

# Effects of sulfate and selenite on mercury methylation in a mercury-contaminated rice paddy soil under anoxic conditions

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**Abstract** Biogeochemical cycling of sulfur and selenium (Se) could play an important role in methylmercury (MeHg) dynamics in soil, while their potential effects on MeHg production in rice paddy soil are less understood. The main objective of this study was to explore the effects of sulfate and selenite on net MeHg production in contaminated rice paddy soil, characterized with massive MeHg production and thus MeHg accumulation in rice. A series of microcosm incubation experiments were conducted using a contaminated paddy soil amended with sulfate and/or selenite, in which sulfate-reducing bacteria were mainly responsible for MeHg production. Our results demonstrated that sulfate addition reduced solid and dissolved MeHg levels in soils by  $\leq 18$  and  $\leq 25$  %, respectively. Compared to sulfate, selenite was more effective in inhibiting net MeHg production, and the inhibitory effect

depended largely on amended selenite doses. Moreover, sulfate input played a dual role in affecting Hg-Se interactions in soil, which could be explained by the dynamics of sulfate under anoxic conditions. Therefore, the effects of sulfate and selenium input should be carefully considered when assessing risk of Hg in anoxic environments (e.g., rice paddy field and wetland).

**Keywords** Methylmercury · Sulfate · Selenium · Paddy soil

## Introduction

Methylmercury (MeHg), an extremely toxic species of mercury (Hg), tends to accumulate along the food chain and poses a potential threat to human and wildlife health (Mergler et al. 2007; Li and Tse 2015). Recent studies reveal that MeHg could accumulate in rice plants (Feng et al. 2008; Qiu et al. 2012), especially in rice grain (Zhang et al. 2010). Grain MeHg derives mainly from rice paddy soil (Meng et al. 2010; Zhou et al. 2015) because the flooding conditions during rice growth favor mercury methylation by anaerobic bacteria (e.g., sulfate-reducing, iron-reducing bacteria and/or methanogens, Liu et al. 2014; Wang et al. 2014a; Goñi-Urriza et al. 2015). Therefore, it is of importance to investigate the key factors affecting MeHg production in paddy soil.

Selenium (Se) is an essential trace element for plants, animals, and human beings (Zhu et al. 2009). Previous pot or hydroponic experiments demonstrate that Se addition could effectively reduce inorganic mercury (IHg) accumulation in plants such as radish and garlic (Shanker et al. 1996; Zhao et al. 2013). Recently, a field study shows that translocation of both MeHg and IHg into aboveground tissues of rice decrease with increasing Se concentrations in soil (Zhang et al. 2012). The observed inhibitory effect of Se on both Hg

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accumulations in rice plants has also been demonstrated elsewhere (Wang et al. 2014b; Zhao et al. 2014). The authors propose that the formation of less bioavailable Hg-Se complexes in the rhizosphere and/or root may explain the observed Hg-Se antagonisms (Zhang et al. 2012; Wang et al. 2014b; Zhao et al. 2014). Thus, Se amendment into soils might be an effective approach to reduce soil MeHg concentrations and thus MeHg accumulation in rice.

Sulfate also plays an important role in mercury methylation. At low levels, sulfate could enhance activities of sulfate-reducing bacteria (SRB), the principal methylator under anoxic conditions, and consequently increase net MeHg production in soil (Gilmour et al. 1992). However, at high levels, the products of sulfate reduction (e.g., sulfides, polysulfides, and organic thiols) could inhibit IHg methylation via formation of Hg-Se complexes (Gilmour et al. 1998; Han et al. 2008). Although paddy soils often contain low or moderate levels of sulfate (5.8–633 mg kg<sup>-1</sup>, Fulda et al. 2013), sulfate could be introduced into paddy soils through fertilization (Liu et al. 1990), atmospheric deposition, and/or irrigation (Hu et al. 2002). Therefore, the effects of sulfate input on mercury methylation in contaminated paddy soils warrant investigation. Especially, it remains unclear how sulfate input affects Hg-Se interactions in paddy soil.

The main objective of this study was to explore the effects of sulfate and/or selenite addition on net MeHg production in soil under anoxic conditions. A series of microcosm incubation experiments were conducted using a field-collected contaminated paddy soil amended with sulfate and/or selenite. And MeHg concentrations in the solid and dissolved phases of soils were monitored at different time.

