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Increased Methylmercury Accumulation in Rice after Straw Amendment

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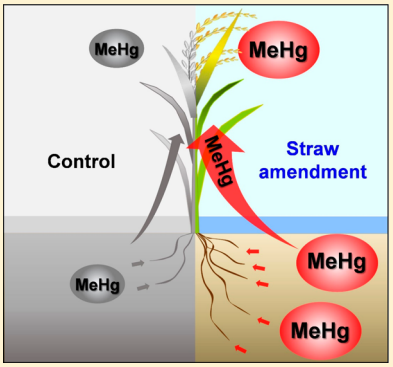
Supporting Information

ABSTRACT: Consumption of rice has been shown to be an important route of dietary exposure to methylmercury (MeHg, a neurotoxin) for Asians having a low fish but high rice diet. Therefore, factors that increase MeHg production and bioaccumulation in soil−rice systems, could enhance the risk of MeHg exposure. On the basis of a national-scale survey in China (64 sites in 12 provinces) and rice cultivation experiments, we report that straw amendment, a globally prevalent farming practice, could increase MeHg concentrations in paddy soils (11−1043%) and rice grains (95%). By carrying out a series of batch incubation, seedling uptake and sand culture experiments, we demonstrate that these increases could be attributed to (1) enhanced abundances/activities of microbial methylators and the transformation of refractory HgS to organic matter-complexed Hg, facilitating microbial Hg methylation in soils; (2) enhanced MeHg mobility, and increased root lengths (35−41%) and tip numbers (60−105%), increasing MeHg uptake by rice roots; and (3) enhanced MeHg translocation to rice grains from other tissues. Results of this study emphasize fresh organic matter-enhanced MeHg production and bioaccumulation, and highlight the increased risk of MeHg after straw amendment and thus the need for new policies concerning straw management.



INTRODUCTION

Methylmercury (MeHg), a highly toxic and bioaccumulative Hg species, is currently a global concern. While fish consumption has long been recognized as the main source of MeHg intake by humans, recent studies have further identified rice contributes a significant proportion of dietary MeHg.[1] For instance, rice consumption accounted for 82−96% of dietary MeHg exposure for residents in Sichuan and Guizhou provinces, China,[2] which have 1.7% of the world's population. Consequently, factors that may increase MeHg production in soils and its accumulation in rice, would in turn enhance human exposure to MeHg, especially in Asia where rice is the staple food for most people.

Annual or biannual incorporation of crop residues such as straw into soils contributes >65% of the organic matter (OM) input into paddy soils.[3] This large input of straw OM has raised global interest in exploring the impacts of straw return on soil properties such as organic C, N, P, and Si content,[4,5] microbial communities,[6] and greenhouse gas emissions from soils,[7] as well as metal mobility/bioavailability,[8] while the potential effects of straw return on Hg biogeochemistry are largly ignored. However, straw OM might impact the risk of MeHg bioaccumulation in contaminated soil−rice systems, considering that (1) OM is a key factor controlling Hg bioavailability and methylation,[9−15] and (2) compared with aged soil OM (e.g., humic substances), straw OM (mainly composed of cellulose, hemicellulose, lignin, and crude

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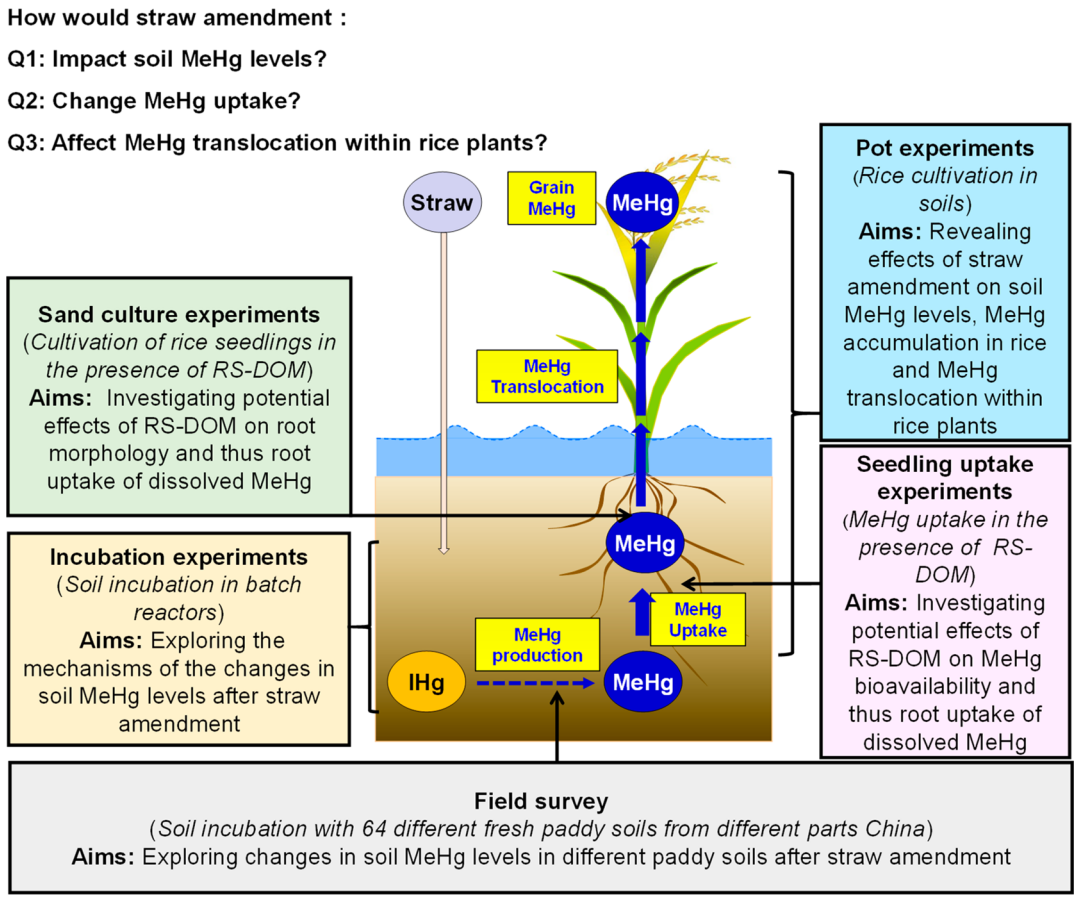

