Response to comments from reviewers 1

The study conducted by Zhao et al. takes on a challenging set of important questions regarding mercury in rice paddy soils. The study looks at two different systems, a point source polluted rice paddy at Gouxi, where artisanal mining of Hg occurs, and Wukeng, an abandoned mine with reduced atmospheric deposition of Hg. These two sites are commonly compared to Hauxi, a 'control site' of regional pollution. The design of the study is less-than ideal (see below in specific comments) but the temporal measurements of MeHg makes this study an asset for those interested in Hg in rice paddies.

On behalf of my co-authors, I would like to thank the anonymous reviewer for dedicating time to provide comments and criticism. The reviewer raises important issues, which have helped us to improve the manuscript. We have completed a substantial revision and our changes are highlighted in red color in the revised manuscript. Our point-by-point response to the reviewer's comments is as follows:

The manuscript falls short in a few areas. First, the paper lacks a strong, clear statement of hypotheses and the biological/chemical mechanism. By clearly stating the hypotheses, this will greatly aid in organizing the discussion and determine which mechanisms need to be specifically addressed in greater detail in the discussion. From my understanding of the manuscript one hypothesis should be "We hypothesize that theGouxi site will have greater MeHg than the Wukeng site because greater atmospheric deposition of 'new Hg' is more susceptible to methylation". A second hypothesis could be "We expect greater MeHg in the upper mineral soil horizons at the Gouxi site than Wukeng site because 'old Hg' is less susceptible to methylation". These are just suggestions but explicitly stating them is needed to guide the discussion.

We agree with the reviewer's comment that current manuscript needs a strong and clear-statement hypothese. On the basis of the reviewer's suggestion we have added hypotheses in the revised manuscript as follows: "on the basis of the previous studies we hypothesize that, 1) the active Hg methylation process occurs in rice paddy in artisanal Hg mining area; 2) in contrast, production of MeHg is limited in rice paddy in abandoned Hg mining area" (see page 3 lines 17-20)

In addition to the missing hypotheses, the inclusion of the water-atmospheric model was unnecessary inlieu of a more simplistic comparison of fluxes. The modeling made many assumptions that also made it unreasonable to apply (see specific comments).

Thank you for this suggestion. The authors provide the detailed response in the specific comments.

Lastly, Hg in Oryza sativa data should be presented in this study. Since the uptake of MeHg and Hg byrice is central to this study, linking the belowground processes to the plant would be agreat addition to the study.

We agree with the reviewer's comment herein. Current study focused on the speciation, distribution of Hg in the paddy soil. The rice plant sample was unavailable in this study. We previously collected twenty-two and thirteen whole rice plant samples from the abandoned Hg mining sites and the artisanal Hg mining site during the harvest seasons, respectively. Our results showed that the concentration of MeHg in rice grain collected from artisanal Hg mining areas (32±14 ng g^{-1}) was shown to be significantly higher than that in rice grain collected from abandoned Hg mining areas (7.0±3.2 ng g^{-1}). These sentences shown above were added in the revised manuscript (see page 3 lines 13-17).

Specific comments

The introduction lacks discussion of microbial and chemical mechanisms responsible for methylation. Even if you are not testing for methylating bacteria, the mechanisms of methylation should not be absent.

We agree with the reviewer's comments herein. The discussion concerning microbial and chemical mechanisms responsible for methylation was added in the revised manuscript as follows:

Current understanding is that the mobility and methylation of Hg in ephemeral flooded soil is determined by a range of factors, such as redox potential, pH, dissolved organic carbon, sulfur, iron, and dissolved Hg content (e.g., Ullrich et al., 2001; Benoit et al., 2001). Moreover, Hg methylation is largely facilitated by a subset of sulfate-reducing bacteria (SRB) (Gilmour et al., 1992) and/or iron-reducing bacteria (Fleming et al., 2006) in anoxic conditions. Specially, the methylation of inorganic Hg (IHg) in paddy soil primarily occurs under reducing conditions through a process mediated by sulfate-reducing bacteria (Peng et al., 2012; Rothenberg and Feng, 2012; Wang et 1 al., 2014; Liu et al., 2014; Liu et al., 2009; Somenahally et al., 2011) (see page 2 lines 23-27 and page 3 lines 1-3).

In the experimental design, I understand the practicality of monitoring two, 10 X 10 meter rice paddies. However, sampling one plot multiple times to assess an treatment/affect is potentially pseudoreplication.