## Materials and methods

### Soil collection and preparation

Soil samples were collected from the surface (0–20 cm) of rice paddy fields in a mercury mining area in Xunyang, Shanxi Province, China. Detailed information of the mining area is given in previous studies (Zhang et al. 2009; Qiu et al. 2012). The soil samples were air-dried, sieved to an effective diameter of  $\leq 0.36$  mm, and mixed homogeneously before being used in the microcosm experiments. The soil was neutral (pH  $6.5 \pm 0.1$ ) and contained  $332 \pm 5$  mg kg<sup>-1</sup> total sulfur and  $158 \pm 5$  mg kg<sup>-1</sup> exchangeable sulfate, respectively. The measured selenium, total mercury, and methylmercury concentrations in the paddy soil were  $0.51 \pm 0.22$  mg kg<sup>-1</sup>,  $32.9 \pm 0.4$  mg kg<sup>-1</sup>, and  $4.35 \pm 0.07$   $\mu$ g kg<sup>-1</sup>, respectively, with  $2.6 \pm 0.2$  % total organic carbon.

### Experimental design

There were totally nine treatments (Table 1) with triplicate per treatment. To enhance microbial activity, 5 mM sodium lactate (Fulda et al. 2013) was added (referred as control hereafter), in companion with a blank treatment without lactate addition. In this study, selenite was chosen because it was one of the dominant Se species in soil (Jayaweera and Biggar 1996), and a high dose (3.0 mg kg<sup>-1</sup> Se, referred to as 3.0Se) or low dose (0.5 mg kg<sup>-1</sup> Se, referred to as 0.5Se) of selenite was amended into the soil to evaluate the potential effect of variations in Se concentrations on MeHg production. The amended Se levels (0.5 or 3 mg kg<sup>-1</sup>) were within the range of the reported soil Se levels in the field (0.16–36.6 mg kg<sup>-1</sup>, Zhang et al. 2012). Sulfate was amended at 800 mg kg<sup>-1</sup>, which was environmentally relevant (Michael et al. 2015) and has been reported to affect Hg biogeochemistry in sediments (Gilmour et al. 1998; Han et al. 2008), in the absence or presence of 0.5Se or 3.0Se (referred to as S, 0.5Se+S, or 3.0Se+S). In addition, the role of methanogens (MPA) or SRB in mercury methylation in the presence of sulfate was examined by adding sodium-2-bromoethanesulfonate (BES, 30 mM in soil solution, inhibiting MPA activity) or sodium molybdate (Na<sub>2</sub>MoO<sub>4</sub>, 20 mM in soil solution, inhibiting SRB activity) (St. Pierre et al. 2014). And the two treatments were referred to as S-MPA and S-SRB.

### Soil incubation and sampling

Incubation experiments were conducted in the laboratory using 50-mL centrifuge tubes (Corning, USA) as batch reactors. Briefly, 10-g air-dried soil was amended with different volumes of stock solutions of Na<sub>2</sub>SO<sub>4</sub> (2000 mg L<sup>-1</sup>), Na<sub>2</sub>SeO<sub>3</sub> (50 mg L<sup>-1</sup>), Na<sub>2</sub>MoO<sub>4</sub> (0.5 M), BES (0.5 M), and/or Na-lactate (1.5 M). Prior to incubation, pH in all solutions was adjusted to 6.5 using 0.1 M NaOH, and the tubes were filled with Milli-Q water to a total volume of 30 mL. All tubes were sealed and incubated in the dark at 28 °C for 20 days. The tubes were mixed manually twice per day. Both solution and soil were sampled on days 0 (4 h after equilibrium), 5, 10, and 20. The redox potential relative to the Ag/AgCl reference electrode (Eh), pH, and dissolved oxygen in the solutions were measured using HQ30d multi-parameter meters (HACH, USA). After that, mixtures were centrifuged at 2900×g for 20 min, and the supernatants were filtered through 0.45- $\mu$ m polyethersulfone filter capsules. For dissolved MeHg and sulfate analysis, the aliquots of the filtered supernatants were preserved with 0.4 % HCl (v/v) at -20 °C. Aliquots for total dissolved Se determination were preserved with 2 % HNO<sub>3</sub> (v/v) at -20 °C. Soil subsamples were frozen at -20 °C for MeHg measurement. All laboratory operations, except centrifugation, were conducted in a glovebag (AtmosBag, Aldrich) filled with nitrogen gas unless otherwise specified.