Figure 1. Conceptual framework for this study.

protein) is an important carbon source for microbes,[16] including sulfate-reducing bacteria (SRB) and other microbial methylators of Hg. Given that straw return is a prevalent farming practice globally in both pristine and Hg-contaminated areas, such as California's Central Valley, U.S.A.,[9,17] Mindanao, Philippines,[18] and the Xunyang and Tongren regions of China,[19,20] a better understanding of MeHg bioaccumulation following straw amendment is needed. Recently we reported large increases (~40−700%) in soil MeHg concentrations of two mining-impacted soils following rice straw amendment,[11,21] however, little has been done to address the larger questions concerning the generality of increased MeHg production after straw amendment and more importantly, the underlying mechanisms. Furthermore, little is known about the changes in MeHg uptake by crop plants in response to straw amendment,[22] which is important considering potential impacts of straw OM on MeHg mobility or plant physiology. These knowledge gaps hinder a comprehensive understanding of the risk of MeHg bioaccumulation in soil−plant systems.

Thus, the aim of this study was to investigate MeHg accumulation in rice grains after straw amendment and the mechanisms responsible for this process. Specifically, we explored: (1) the impacts of straw amendment on soil MeHg levels, (2) the changes in root uptake of dissolved MeHg in response to straw amendment, and (3) the effects of straw amendment on MeHg translocation within rice plants. The three processes were examined in field and laboratory experiments. A summary of these experiments, as well as their respective objectives, is presented in Figure 1. These results provide new insights into the processes leading to elevated MeHg production and bioaccumulation in soil−rice systems, and help better predict the risk of dietary exposure to MeHg for Asians.

MATERIALS AND METHODS

Paddy Soil and Rice Straw. Surface soil (0−20 cm) was collected from a contaminated paddy field in Xunyang (referred to as XUN soil), one of the largest mercury mining areas in China. After return to the laboratory, the XUN soil was air-dried, ground, sieved through a 2 mm mesh, and used in the pot and incubation experiments described below. Total Hg and MeHg concentrations in XUN soil were 44.0 ± 3.3 mg/kg and 6.0 ± 0.1 μg/kg, respectively. Other soil characteristics are listed in Table S1 of the Supporting Information (SI).

Rice straw was collected from a site free of any known mercury contamination, and was washed, air-dried, and ground. In China, before straw is returned to the fields, it is commonly chopped up to enhance its decomposition in situ. Thus, in our study, the straw was ground prior to its use. Low-Hg straw was used in all experiments to minimize potential interference by Hg-containing straw on soil MeHg levels, which was not examined in this study. The concentrations of total Hg and MeHg in the straw were 30.9 ± 0.2 and 2.7 ± 0.1 μg/kg, respectively.

The chemicals used in this study are listed in Table S2.

Field Survey. Net MeHg production in 64 field-collected paddy soils after straw amendment was quantified, to explore changes in soil MeHg levels in different soils after straw amendment. Wet paddy soils were freshly collected from 64

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sites in 12 provinces of China (Figure S1). The rice planting area of those provinces together accounts for 82% of that in China (China Statistical Yearbook 2017), and these soils are therefore representative of paddy soils in China. Total Hg levels in most soils range from 24 to 446 μg/kg, while that in site 52 (mining-contaminated) reaches 74 mg/kg (Table S3). Specifically, surface soils (2−15 cm) were collected from rice paddy fields in 2017, immediately after rice harvest. Thus, the microbial communities in those wet soils could represent those in different paddy soils. These soil samples were immediately placed in an icebox, brought back to the lab, and used in an incubation experiments: All soils were subjected to 21 days of batch incubation at 28 °C. Two treatments, CK (soil without rice straw) and RS (soil +1% rice straw amendment, i.e., 10 g/kg soil), were established, with soil MeHg levels determined on day 21 to examine the effects of straw amendment on net MeHg production in soils (details in SI). The amount of incorporated straw (i.e., 1%, w/w), which is the same in all experiments of this study, is comparable with those commonly used in farming practice.[23] Soil incubation was conducted in batch reactor instead of in field, because the ambient temperature at some of those sampling sites was not representative of the temperature during rice cultivation. A temperature of 28 °C was used for the soil incubation as it is typical of the temperature during the rice cultivation period in the above-mentioned major rice production regions of China.

Pot Experiments. Rice cultivation was conducted in XUN soil to quantify the changes in soil MeHg levels and MeHg accumulation in rice in the presence (RS: XUN soil + rice straw) or absence (CK: XUN soil) of straw amendment. For each treatment, triplicate pots were used, filled with 2 kg of XUN soil and planted with rice (2 plants/pot). All pots were placed in a greenhouse (Nanjing, Jiangsu Province, China) at ambient temperature (14−36 °C). On day 0, straw was mixed with XUN soil (for RS only). Soils in both treatments were then amended with basic fertilizers and flooded with DI to a level of 3−5 cm above the soil surface. On day 31, 30-day-old rice seedlings of Wufeng (*Oryza sativa* L. *indica* cultivated in a low-Hg soil) were transplanted into these soils. On day 129, the plants were harvested. The roots (iron plaque removed),[24] straw (stem and leave), and grains (brown rice) were washed, oven-dried, weighed, and ground into powder for determination of MeHg, TAA (total amino acids), Cys (cysteine), Glu (glutamic acid), total N, and total S. Amino acids were measured using an L-8900 automatic amino acid analyzer (HITACHI, Japan), and N and S on an Elementar Analysensysteme (GmbH, Germany).