As summarized in the manuscript "Changing redox parameters over the rice growing season may affect the process of Hg methylation. Previous studies have observed that in artificially Hg-polluted soil, Hg bioavailability to methylation can be significantly affected by the level of water saturation (Rothenberg and Feng, 2012; Wang et al., 2014; Peng et al., 2012). Peng et al. (2012) specified that intermittent flooding, as opposed to continuous flooding, could reduce soluble Hg concentrations and inhibit Hg methylation in the rice rhizosphere, subsequently decreasing the accumulation of MeHg in rice grain. Flooded conditions enhance anaerobic microbial activities and increase MeHg yields. Drying of a paddy field is an important cultivation step to control rice plant tillering and increase yield" (page 14 lines 9-15).

In this study the two selected experimental rice paddy were cultivated during the period 1st June through 10th September (100 days) 2012.Standing water (2-8 cm) was maintained above the soil surface (flooded condition) throughout the growing period, from Day 0 to Day 80. The paddy fields were thereafter drained from Day 80, prior to harvest between Days 90 to 100. During the 10 days draining period, approximately 2-4 cm depth of water was maintained above the soil surface (page 5 lines 12-16). In order to investigate the speciation, distribution, and Hg methylation in the paddy soil during the whole rice growing season, five consecutive sampling campaigns were conducted (1st June-10th September, 2012) (page 5 lines 20-21). Therefore, the authors think that sampling one plot multiple times is necessary, but not pseudoreplication.

For Section 3.3.2 on Soil Cores, the physical and chemical data of the soil cores shouldbe included in the discussion.

The authors agree with the reviewer's comments herein. Main physical and chemical parameters including pH and organic matter content in soil cores, were analyzed in this study and available in a companion paper (Zhao et al., 2016). Briefly, organic matter in soil cores averaged 4.8±0.75% and 3.5±0.59% at Gouxi and Wukeng, respectively. The pH values in soil samples, which averaged 6.7±0.10 at Gouxi and averaged 6.6±0.14 at Wukeng, were nearly

neutral during the rice growing season. Although the pH values of the irrigation water and paddy water at Wukeng being significantly higher than those at Gouxi (Table 5), no significant difference of pH levels in soil cores was observed between these two sampling sites throughout the five sampling campaigns, indicating that the irrigation water and paddy water have little influence on the values and distributions of pH in soil cores. Statistical analysis revealed that there is no direct impact of pH and organic matter content on MeHg concentrations in soil core across the two sampling sites, indicating that absolute pH and organic matter might not be the most important factors regulating Hg methylation activity (Zhao et al., 2016).The information shown above was added in the revised manuscript (see page 13 lines 24-27 and page 14 lines 1-8).

Was dissolved oxygen, sulfate, Fe or other important electron acceptors measured and comparable through time?

General Water Quality Characteristics of irrigation water and paddy water including pH, dissolved oxygen (DO) concentration, and temperature (T) were measured in situ and these data was listed in Table 4 (page 31 lines 1-2). In order to reveal the factors controlling Hg methylation in rice paddy, concentrations of Fe2+, Fe^{3+}, S^{2-}, and SO_4^{2-} in soil pore water were analyzed and were available in a companion paper (Zhao et al., 2016). Briefly, no discernible vertical trend in Fe^{3+} distribution was observed in the soil pore water across the two sampling sites during our sampling periods. The Fe^{2+} concentrations in soil pore water at Gouxi exhibited a relative narrow range (41~417 μM), whereas they ranged widely from 2.3 μM to 843 μM at Wukeng during the sampling periods. S^{2-} concentration in the soil pore water of Wukeng (0.70±0.36μM) showed a narrow scale variation with depth. A more obvious vertical variation was present in the corresponding data at Gouxi (1.8±0.79 μM), with the highest value presented in the surface soil layer. The relative temporal variation of sulfide concentrations among the three sampling campaigns at both Wukeng and Gouxi are less pronounced (K-W test, p=0.73 and p=0.33 for Wukeng and Gouxi, respectively). The highest SO_4^{2-} concentrations were present in the surface soil layer and decreased with depth across the two sampling sites. As concluded in the companion paper(Zhao et al., 2016), SO_4^{2-} stimulating the activity of SRB was a potentially important metabolic pathway for Hg methylation in rice paddy soil in Hg mining

area. Furthermore, the iron cycling in rice paddies could impact the availability of Hg for methylation in pore water. This information was added in the revised manuscript (see page 12 lines 16-28 and page 13 lines 1-2).

Page 13 Line 1 – 9: The results should be better integrated with existing knowledge about the effect of microbial production of MeHg in flooded soils.