**Table 1** Experimental design

Description of treatment	Name of treatment
No carbon source	Blank
Lactate addition as carbon source	Control
Lactate+Na <sub>2</sub> SeO <sub>3</sub> (0.5 mg kg <sup>-1</sup> )	0.5Se
Lactate+Na <sub>2</sub> SeO <sub>3</sub> (3.0 mg kg <sup>-1</sup> )	3.0Se
Lactate+Na <sub>2</sub> SeO <sub>3</sub> (0.5 mg kg <sup>-1</sup> )+Na <sub>2</sub> SO <sub>4</sub>	0.5Se+S
Lactate+Na <sub>2</sub> SeO <sub>3</sub> (3.0 mg kg <sup>-1</sup> )+Na <sub>2</sub> SO <sub>4</sub>	3.0Se+S
Lactate+Na <sub>2</sub> SO <sub>4</sub>	S
Lactate+Na <sub>2</sub> SO <sub>4</sub> +Na-BES (methanogens/MPA inhibitor)	S-MPA
Lactate+Na <sub>2</sub> SO <sub>4</sub> +Na <sub>2</sub> MoO <sub>4</sub> (sulfate-reducing bacteria/SRB inhibitor)	S-SRB

### Chemical and statistical analysis

The total Hg concentrations in the soil samples were analyzed by Milestone DMA-80 direct mercury analyzer (Milestone, Italy) according to the US Environmental Protection Agency (EPA) Method 7473. Methylmercury in the soil was extracted with HNO<sub>3</sub>/CuSO<sub>4</sub>-CH<sub>2</sub>Cl<sub>2</sub> for 30 min (Liang et al. 2004). After that, an aliquot of the CH<sub>2</sub>Cl<sub>2</sub> was transferred to a centrifuge tube filled with Milli-Q water. The tubes were heated at 45 °C for 45 min and then purged with N<sub>2</sub> (80–100 mL min<sup>-1</sup>) for 10 min. Finally, an appropriate aliquot of the extractant (0.2–0.5 mL), the citrate buffer (0.5 mL), and the ethylation reagent (NaBEt<sub>4</sub>, 50 µL) were added into the vial containing Milli-Q water and determined for MeHg by cold vapor atomic fluorescence spectrometry (CVAFS, Brooks Rand, USA) following the US EPA Method 1630. Methylmercury in liquid samples was directly determined by CVAFS. For total Se analysis, the soil samples were digested by microwave digestion system (Ethos EZ, Milestone, Italy) using 8 mL HNO<sub>3</sub> and HF mixture (1:1 v/v) at 120 °C for 2.5 h. Concentrations of Se were measured by inductively coupled plasma mass spectrometry (NexION-300 ICP-MS, PerkinElmer, USA) with kinetic energy discrimination mode. Indium (In) was used as an internal standard during the analysis. Standard reference materials were used to ensure the quality of digestion and analysis. The measured concentrations of the total Hg and Se in reference soils (GBW07423 and GBW07405, National Research Center for Certified Reference Materials, China) were 34±1 µg kg<sup>-1</sup> and 1.78±0.16 mg kg<sup>-1</sup>, respectively, which were in good agreement with the certified values of 32±3 µg kg<sup>-1</sup> and 1.60±0.20 mg kg<sup>-1</sup>. The determined MeHg concentration was 70±4 µg kg<sup>-1</sup> in estuarine sediment (ERM-CC580, Institute for Reference Materials and Measurements, Belgium), with the certified values of 75±4 µg kg<sup>-1</sup>. The recoveries of Hg, Se, and MeHg in the soil were 106±2, 111±10 and 94±6 %, respectively (means±SD, n=3).

Soil pH was measured using HQ30d following the method described in Fulda et al. 2013. The total organic carbon and

sulfur in soils were determined by an element analyzer (vario TOC cube, Elementar, Germany). The exchangeable sulfate in soils was extracted using 0.5 M NaHCO<sub>3</sub> (Weber et al. 2009). And sulfate in the extracts as well as the filtered supernatants (i.e., soil solution) were analyzed by ion chromatography (Dionex ICS1100, Thermo Fisher Scientific, USA). Water content in the soils was determined by drying the soils at 105 °C for 48 h. And all solid phase concentrations are reported on a dry weight basis.