Soils (1−11 cm) were sampled on days 0 (4 h after straw amendment), 31 (right before seedling transplantation), and 126 (3 days before harvest). Soil samples were immediately centrifuged at 4000 rpm for 20 min to remove the overlying water and then separated into subsamples in an anoxic glovebag filled with N_2 (AtmosBag, Sigma-Aldrich) for the determination of soil moisture and MeHg and DOC (dissolved organic carbon, Shimadzu TOC-5000A, Japan) concentrations.

Incubation Experiments. XUN soil (with or without straw amendment) was incubated in batch reactors and potential microbial methylators and Hg speciation in soils were then quantified, to elucidate the effects of straw addition on microbial MeHg production. Incubation experiments were conducted in addition to pot experiments to enable the exploration of the early, more dynamic phases of straw decomposition, sulfate/iron reduction, and microbial MeHg production. Five treatments were used for soil incubation experiments: (1) control without straw amendment (CK), (2) rice straw amendment (1% rice straw: RS), (3) rice straw amendment with SRB inhibited (1% rice straw and Na_2MoO_4, a specific inhibitor of SRB:[25] RS-SRB), (4) lactate amendment ($C_3H_5O_3Na$, as a labile carbon source: L), and (5) rice straw and lactate amendment (1% rice straw and $C_3H_5O_3Na$: RS+L). Lactate is commonly used in incubation experiments as a microbial electron donor, including for SRB.[26] Inhibitor of methanogens, i.e., bromoethanesulfonate (BES), was not used in this study, mainly because SRB are capable of using sulfonates.[12] For instance, results of our preliminary experiments indicate that BES addition could enhance net MeHg production in the examined soils.

The soils were incubated in batch reactors anoxically for 40 days followed by 5-days of reoxidation. The incubation scheme was designed to simulate the generally anoxic conditions in flooded paddy soils as well as the fluctuating redox potential typically occurring in these soils. Specifically, 10 g of amended or unamended soil were resuspended in 30 mL DI (for CK and RS treatments) and Na_2MoO_4 (for RS-SRB, 20 mM final concentration) or lactate (for L and RS+L treatments, 10 mM final concentration) was added. All tubes were sealed and incubated in the dark under anoxic conditions (Eh values shown in Figure S2) in an environmental chamber (28 °C). After 40 days, all tubes were uncapped for 1 h every day to evaluate MeHg dynamics under subanoxic conditions (Eh = −41−46 mV).

After 4 h and 5, 10, 15, 30, and 45 days, three tubes in each treatment (total of 18 tubes/treatment) were centrifuged to separate soil from overlying water. And then overlying water (simulating porewater in paddy soils) was filtered through a 0.45-μm polyether sulfone filter capsule (Anpel, China). MeHg levels in soils were then measured and overlying water was analyzed for MeHg, Eh and pH (HACH, HQ30D, U.S.A.), dissolved Fe (flame atomic absorption spectroscopy, Thermo, U.S.A.), DOC, and SO_4^{2-} (ion chromatography, Dionex, ICS-1000, U.S.A.). In addition, Hg speciation in soils, determined in XANES (X-ray absorption near edge structure) analysis on days 5, 10 and 30, was monitored (details in SI). Abundances of SRB (the principle Hg methylators in XUN soil and many other soils)[25,27] on days 5, 10, 15, and 30 were determined (details in SI) based on the most probable number method.[28] The *hgcA* gene (encoding an Hg-methylating protein and indicative of the abundance of potential microbial methylators of Hg)[29] copy numbers in soils were assessed on day 15 (details in SI), when the difference in soil MeHg levels between RS and CK was the largest.

Seedling Uptake and Sand Culture Experiments. Seeding uptake of dissolved MeHg in the presence or absence of RS-DOM (rice straw-derived dissolved organic matter, of different molecular sizes and concentrations) was quantified, to explore potential effects of RS-DOM on MeHg bioavailability and thus roots uptake of dissolved MeHg. Details of the experiments are presented in SI.

The potential effects of RS-DOM on root morphology and on root uptake of dissolved MeHg were investigated in the sand culture experiment. Five germinated rice seeds were sowed into a polyethylene bottle that had been filled to the top with 250 g of quartz sand (<1 mm, acid-washed to remove surface-sorbed OM). Five replicate bottles (25 rice seedlings) were used for each treatment: two for quantifying root length and tip number (indicative of root growth) and three for

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determining the uptake of dissolved MeHg. For RS treatment, to simulate porewater in straw-amended paddy soil, RS-DOM was added to culture solution (complete Hoagland solution) at a concentration of 400 mg DOC/L, comparable to those DOC levels in paddy soils[30] and to that in the straw-amendment-induced-increases in DOC in pot experiments (Figure S3A). The culture solutions for both treatments were renewed every other day to maintain relatively constant nutrient and RS-DOM levels. On days 10 and 20, five seedlings each were removed, and the root tip number and root length were determined (winRHIZO 2013e-Expression 11000XL). The rice seedlings remaining after 20 days of cultivation (in the presence or absence of RS-DOM) were removed from sand, rinsed thoroughly with DI, and then used in the determination of seedling uptake of the dissolved MeHg in a solution containing 20 ng MeHg/L, prepared in 0.01 M $CaCl_2$ (without RS-DOM).

Statistics. Differences between treatments were determined using one-way analysis of variance (ANOVA, SPSS, version 16.0). Posthoc tests were used to identify variances among groups ($p \leq 0.05$).

