (PF) conditions. CF = continuously flooded, F-A = flooded−aerobic, A-F = aerobic−flooded, CA = continuously aerobic.

in the CA treatment was 3.6-fold lower than that in the CF treatment, whereas under paddy field conditions it was 3.0-fold lower. Moreover, there were significant positive correlations between *dsrAB* gene copy numbers and MeHg concentrations in brown rice ($R^2 = 0.62$, $P < 0.01$), root tissues ($R^2 = 0.31$, $P < 0.05$), and rhizosphere soil ($R^2 = 0.41$, $P < 0.05$) of rice plants (Figure 4a−c).

3.4. Phylogenetic Analysis of the *dsrAB* Sequences. Full-length *dsrAB* sequence clone libraries were established from four different treatments: the CF and CA treatments

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Table 2. *dsrAB* Gene Copy Numbers in Rhizosphere Soils of Two Rice Cultivars (NF: Nanfeng; ZX: Zixiang) Grown under Glasshouse (GH) and Paddy Field (PF) Conditions[a]

water treatment	*dsrAB* gene copy number (copies g^{-1} dry soil)			
	GH-NF	GH-ZX	PF-NF	PF-ZX
CF	$2.5 \times 10^8 \pm 5.4 \times 10^7$ a	$3.5 \times 10^8 \pm 6.5 \times 10^7$ a	$2.0 \times 10^8 \pm 4.3 \times 10^7$ a	$2.6 \times 10^8 \pm 4.3 \times 10^7$ a
F-A	$7.6 \times 10^7 \pm 7.8 \times 10^6$ b	$2.6 \times 10^8 \pm 3.8 \times 10^7$ a	–	–
A-F	$7.8 \times 10^7 \pm 1.2 \times 10^7$ b	$1.2 \times 10^8 \pm 1.8 \times 10^7$ b	–	–
CA	$7.0 \times 10^7 \pm 8.1 \times 10^6$ b	$9.3 \times 10^7 \pm 3.9 \times 10^6$ b	$6.0 \times 10^7 \pm 7.8 \times 10^6$ b	$9.5 \times 10^7 \pm 1.2 \times 10^7$ b

[a]Data are means ± SE (n = 4). Different letters within the same column indicate significant difference between the water treatments at the level of $P < 0.05$. CF = continuously flooding, F-A = flooding–aerobic, A-F = aerobic–flooding, CA = continuously aerobic.

under both glasshouse (GHCF, GHCA) and paddy field (PFCF, PFCA) conditions. Each clone library was constructed with combined PCR products from the two cultivars. Prior to treeing, 63, 68, 53, and 54 sequences obtained from the four libraries were grouped (based on 97% amino acid identity threshold on the first 800 bp) into 13, 13, 16, and 14 operational taxonomic units (OTU), respectively. Calculation of coverage rates (Table 3) showed that sufficient clones were analyzed to cover the major part of the *dsrAB* gene diversity for these four libraries. Notably, the Shannon–Wiener and Simpson diversity indices showed that the SRB communities in the CA treatments (GHCA, PFCA) had a higher diversity than those in the CF treatments (GHCF, PFCF) (Table 3).

Based on the deduced *dsrAB* sequences, a phylogenetic consensus tree was constructed (Figure S2, Supporting Information). The SRB OTUs were grouped into nine groups according to their closest affiliations. Groups I–VI belonged to *Deltaproteobacteria*, the class to which most Hg-methylating sulfate reducing bacteria belong. Group VII was closely related to the sulfite reducing genera *Desulfosporosinus* and *Desulfitobacterium*, which belong to family Peptococcaceae. Sequences from the remaining two groups (groups VIII and IX) had very low similarities with known *dsrAB* sequences and formed separate, deeply branching *dsrAB* lineages in the phylogenetic tree.

Taxa from unidentified SRB and Desulfobacteraceae dominated the CF libraries, accounting for 70% and 89% of the SRB communities under glasshouse and paddy field conditions, respectively (Table 3). However, this trend changed in the CA treatments, with taxa from Syntrophaceae, Syntrophobacteraceae, and Peptococcaceae dominating the *dsrAB* libraries.