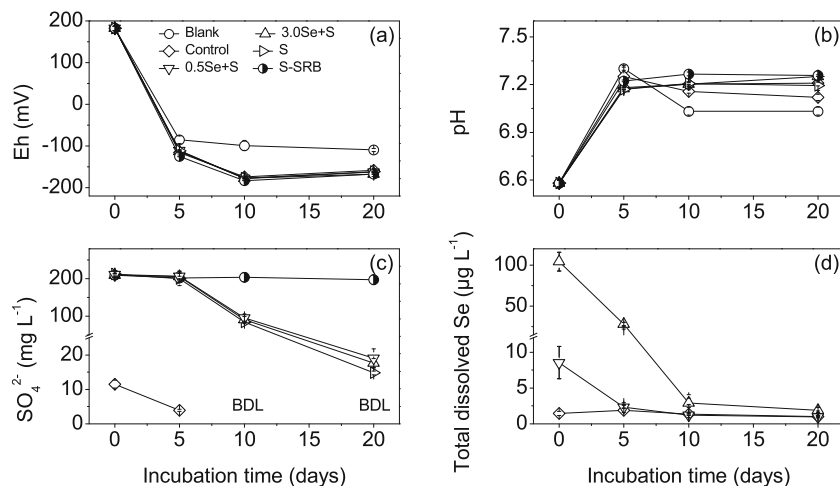
Statistical analysis was performed using SPSS 16.0. One-way analysis of variance (ANOVA) was used to test for differences among the treatments followed by Tukey's HSD test ( $p < 0.05$ ).

## Results and discussion

### Soil reduction under flooding condition

Changes in Eh, pH, sulfate, and total dissolved Se in the dissolved phase of soil were monitored throughout the experiment and shown in Fig. 1 and supplementary information Fig S1. Eh decreased rapidly within the first 5 days (from 180 to ~-110 mV in all lactate-amended treatments and -85 mV in the blank), followed by a slight decrease from days 5 to 10. After that, Eh remained at a relatively constant level (~-170 mV in all lactate-amended treatments and -110 mV in blank) until the end of the experiment (Fig. 1a and S1a). Meanwhile, pH in all treatments increased within the first 5 days (from ~6.5 to ~7.2) and remained relatively constant at ~7.2 after that (Fig. 1b and S1b). Dissolved sulfate levels decreased sharply after day 5 in sulfate-added treatments (i.e., S, 0.5Se+S, and 3.0Se+S, Fig. 1c) and became relatively low on day 20 (~20 mg L<sup>-1</sup>). Moreover, total dissolved Se levels decreased rapidly from ~104 to ~3 µg L<sup>-1</sup> in 3.0Se and 3.0Se+S within 10 days (Fig. 1d and S1c). Similar decreasing trends were also observed in 0.5Se and 0.5Se+S (Fig. 1d and S1c).

Lactate amendment affected soil characteristics markedly (e.g., Eh and dissolved oxygen) and thus MeHg levels, when



**Fig. 1** Temporal changes of Eh (a), pH (b), sulfate (c), and total dissolved Se (d) concentrations in the dissolved phase of soils throughout the experiment period. Sulfate levels were measured in the control and sulfate-amended treatments, including S, 0.5Se+S, 3.0Se+S, and S-SRB. Total dissolved Se levels were measured in the control and

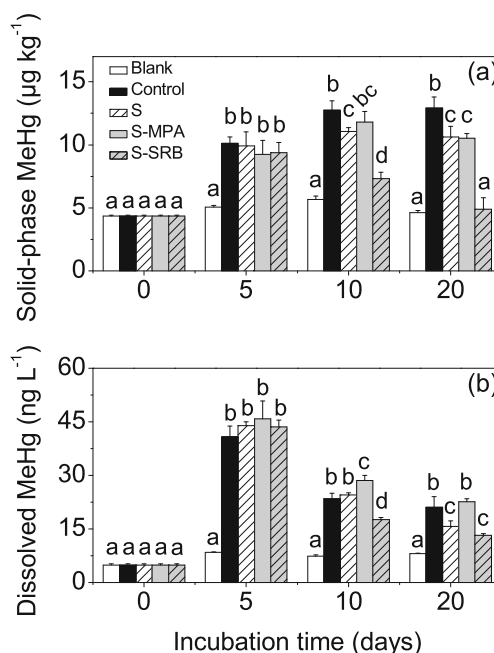
Se-amended treatments, including 0.5Se, 0.5Se+S, 3.0Se, and 3.0Se+S. BDL: levels of sulfate in solution below detection limits (0.1 mg L<sup>-1</sup>) from day 10 to 20 in the control. Data are shown as mean±standard deviation (n=3). Parts of data were presented in supplementary information Fig. S1