RESULTS

Field and Pot Experiments: Enhanced MeHg Levels in Soils and Plants after Straw Amendment. Net MeHg production in different wet paddy soils (field-collected from major rice production areas of China) in the presence (RS) or absence (CK) of rice straw is depicted in Figure S1. Soil incubation with straw resulted in significantly higher net MeHg production in 61 out of 64 paddy soils (13−1043% higher, average 170%, RS vs CK; $p \leq 0.05$; Figure S1).

MeHg concentrations in XUN soil and MeHg accumulation in rice plants, in response to straw amendment, were quantified in pot experiments. MeHg level in RS soil was 64% higher than in CK soil on day 31, at the time of seedling transplant, and still 11% higher on day 126, 3 days before harvest (Figure 2A). DOC levels were also 32−391% higher in RS than in CK soil during rice cultivation (Figure S3A) and were positively and linearly related to soil MeHg levels (Figure S3B). Increases in MeHg concentrations were higher in harvested rice tissues (RS vs CK: 95% higher in grains and 73% in roots, Figure 2B) than in soils (11−64% higher in RS vs CK treatments during rice growth). This was reflected in MeHg bioaccumulation factor (BAF, calculated as the tissue MeHg concentration/soil MeHg concentration on day 126), which was 43−74% higher in root, grain, and the whole plant following straw amendment (Figure 2C). This result suggests that straw amendment facilitated not only MeHg production in soils but also the uptake of MeHg by rice plants. Besides, tissue biomasses were comparable between treatments (Figure S4). Consequently, total MeHg uptake by rice plants was significantly higher (Figure 2D) in straw amended treatment (0.99 μg MeHg/plant) than in control (0.62 μg MeHg/plant).

The translocations of MeHg and other compounds from roots and straw to grains were also examined. The distributions (%, content in grain/summed content in root + straw + grain) of MeHg, biomass, TAA, Cys, Glu, N, and S were 46%, 28%, 23%, 48%, 22%, 63%, and 34% higher in RS than in CK rice grains (Figure S5), suggesting their facilitated translocation (to grains) in rice plants grown after straw amendment.

Incubation Experiments: Increased Microbial Abundances/Activities and Modified Hg Speciation in Soils after Straw Amendment. The two key factors controlling microbial Hg methylation in soils, i.e., microbial methylators and Hg speciation in soils were quantified in laboratory incubation experiments conducted in XUN soil. MeHg levels were consistently higher in RS than in CK soils during the 45-day incubation period (Figure 3). By contrast, MeHg levels in RS-SRB soils were comparable to those in CK soils, indicating the key role of SRB in controlling net MeHg production in straw-amended XUN soil. Similar with straw amendment, amendment of lactate (L treatment) resulted in soil MeHg levels 29−139% higher than those in CK soils. This result demonstrates that labile carbon could be a limiting factor of microbial methylator activity and thus also of MeHg production. The coamendment of lactate and straw (RS+L) did not lead to a further increase in soil MeHg level.

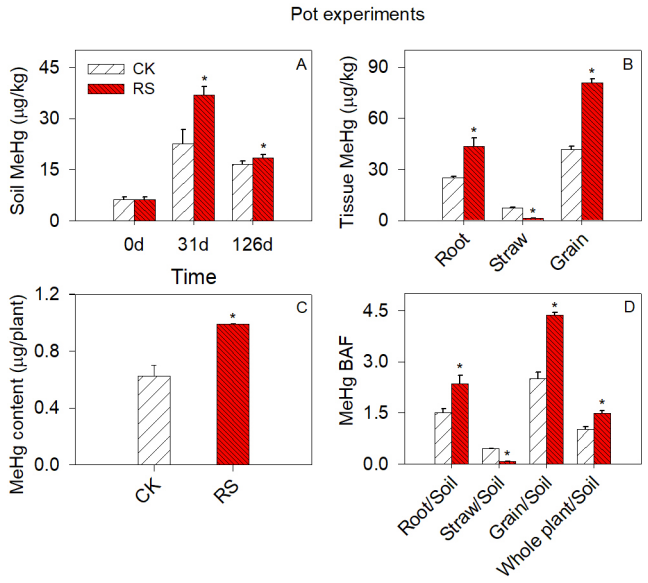

Figure 2. Pot experiments: MeHg concentrations in soils (A), plant tissues at harvest (B), MeHg uptake (μg MeHg/plant, (C) and bioaccumulation factor (BAF) in root, straw, grain, and the whole plant (calculated as tissue or whole plant MeHg concentration/soil MeHg concentration on day 126, (D). CK and RS represent the control and rice straw amendment, respectively. Asterisks (*) indicate significant differences from CK ($p \leq 0.05$, one-way ANOVA). Mean ± SD, $n = 3$.

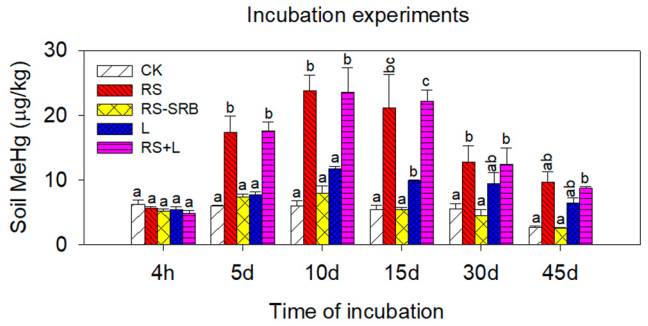

Figure 3. Incubation experiments: Soil MeHg concentrations in different treatments. CK, RS, RS-SRB, L, and RS+L represent control, rice straw amendment, rice straw amendment with SRB inhibited, lactate amendment, and rice straw and lactate amendment, respectively. The different letters (i.e., a−c) above the bars indicate significant differences among treatments (one-way ANOVA, $p \leq 0.05$). Mean ± SD, $n = 3$.