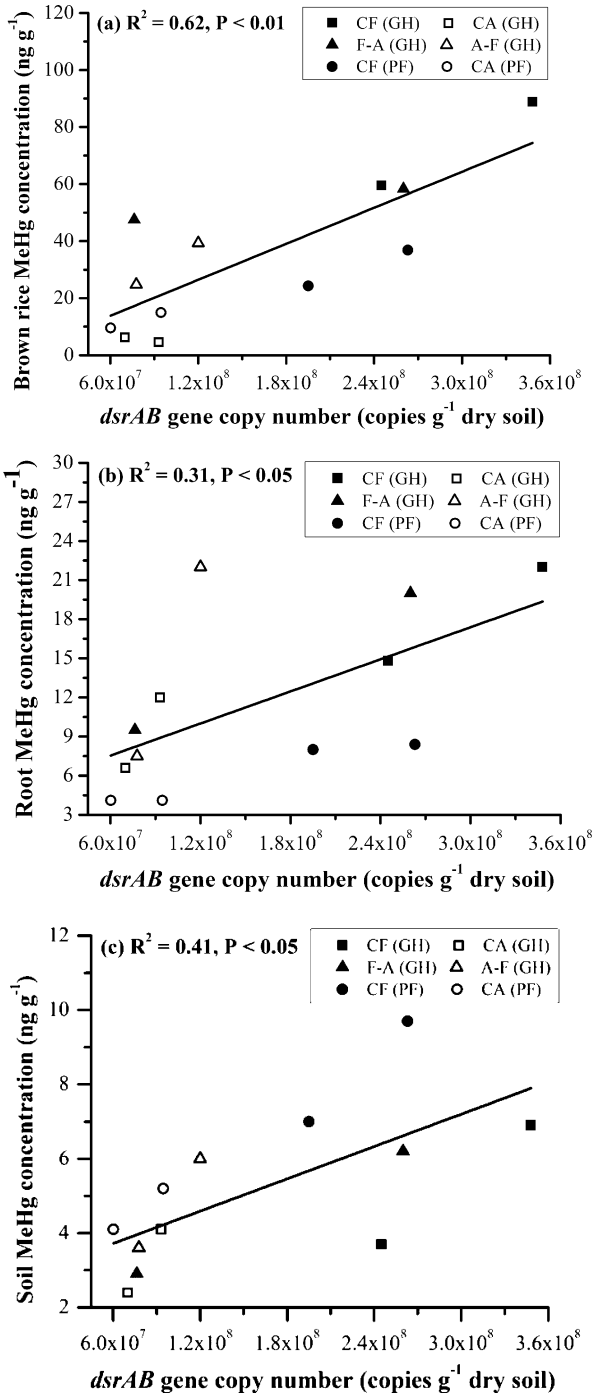

Figure 4. Relationships between *dsrAB* gene numbers and MeHg concentrations in brown rice (a), root (b) tissues, and rhizosphere soil (c) of rice plant grown under glasshouse (GH) and paddy field (PF) conditions. CF = continuously flooded, F-A = flooded–aerobic, A-F = aerobic–flooded, CA = continuously aerobic.

4. DISCUSSION

4.1. Water Management. The present results clearly indicate that both total Hg and MeHg bioaccumulations in rice tissues were greatly reduced in the aerobic treatment (Figure 1; Table S2, Supporting Information), whether under glasshouse or paddy field conditions. This observation is consistent with previous studies.[25,26] The significant differences of THg and MeHg concentrations between the two rice cultivars in the CF treatment (Figure 1) reflect variations in the capacity for accumulation. This may be related to several factors, such as genome, properties of translocation of Hg in plants,[38] and the Hg-binding efficiency by root surfaces.[39]