compared to the blank. For instance, lactate-amended treatments were lower in Eh, indicating a stronger anoxic condition and higher consumption rate of dissolved oxygen (~1.7 vs 1.4 mg L<sup>-1</sup> day<sup>-1</sup>) than the blank. Meanwhile, lactate served as an important carbon source for microorganisms (Fulda et al. 2013), and lactate addition may enhance the activity of methylation microorganisms (Ullrich et al. 2001) as well, which subsequently resulted in higher levels of MeHg (Fig. 2, control vs blank). Furthermore, MeHg levels in the solid and dissolved phases increased rapidly within 5 days in all lactate-amended treatments but became less variable after that (Figs. 2 and 3), suggesting the key role of lactate in enhancing microbial production of MeHg in the early stage of soil reduction.

**The potential methylators in the soil**

In the presence of sulfate, temporal changes of MeHg levels in the solid and dissolved phases are demonstrated in Fig. 2. When compared to the S treatment, the addition of molybdate (SRB inhibitor) in the S-SRB treatment reduced MeHg levels on days 10 and 20. For example, MeHg levels in the solid phase decreased by 34 % (day 10) and 54 % (day 20) compared to the S treatment. Meanwhile, dissolved MeHg levels declined by 25 % (day 10) and 37 % (day 20) under molybdate addition. In parallel, molybdate inhibited sulfate reduction by more than 93 % (Fig. 1c). These results suggested that SRB played a critical role in MeHg production between days 10 and 20. Therefore, it would be interesting to examine the potential effects of sulfate addition on microbial production of MeHg in the tested soil. In contrast, MPA inhibitor did not affect soil/dissolved MeHg levels significantly, implying that methanogens played a minor role in mercury methylation.

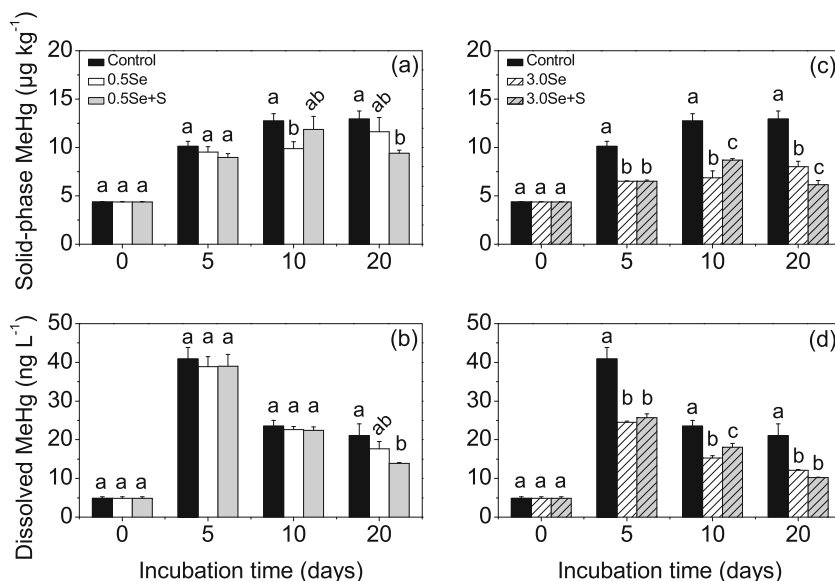
Differently, molybdate had no significant effects on net MeHg production within the first 5 days (Fig. 2). Although SRB activity was not determined in this study, the relatively slow decrease in sulfate concentrations (Fig. 1c) may suggest low activity of SRB in this stage. Under this circumstance, other microbial methylators (e.g., iron-reducing bacteria), which was also reported in previous studies (Fleming et al.