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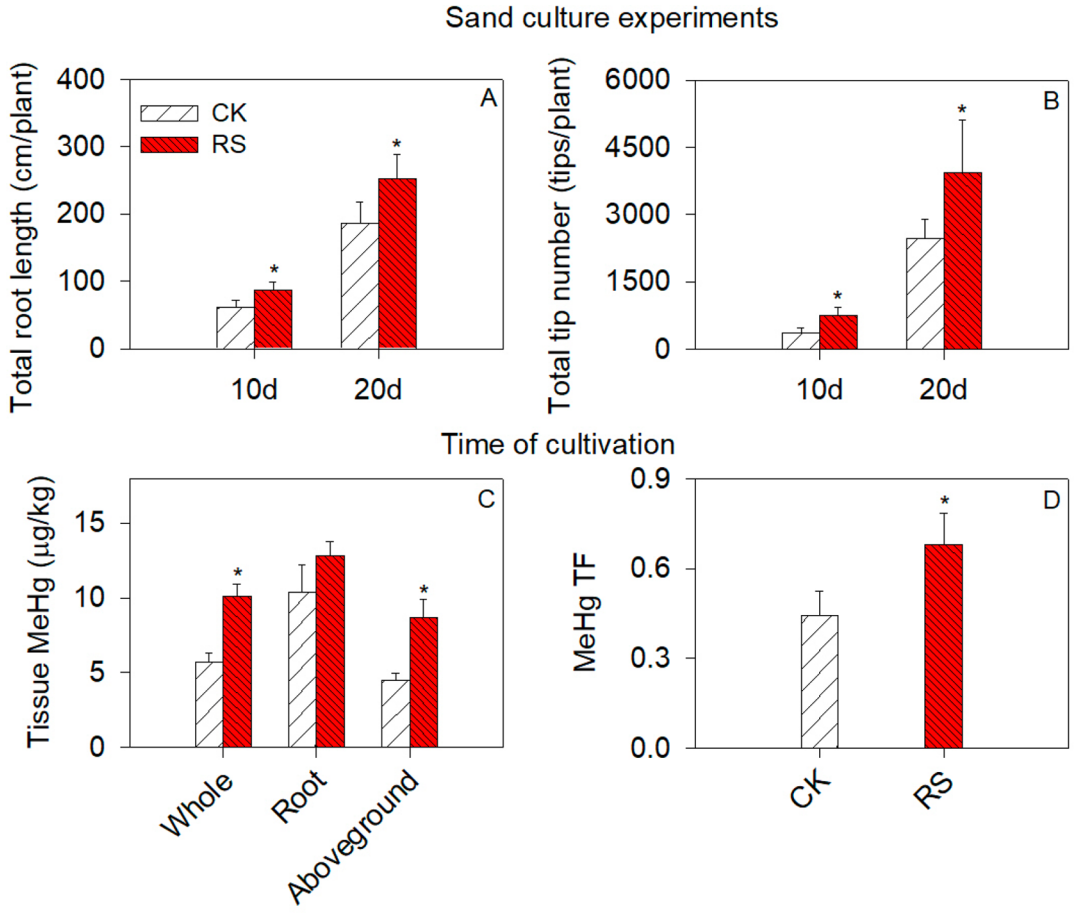

Figure 4. Sand culture experiments: Total root length (A) and tip number (B) of rice seedlings cultivated in quartz sand with or without rice straw dissolved organic matter (RS-DOM). MeHg concentration in seedlings (C) and the translocation factor (TF) of MeHg from root to aboveground tissue (D, aboveground MeHg concentration/root MeHg concentration). CK and RS represent rice seedlings planted in nutrition solution and nutrition solution with RS-DOM, respectively. Asterisks (*) indicate significant differences from CK ($p \leq 0.05$, one-way ANOVA). Mean ± SD, for root lengths and tip numbers, $n = 5$; for MeHg uptake, $n = 3$.

Changes in the abundances/activities of potential microbial methylators, in response to straw amendment, were also quantified: (1) Potential microbial methylators of Hg were assessed by quantifying the abundance of SRB and the copy number of the *hgcA* gene, responsible for Hg methylation, in soils. Compared with CK soils, straw amendment increased SRB abundance 21- to 2500-fold and 25- to 5000-fold in RS and RS+L treatments, respectively (Figure S6A). The increases in DOC level (Figure S6B) after straw amendment were also significant, and SRB abundances correlated positively with DOC levels (Figure S7). The copy number of *hgcA* was also higher (5.6-fold) in RS than in CK soil (Figure S8). (2) Enhanced reduction of SO_4^{2-} (e.g., from SO_4^{2-} to S^{2-}), as well as enhanced iron reduction [e.g., from insoluble Fe(III) to dissoluble Fe(II)] were observed after straw amendment (Figure S9), indicating increased activities of potential microbial methylators of Hg (i.e., sulfate/iron-reducing bacteria). Specifically, sulfate levels remained relatively constant in CK and RS-SRB soils but decreased with time after straw or lactate amendment. Meanwhile, both Eh (Figure S3) and pH (Figure S10) values were lower in the straw-amended (RS, RS-SRB, and RS+L) than those in CK and L treatments.

In addition to increasing the abundances/activities of potential microbial methylators of Hg, straw amendment could modify Hg speciation in soils, with implications for microbial Hg methylation: XANES analysis (Figure S11, Table S4) reveals a shift from more refractory (HgS: 61.2−68.4% in CK vs 41.2−59.2% in RS soils) to more mobile species (organically complexed Hg: 0−6.5% in CK vs 13.5−21.7% in RS soils) and thus a potential increase in the bioavailability of Hg to microbial methylators in soils after straw amendment.

Dissolved MeHg levels were 7−59, 4−11, and 7−84 times higher in RS, RS-SRB, and RS+L than in CK treatments during the 45-day incubation period (Figure S12A). The lower MeHg partition coefficients (Kds, Figure S12B) indicate the increased mobility of MeHg in the respective straw-amended soils.