Under paddy field conditions, the growth of rice plants was not affected by the aerobic treatment (Table S1, Supporting Information), whereas THg concentrations in brown rice declined below the National Guidance Limit for crops (<20 ng g^{-1}), although the paddy soil is heavily contaminated with Hg (Figure 1). This result suggests that water management could help to obtain safe brown rice from Hg-contaminated paddy

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Table 3. Distribution and Diversity of the *dsrAB* Sequences in Four Clone Libraries from Rhizosphere Soils of Rice Plants under Glasshouse (GH) and Paddy Field (PF) Conditions[a]

	GHCF	GHCA	PFCF	PFCA
no. of OTUs	13	13	16	14
group distribution (% clones)				
Desulfobacteraceae	14.3	8.8	43.4	18.5
Desulfovibrionaceae	12.7	0	0	5.6
Desulfobulbaceae	0	7.4	0	0
Syntrophaceae	4.8	36.8	0	0
Syntrophobacteraceae	4.8	25.0	1.9	0
Peptococcaceae	7.9	22.0	9.4	33.3
unidentified SRB-1	55.5	0	37.7	27.8
unidentified SRB-2	0	0	7.6	14.8
diversity indices				
coverage (%)	100	100	98.1	100
Shannon–Wiener index	1.96	2.10	1.76	2.13
Simpson index	0.64	0.74	0.65	0.75

[a]Proportion of sequences is calculated as ratio of analyzed sequences affiliated with different groups to the total sequences number in clone library. CF = continuously flooded, CA = continuously aerobic.

fields, simultaneously guaranteeing the yields. Furthermore, THg and MeHg concentrations in brown rice under paddy field conditions were lower than those under glasshouse conditions. This may be due to the role of dilution, because rice plant biomass is higher under field conditions than under glasshouse conditions. For the latter, growing rice aerobically during either the vegetative or the reproductive stage also markedly decreased THg and MeHg accumulation in brown rice (Figure 1). These results suggest that the uptake processes of THg and MeHg proceed throughout the entire growth period, and that THg and MeHg accumulations can be reduced if the period of flooding is shortened.

The distribution of MeHg content (ng plant^{-1}) in the tissues of the rice plants was greatly affected by water management (Figure 2). First, the total content of MeHg accumulated by rice plants was greatly decreased in the CA treatment, which is likely due to the diminished concentrations of MeHg in all tissues. Second, less MeHg was accumulated in brown rice in the CA treatment. In the CF treatment, up to 88% of the MeHg content could be transported and stored in brown rice, whereas the proportion declined to 38% in the CA treatment. Meng et al.[12] have reported that in the young rice plant, the majority of MeHg taken up by roots is located in the leaves and stalks. However, most of this MeHg is transferred to the grain during the ripening period. Thus, our results suggest that the aerobic treatment could not only reduce the total amount of MeHg taken up by rice plants but may also affect the ways of transferring MeHg to brown rice, leading to less MeHg accumulated.

4.2. Hg Bioavailability and SRB Communities. Bioavailability refers to the amount of a compound that can potentially dissolve from a sample and become an aqueous exposure route to an organism.[32] In a general way, compared with the other Hg fractions (the acid-soluble, the metal oxides-bound, the organic, and the residual fractions), the water-soluble and exchangeable fractions are the most mobile and bioavailable fractions. These can easily be transported by natural processes and absorbed by plants.[1,40] In our study, the sum of the water-soluble fraction and the "human stomach acid"-soluble fraction were considered to be the "bioavailable inorganic Hg".[32,33] Our data suggested that the "bioavailable inorganic Hg" accounted for 0.02–0.18% of the THg amount in soil. This is in agreement with the previous findings,[41] because Hg is usually not present in the form of water-soluble ionic species in the aqueous phase but as species bound to organic matter (without a Hg–carbon bond) or suspended mineral particles.[42] However, the inorganic Hg absorbed from the soil accounts for the major part of THg accumulated in rice plants.[11,12] In the present study, whether under glasshouse or paddy field conditions, "bioavailable inorganic Hg" concentrations were markedly decreased in the aerobic treatment (Table S3, Supporting Information) and were positively related to THg concentrations in brown rice and root tissues (Figure 3). These findings suggest that aerobic conditions contribute to the immobilization of Hg in soil, resulting in lower Hg bioavailability and less Hg to be taken up by rice plants. Peng et al.[26] also observed that the THg concentration in soil solutions declined dramatically in an aerobic treatment. The immobilization of Hg may be due to the iron and manganese oxides soil components which can adsorb or coprecipitate Hg from the soil solution.[1] More importantly, the water-soluble fraction of Hg may serve as the substrate for the Hg methylation process.[1,43] The decreased availability of inorganic Hg for methylation may help reduce the production of MeHg in the rhizosphere, which was also been detected in our study (Table 1).