**Fig. 2** Temporal changes of MeHg in the solid and dissolved phases of soils throughout the experiment period. **a** MeHg in the solid phase. **b** MeHg in the dissolved phase. Data are shown as mean±standard deviation (n=3). Different letters within the same day indicate significant differences among treatments (p<0.05)



**Fig. 3** Temporal changes of MeHg in the solid (a, b) and dissolved (c, d) phases of soils throughout the experiment in the presence of selenite or sulfate. a, b MeHg in the control, 0.5Se, and 0.5Se+S treatments. c, d MeHg in the control, 3.0Se, and 3.0Se+S treatments. Data are shown as mean±standard deviation ( $n=3$ ). Different letters within the same day indicate the significant differences among treatments ( $p<0.05$ )



2006; Kerin et al. 2006), rather than SRB could also be responsible for IHg methylation.

### Effects of sulfate on net MeHg production

Addition of sulfate had no significant effects on net MeHg production within the first 5 days relative to the control (Fig. 2). Similarly, previous studies also demonstrated that sulfate addition had a limited effect on net MeHg production within a short time (Gilmour et al. 1992; Han et al. 2008). Possible explanation is that sulfate was not a limiting factor at this stage, indicated by the low sulfate reduction rate: sulfate reduction rate in the S treatment was much lower within the first 5 days ( $2.4 \text{ mg L}^{-1} \text{ day}^{-1}$ ) than that from days 5 to 10 ( $23 \text{ mg L}^{-1} \text{ day}^{-1}$ ) (Fig. 1c). Previous studies demonstrated that sulfate was reduced after the depletion of oxygen, nitrate, and Mn(IV/III)-/Fe(III)-(hydr)oxides (Weber et al. 2009; Fulda et al. 2013), which may explain the observed slow reduction of sulfate within the first 5 days.

With a rapid decrease in sulfate levels from days 5 to 20 (Fig. 1c), the soil MeHg levels declined significantly by 13 % (day 10) and 18 % (day 20) compared to the control ( $p<0.05$ , Fig. 2a). The inhibitory effects of sulfate on net MeHg production could be attributed to the reduced availability of IHg for methylating microbes. Iron sulfides were observed in this study, as indicated by the grayish black color on the surface of the soil samples. Iron sulfides (FeS(s)) could either absorb dissolved IHg to form less bioavailable FeS-Hg surface complexes or react with dissolved IHg to form  $\beta$ -HgS(s) precipitation, which may result in decline in IHg availability to the methylating microorganisms (Skylberg and Drott 2010; Jonsson et al. 2012). Thus, formation of FeS(s) could play a key role in inhibiting IHg methylation. Alternatively, the reduced sulfate species, e.g., polysulfides and organic thiols,

had high affinity for IHg (Skylberg and Drott 2010) and may also reduce IHg availability to methylators. Although sulfate may facilitate MeHg production by enhancing SRB activity (King et al. 2000), this positive effect of sulfate addition on MeHg levels could be less important compared to the inhibitory effects mentioned above.

### Effects of Se on net MeHg production

In line with previous studies that MeHg levels declined with increasing Se amendment in sediment or rice paddy soil (Jin et al. 1999; Wang et al. 2014b), our study demonstrated that the inhibitory effects of Se addition on net MeHg production depended on Se doses (Fig. 3). For instance, MeHg levels in the solid or dissolved phases decreased by 36–46 % (Fig. 3c) or by 37–51 % (Fig. 3d) following  $3 \text{ mg kg}^{-1}$  Se amendment (i.e., 3.0Se) relative to the control, while MeHg levels were rather comparable in the 0.5Se treatment and the control (Fig. 3a and b).