Seedling Uptake Experiments: Less Variable MeHg Bioavailability in the Presence of RS-DOM. Effects of RS-DOM on the bioavailability of dissolved MeHg (e.g., by complexing MeHg) to rice seedlings were explored, by quantifying root uptake of dissolved MeHg in the presence or absence of RS-DOM (Figure S13). Although larger-molecular-mass fractions (3−100 kDa and >100 kDa) of RS-DOM significantly inhibited seedling uptake of dissolved MeHg, they comprised only a small proportion of pristine RS-DOM (~11%, Figure S13A). By contrast, the effect of the smaller-molecular-mass fraction (<3 kDa) of RS-DOM, accounting for ~89% of pristine RS-DOM, on MeHg uptake was insignificant. Consequently, pristine RS-DOM (0−200

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mg/L) had only minor effects on the uptake of dissolved MeHg by rice seedlings (Figure S13B).

Sand Culture Experiments: Modified Root Morphology and Enhanced MeHg Uptake Following Seedling Cultivation with RS-DOM. Root morphology (indicated by root length and tip number) was compared in rice seedlings cultured in quartz sand with or without RS-DOM. Seedling cultivation with RS-DOM resulted in significantly longer root length (41% and 36% longer on day 10 and day 20, respectively; Figure 4A) and higher tip number (105% and 60%, respectively; Figure 4B) in RS than in CK seedlings. Meanwhile, cultivation of seedlings with RS-DOM for 20 days facilitated their uptake of dissolved MeHg (in 0.01 M $CaCl_2$ solution), with increases of 76%, 24%, and 94% in whole plants, roots, and aboveground parts, respectively (Figure 4C). Similar to the results of pot experiments, translocation factors for MeHg (aboveground MeHg concentrations/root MeHg concentrations) were 54% higher in RS than in CK seedlings (Figure 4D).

DISCUSSION

Our experiments provide evidence of increase in grain MeHg level as high as 95% following soil amendment with rice straw. MeHg level in rice grains (80.9 μg/kg) harvested from plants cultivated after straw amendment was approximately four times higher than the amount permitted by the Chinese government (20 μg total Hg/kg), indicating increased risk of dietary MeHg exposure in Hg mining-impacted areas. Further studies on quantifying grain MeHg levels following straw amendment in different areas are necessary. Furthermore, our field survey and pot experiments demonstrate enhanced MeHg production in rice straw-amended paddy soils (11−1043% higher, RS vs CK). While all of the examined soils in the field survey (except site 52) were not from Hg mining areas, MeHg levels in some straw-amended soils (e.g., 2−4.4 μg/kg in 20 sites, Figure S1) were comparable to the amounts in some Hg mining-impacted soils, e.g., Gouxi, Guizhou (4.1−4.4 μg/kg)[31] and Xunyang, Shaanxi (1.2−11 μg/kg)[32] in China, and Oregon (1.9−2.4 μg/kg) in United States.[33] These increases might lead to greater uptake and accumulation of MeHg in rice and presumably in other crops (e.g., wheat) cultivated in rotation systems, considering that uptake of MeHg from soil is the dominant route of MeHg accumulation in crop plants[34] and MeHg accumulation in plants were found to be positively related to soil MeHg levels.[35] Our results highlight the need to reconsider straw management policies to account for the potentially higher ecological and health risk of MeHg due to straw amendment.

To obtain a mechanistic understanding of MeHg dynamics and the resulting risk of amending soil−rice systems with straw, we explored three processes that could contribute to the enhanced MeHg level in rice grains.

Increased Soil MeHg Levels. Enhanced abundances/activities of microbial methylators and mobilization of refractory HgS after straw amendment could be mainly responsible for the increased MeHg levels in soils, as discussed below.

The results of our incubation experiments provide multiple lines of evidence that increased abundances/activities of microbial methylators of Hg, e.g., SRB, play a key role in the elevated MeHg levels in soils after straw amendment. First, *hgcA* gene copy number was significantly higher in RS than in CK soil, indicating higher abundances of potential microbial methylators in soils after straw amendment. Second, the significantly higher SRB abundances in RS than in CK soils were accompanied by decreases in SO_4^{2-} levels over time in RS and RS+L but not in CK soils (Figure S9A). This suggests that straw amendment enhanced SRB activity and thus SO_4^{2-} reduction by SRB, a process previously shown to correlate with MeHg production.[36,37] Third, soil MeHg levels were 133−300% higher in RS than in CK soils (Figure 3), but comparable between RS-SRB (SRB-inhibited) and CK soils, again implicating SRB are involved in RS-induced MeHg production in soil. Therefore, we propose that enhanced SRB abundances/activities and thus microbial production of MeHg could be a key factor for the elevated MeHg levels in XUN soil after straw amendment. It should be noted that the abundance of SRB on day 15 was ~2500-fold higher in RS than in CK soil, while *hgcA* gene copy number was only 5.6-fold higher and more in line with the increase in soil MeHg level (3-fold on day 15) in response to straw amendment. The discrepancy in the relationship between soil MeHg levels and SRB abundances could be explained by the fact that not all SRB could methylate mercury.[38] Besides, iron-reducing bacteria (FeRB) could also play a role in MeHg production under straw amendment, which is discussed in SI. Meanwhile, straw amendment had different effects on soil MeHg levels in different paddy soils in this (Figure S1) and previous studies,[39] which could be attributed to differences in microbial communities and should be further explored in future studies.