Our real-time PCR results showed that SRB abundance ranged from 0.6×10^8 to 3.5×10^8 copies g^{-1} dry soil in the rhizosphere (Table 2). Similar target SRB numbers have been reported for paddy soils and other freshwater environments. For example, Liu et al.[27] observed SRB abundance ranged from 3.60×10^8 to 8.02×10^8 copies g^{-1} dry soil in paddy soil. SRB abundances ranging from 0.2×10^8 to 5.7×10^8 copies mL^{-1} have been detected in estuarine sediments by using competitive PCR.[44] Our results also demonstrated that SRB abundance was significantly influenced by water management. The lower abundance of SRB in the CA treatment may be due to higher oxygen intrusion into the rhizosphere, because SRB are anaerobic microorganisms.[28,34] SRB have been recognized as the primary Hg methylators in estuarine and freshwater ecosystems.[13,45] In a previous study, Meng et al.[12] also indicated that soil is the dominant source for MeHg to the rice plant. From this perspective, a lower abundance of SRB may bring about (1) lower levels of MeHg produced in the rhizosphere, and (2) less MeHg to be absorbed and accumulated in plants. This is further confirmed by the reduced MeHg concentrations in the rhizosphere (Table 1) and the significant positive relationships between *dsrAB* gene copy numbers in the rhizosphere and MeHg concentrations in brown rice, root tissues, and rhizosphere soil (Figure 4) detected in our study.

Our *dsrAB* clone sequencing further revealed that the SRB community composition was influenced by water management. The appearance of families Desulfobacteraceae and Peptococcaceae in each *dsrAB* library indicates their widespread distribution in the paddy field soil, which is consistent with previous studies.[46,47] However, the proportion of Desulfobacteraceae declined under aerobic conditions, with Peptococcaceae accounting for a larger proportion (Table 3). Together with the alterative group distribution, *dsrAB* diversity indices were higher in the aerobic treatment than those in the flooded treatment. Because the local environment in the rhizosphere could be influenced by the intrusion of O_2 under aerobic

conditions (as detected in our study Figure S1, Supporting Information), Peptococcaceae may be more adaptable to the aerobic conditions, owing to their capacity to form endospores and switch to an energy-conserving metabolism, such as syntrophic fermentation.[48] In addition, the groups related to Desulfobacteraceae, Desulfovibrionaceae, and Desulfobulbaceae may play a more important role in the methylation process, because strains belonging to those families have been found to possess a stronger ability for methylation.[49−51] The lowered proportions of these families in the CA treatment may contribute to the weakened production of MeHg in the rhizosphere and lower accumulation of MeHg in rice plants.

This study systematically explored the reasons why different water treatments have different effects on Hg/MeHg accumulation in rice and elucidates the possible factors involved in these effects from a new perspective. Our results demonstrate that the significantly decreased bioavailability of Hg and production of MeHg are due to reduced SRB numbers and lower proportions of Hg methylators in the rhizosphere under aerobic conditions. These are the main reasons for the reduced Hg and MeHg accumulation in aerobically grown rice. This points to an explicit strategy for the production of safe rice from Hg-contaminated paddy fields.

■ ASSOCIATED CONTENT

Supporting Information

Additional text, figures, and tables. 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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