The inhibitory effect could be partially attributed to the formation of less bioavailable Hg-Se complexes, which would reduce IHg availability to microbial methylators. Indeed, rapid Se transformation in soils was observed in previous studies (Oremland et al. 1989; Jayaweera and Biggar 1996; Jain et al. 2015), and the products such as selenide, elemental Se, and organic Se could thermodynamically react with IHg to form HgSe precipitation ( $\log K_{sp}=-58$ , Björnberg et al. 1988), and thus reduce IHg availability to methylators (Yang et al. 2008). Similarly, selenite addition resulted in decreased MeHg concentrations in the culture of SRB *Desulfovibrio desulfuricans* (Truong et al. 2013), and inert solid HgSe was identified by X-ray absorption spectroscopy (Truong et al. 2014). Moreover, the inhibitory effects of selenite addition at higher level (i.e.,  $3.0 \text{ mg kg}^{-1}$  Se) on net MeHg production were more evident

than that of sulfate over the course of experiment (Figs. 2 and 3c and d). Possible reasons are that selenite reduction could be more sensitive to redox changes than sulfate reduction (Oremland et al. 1989) and the reduced Se species have a higher affinity with IHg than the reduced S species (Yang et al. 2008), leading to the higher ability of Se to reduce IHg availability to microbial methylators. Nevertheless, future study is warranted to further clarify the underlying mechanisms.

### Effects of sulfate on Hg-Se interactions

When compared to those added with selenite only, as shown in Fig. 3, sulfate addition exhibited no evident effects on net MeHg production within the first 5 days. The insignificant effect of sulfate on net MeHg production in the presence of Se may be due to the less important role of sulfate and thus SRB in controlling MeHg production within 5 days, as discussed above.

After 5 days, sulfate played a dual role in affecting MeHg levels in the dissolved and solid phases of soils. On day 10, MeHg levels were moderately higher in treatments added with both selenite and sulfate (0.5Se+S and 3.0Se+S) than those with selenite only (0.5Se and 3.0Se, Fig. 3), indicating that sulfate addition could partly offset the inhibitory effects of Se on MeHg production. It was possible that sulfate input may increase activity of methylation microorganisms (e.g., SRB, Gilmour et al. 1992). Besides, formation of neutral Hg-sulfides (e.g.,  $\text{HgS}_{\text{nanoparticles}}$ ) has been reported (Graham et al. 2012). Neutral Hg-sulfides were believed to be available to microbial methylators (Jonsson et al. 2012; Zhang et al. 2014), while HgSe was less bioavailable (Yang et al. 2008). And this may also explain the generally higher MeHg levels in the Se+S treatments than the Se only treatments. However in the dissolved phase, MeHg levels were comparable in the 0.5Se and 0.5Se+S treatments (Fig. 3b). Inconsistent trends between MeHg in the solid and dissolved phases could possibly be attributed to MeHg partitioning between the two phases. Meanwhile, MeHg levels were significantly lower in 3.0Se+S than the control, probably because that the sulfate addition was insufficient to offset the inhibitory effects of high level of Se on IHg methylation.

In contrast, MeHg levels were generally lower (16–23 %) in soils added with selenite and sulfate (0.5Se+S and 3.0Se+S) than those with selenite only (0.5Se and 3.0Se, Fig. 3) on day 20. This may because large amount of iron sulfides and other reduced sulfate species could be produced following sulfate reduction, suggested by the relatively low sulfate levels on day 20 ( $\sim 20 \text{ mg L}^{-1}$ , Fig. 1c). And this would largely reduce IHg bioavailability and MeHg production (Skylberg and Drott 2010; Jonsson et al. 2012). Meanwhile, sulfate was gradually exhausted over time, and thus sulfate concentrations became extremely low on day 20. Therefore, the promoting

effects of sulfate addition on SRB might be minimal at this stage. Consequently, the combined effects of Se and sulfate addition resulted in further decrease in MeHg concentrations compared to Se-added soils.

### Conclusion

Our data demonstrated that selenite addition could effectively reduce net MeHg production through the experiment, depending on Se doses. Meanwhile, sulfate addition played a dual role in affecting Hg-Se interactions in soil and thus soil MeHg concentrations: (1) the formation of iron sulfides and other reduced sulfur species under anoxic conditions may bind strongly with IHg and further reduce IHg methylation in the presence of Se; (2) sulfate could partly offset the inhibitory effects of Se on net MeHg production, probably by enhancing SRB activity and facilitating microbial production of MeHg. Our results emphasized the necessity to consider both Se and sulfate dynamics when investigating MeHg production in soils under anoxic conditions. And future studies quantifying temporal changes in microbial activities under sulfate and/or Se amendment would help better understand MeHg dynamics in anoxic soil, e.g., rice paddy soil.

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