The enhanced microbial abundances/activities and thus the net increases in MeHg production may have been associated with the input of RS-OM, as a supply of labile carbon for methylating bacteria. Consistent with previous studies,[11,17] DOC levels were significantly higher after straw amendment (RS vs CK, pot and incubation experiments), most likely due to the dissolution/decomposition of the RS-OM. Positive relationships were found between the abundances of SRB in soils and the DOC levels in the overlying water (incubation experiments: Figure S7), and between soil MeHg and DOC levels (pot experiments: Figure S4B; incubation experiments: Figure S14A), demonstrating an important role for RS-DOM in controlling SRB abundances and thus microbial MeHg production in soils. We hypothesized that the supply of labile carbon was the limiting factor for the abundances/activities of microbial methylators in studied XUN soil and that, by increasing the labile carbon pool, straw amendment increased MeHg production. To test the hypothesis, lactate (a common labile carbon source for bacteria, including SRB and FeRB)[26] was used as a proxy for RS-DOM labile carbon. Similar to RS-DOM, lactate alone increased soil MeHg levels and led to enhanced SO_4^{2-} reduction (L vs CK, Figure S9A), suggesting that labile carbon was the key factor controlling the activities/abundances of microbial methylators. Soil MeHg levels in L were lower than those in RS (Figure 3), which might be explained by different availability of carbon sources (i.e., lactate and straw organic matter) to microbial methylators.[40] The comparable soil MeHg levels in RS and RS+L treatments indicate that excess labile carbon sources, in the form of lactate added to RS-OM, did not further increase microbial MeHg production over that achieved with rice straw. In fact, 89% of RS-DOM was present as low-molecular weight organic matter (<3 kDa, Figure S13A), which may serve as electron donor for bacteria including SRB.[9] For instance, relatively high concentration of acetate (943 ± 14 mg/L, accounting for 15.3% of RS-DOC), a main product of anaerobic decom-

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position of straw,[9,41] was detected in RS-DOM. Acetate is often considered as an indicator for labile carbon supply and used as an electron donor by microbial methylators, including SRB and FeRB.[9] Besides, determination of Hg methylation and demethylation rates, both of which could be impacted by straw amendment and thus input of labile carbon, would help better explain the enhanced MeHg levels. Together, these results highlight the need to consider potential increases in microbial MeHg production following labile carbon inputs from farming-derived sources.

In addition to increasing microbial abundances/activities, straw amendment induced changes in Hg speciation in soils, which may have affected microbial Hg methylation. The XANES analysis indicates decreases in HgS levels in soils after straw amendment (Figure S11). HgS dissolves slowly in the presence of DOM,[42,43] and the sulfide produced following sulfate reduction by RS-DOM-stimulated SRB may have enhanced the solubilization of Hg from HgS minerals.[44] Moreover, straw amendment also resulted in a higher proportion of organically complexed Hg in soils (Table S4), possibly due to the association of Hg with the rice straw-OM. Organically complexed Hg is more mobile than HgS and thus more readily available for microbial Hg methylation.[45] Therefore, the transformation of Hg from more refractory (HgS) to more mobile (organically complexed Hg) species could have further contributed to the enhanced microbial Hg methylation after straw amendment. To our knowledge, this is the first study to use synchrotron radiation analysis to follow the changes in Hg speciation in soils after straw amendment. The results offer new knowledge regarding the interactions between Hg and OM in soil−plant systems. Furthermore, for Hg-contaminated soils (especially mining-contaminated soils), they highlight the need to consider the straw-return-induced mobilization of Hg from refractory HgS and thus the increased risk of Hg methylation and Hg bioaccumulation. In addition to mobilizing refractory HgS probably by RS-DOM complexation, straw amendment may also impact Hg speciation in soils by facilitating microbial sulfate reduction and sulfide production: Sulfate levels correlated negatively with soil MeHg levels (r = 0.8, Figure S14B), possibly due to the formation of HgS in soils. The opposite effects of straw amendment on HgS mobilization and formation may counteract with each other, and their relative importance warrant further investigation.

Increased MeHg Uptake by Rice Plants. Besides enhancing soil MeHg levels, straw amendment facilitated MeHg uptake by rice plants, evidenced by increases in MeHg BAF (Figure 2C). The positive effects of straw amendment on MeHg uptake by plants' roots could be explained by (1) the enhanced mobility of MeHg and (2) the increases in root length and tip number in the presence of RS-DOM.

The results of our incubation experiments indicate that, after straw amendment, MeHg partition coefficients (K_ds) were lower, such that MeHg partitioning was preferentially into the dissolved phase, and DOC levels were much higher (34.7−118.3 mg/L in RS vs 17.1−19.7 mg/L in CK; Figure S6B). Considering the high affinity of Hg for DOM, RS-DOM may mobilize MeHg from soils, bringing it into solution and thus making it more available for root uptake. In addition, the lower pH in soils after straw amendment (Figure S10), which was commonly observed in field,[46] could also facilitate MeHg desorption from soil particles. Consequently, the increased partitioning of MeHg into water phase, together with the increases in soil MeHg production, resulted in 7- to 59-fold higher MeHg levels in the overlying water of RS than of CK treatments in our incubation experiments. The increases in MeHg mobility would facilitate uptake of dissolved MeHg by rice roots.

In addition to promoting MeHg partitioning into the dissolved phase, DOM may complex with dissolved MeHg, resulting in altered bioavailability and thus biological uptake of dissolved MeHg, as reported in a previous study.[47] However, according to our results, RS-DOM had only minor effects on the uptake of dissolved MeHg by rice seedlings (Figure S13B). Since RS-DOM consists of a mixture of natural polymers with a wide-ranging molecular mass distribution, a detailed investigation of different RS-DOM fractions may be needed to better understand the potential effects of RS-DOM on MeHg uptake by plants. For example, we observed differential effects of different RS-DOM fractions on MeHg uptake (Figure S13A): While RS-DOM fractions of higher molecular mass (>3 kDa) significantly inhibited MeHg uptake, those with lower molecular mass (<3 kDa) had no significant impact. Since the majority of the RS-DOM was in the low-molecular-mass fraction (Figure S13A), pristine RS-DOM did not significantly influence the uptake of dissolved MeHg by rice seedlings. The dependency of the uptake of dissolved MeHg on the size of the RS-DOM fraction could perhaps be explained by the latter's composition. Higher-molecular-mass RS-DOM includes humic and fulvic acids that strongly bind (e.g., through thiol groups) with MeHg and thereby reduce its bioavailability.[47] By contrast, lower-molecular-mass RS-DOM is mainly composed of small organic acids, including acetic acid (accounting for ∼15.3% of RS-DOC in this study), citric acid, oxalic acid, and maleic acid,[9,48] with relatively low affinities for MeHg. Therefore, the uptake of dissolved MeHg by seedlings was less affected by lower-molecular-mass RS-DOM. It should be noted that the size distribution and specific composition of RS-DOM may differ following the long-term vs short-term amendment of soil with straw (e.g., due to humification). The effects of aged RS-DOM on plant uptake of dissolved MeHg should therefore be investigated in further studies.

Changes in root growth and morphology may also affect MeHg uptake by root. We showed that RS-DOM had a significant effect on rice root morphology and thus on the uptake of dissolved MeHg by roots. In the sand culture experiments, the root lengths of rice seedlings cultivated with RS-DOM increased significantly, by 41% after 10 days and 36% after 20 days, with tip numbers increasing by 105% and 60%, respectively (Figure 4A,B). The uptake of chemicals by plant roots was previously shown to be proportional to root length and tip number,[49] which would explain the 76% higher uptake of dissolved MeHg (in 0.01 M $CaCl_2$ solution) by RS-DOM-cultured rice seedlings than by CK seedlings. The documented positive effects of straw amendment on plant growth have been attributed to the enhanced microbial production of indole acetic acid by the decomposition of rice straw,[50] the release of dissolved/phytoavailable Si, which would promote root elongation and plant growth,[51] and an increase in nutrient (e.g., Bray-2 P, Olsen-P, N, NH_4OAC extractable N, P, K, Ca, and Mg) availability and utilization by crops as well as a reduction in nutrient loss, especially that of N,[7] in farming soils. All of these may also benefit root growth and thus stimulate MeHg uptake from soil.

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The increased mobility of MeHg in the presence of RS-DOM together with the increased ability of roots to absorb dissolved MeHg likely explain the enhanced MeHg uptake after straw amendment. In addition to RS-DOM, other factors should be considered in future studies. For instance, it has been suggested that amendment with Si-rich material (~5%, mainly present as phytolith) into soil could affect the biogeochemistry and uptake of metals (e.g., As and Cd) by rice plants.[52] Whether the Si content in amended rice straw has a similar effect on the uptake of MeHg by plants remains to be determined.

Increased MeHg Translocation to Grains within Rice Plants. The results of our pot experiments indicate that, following straw amendment, MeHg concentrations in rice grains (95% higher, RS vs CK) increased disproportionally relative to those of the whole plant (58% higher, RS vs CK). Conversely, MeHg levels in the straw of straw-amended soils decreased (Figure 2B), whereas the translocation of MeHg from root to aboveground tissues was enhanced following the cultivation of rice seedlings in RS-DOM (Figure 4D). Thus, straw amendment seems to boost MeHg translocation within rice plants, especially to grains.

The increased MeHg distribution to rice grains may have occurred in tandem with enhanced material (amino acids, N, and S) transport after straw amendment: The increased translocations of TAA, Cys, Glu, N, and S (Figure S5) provide initial evidence of facilitated material transport (including MeHg) to grains. The transport of materials among plant tissues was shown to be enhanced under beneficial growth conditions,[51] such as provided by straw amendment. Since MeHg strongly binds amino acids, especially Cys and Glu,[53] MeHg may have been cotransported with those ligands, and its translocation to rice grains thereby enhanced. For instance, a recent study using XANES analysis showed that the majority of MeHg present in rice grains is in the form of MeHg-Cys complexes.[54] Unfortunately, MeHg levels in rice plant tissues in this study were too low for XANES analysis. Further studies are necessary to explore the underlying mechanisms of increased MeHg translocation to grains after straw amendment, such as by analyzing MeHg speciation in various plant tissues.

Our study provides multiple lines of evidence that straw amendment of soils: (1) increases SRB abundances/activities and modifies Hg speciation, thus facilitating the microbial transformation of Hg to MeHg in soils; (2) increases MeHg mobility, root length, and tip number, and thus root uptake of MeHg; and (3) enhances MeHg translocation to grains, leading to increased grain MeHg levels. Together, these results suggest that the input of fresh OM, such as straw and other crop residues, into soils might increase the risk associated with MeHg. Therefore, straw-return-induced increases in MeHg production and bioaccumulation should be considered when developing policies for straw management, in both Hg-contaminated and noncontaminated areas.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b07145.

Materials and methods; additional discussion; additional references; results of field survey (Figure S1); Eh in incubation experiments (Figure S2); DOC and DOC-MeHg relationship in pot experiments (Figure S3); tissue biomass in pot experiments (Figure S4); material distribution (Figure S5); SRB abundances and DOC levels in incubation experiments (Figure S6); DOC-SRB relationship (Figure.S7); *hgcA* gene copy numbers (Figure S8); sulfate and dissolved Fe (Figure S9); pH (Figure S10); XANES (Figure S11); MeHg in overlying water and Kds (Figure S12); results of RS-DOM on MeHg uptake (Figure S13); DOC-MeHg and sulfate-MeHg relationships (Figure S14); properties of Xunyang soil (Table S1); chemicals (Table S2); information on 64 sites (Table S3); XANES (Table S4); and recoveries of standard reference materials (Table S5) (PDF)

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