The authors agree with the reviewer's comments herein. Therefore, we re-organized this paragraph as follows:

One possible reason for the considerably elevated MeHg concentrations in soil at Gouxi between Day 20 and Day 80 relative to Day 100 is an enhancement of Hg bioavailability and numbers of SRB under flooded conditions that stimulated Hg methylation, and increased the soil MeHg concentration (Wang et al., 2014). As the paddy field dried from Day 80, some degree of net MeHg degradation may have occurred, which could be attributed to the decreased SRB numbers and proportion of Hg methylators in the rhizosphere under aerobic conditions(Wang et al., 2014). This likely contributed to a decreasing trend in soil MeHg concentration during the harvest period (see page 14 lines 16-23)

The model is an interesting thought-experiment based on a number of assumptions such as negligible amounts of Hg volatilizing from the water surface and dynamic equilibrium of the aqueous solution. However, the assumption I find the hardest to justify is that the system is behaving as an unsaturated soil (as cited in Munthe and Hintelmann) and not behaving as a water-sediment system. I understand it simplifies the system to a traditional unsaturated agricultural system but the fact is the water and saturated soil (now behaving as a sediment) are exchanging with each other rather than acting as one system. Instead of the model in 3.4, is it possible to just compare the atmospheric Hg fluxes, irrigation Hg fluxes, and 'Old Hg' pools and find the same conclusion that Hg was primarily from atmospheric deposition and MeHg is produced in situ?

Yes, the authors definitely agree with the reviewer's comments herein. After carefully consideration jointly with the reviewer's suggestion, we simplified the model in the revised manuscript (see detail in section 3.4 page 15 lines 4-24 and page 16 lines 1-26).

Briefly, we estimated the relative flux of different Hg vectors (atmospheric- and irrigation-derived Hg) to the rice paddy soil (depth of 20 cm) during the rice growing season. Furthermore, the amount of native THg and MeHg present in the paddy soil were calculated as

well. Our calculated data showed that the MeHg flux to the rice paddy soil attributable to atmospheric deposition (Gouxi=3.3 mg ha^{-1}; Wukeng=2.1 mg ha^{-1}) and irrigation (Gouxi=1.8 mg ha^{-1}; Wukeng=4.2 mg ha^{-1}) was 3 orders of magnitude smaller than the amount of native MeHg already present in the paddy soil (Gouxi=2026 mg ha^{-1}, Wukeng=1613 mg ha^{-1}). A similar low atmospheric deposition (Gouxi=1.8×10^{-2} mg ha^{-1}; Wukeng=3.1×10^{-3} mg ha^{-1}) and irrigation water (Gouxi=0.39×10^{-3} mg ha^{-1}; Wukeng=1.3×10^{-3} mg ha^{-1}) fluxes were apparent for THg (Table 4), when compared with the soil THg pool (Gouxi=3.2 mg ha^{-1}; Wukeng=32 mg ha^{-1}). Furthermore, the THg flux of the atmospheric deposition at Gouxi was approximately 6 times higher than at Wukeng during the rice growing season. Our calculations therefore suggest that despite the highly elevated THg concentration in atmospheric deposition and irrigation water, the flux of new Hg (MeHg and THg) from external sources was small because of the relatively large pool of old Hg in soil (Dai et al., 2013). Therefore, we propose that the dominant source of MeHg to the surface soil layer is *in situ* methylation of inorganic Hg (see page 16 lines 12-26).

Page 15 Line 25: Could you compare your data on estimated Hg methylation with other rice paddies in Asia to assess how alkaline conditions have slowed or retarded Hg methylation?

Yes we re-organized this paragraph and the following information was added in the revised manuscript:

Rothenberg et al. (2012) reported that the more alkaline condition (pH >11) in paddy water at the highly-contaminated site could restrain the bioavailability of $Hg^{2+}$for Hg methylation, resulting in lower pore water and soil MeHg concentrations despite higher total Hg concentrations, which in agree with our study. (see page 17 lines 10-13)

Technical comments

Page 2 Line 10: a strong bioaccumulator? There is an adjective missing. I might suggest re-writing the sentence.

Yes, we re-worked this sentence in the revised manuscript as follows: The contamination of methyl mercury (MeHg) in rice grain (*Oryza sativa*) has recently focussed scientific attention on this important agricultural crop (see page 2 lines 11-12).

Page 2 Line 15: In which part does the rice uptake Hg?

We re-organized this paragraph in the revised manuscript as follows: numerous studies have reported high MeHg concentrations in rice grain collected from Indonesia (Krisnayanti et al., 2012) and different parts of China (Qiu et al., 2008; Horvat et al., 2003; Meng et al., 2014) and the MeHg concentrations in rice grain (brown rice) can be enhanced even in cases where soil is not significantly elevated in Hg (Zhang et al., 2010a; Horvat et al., 2003). Recently, Meng et al. (2014) specified that the majority (~80%) of the more toxic species MeHg was found in edible white rice. (see page 2 lines 13-18)

Page 2 Line 27: Although its a common term in Hg literature, please define IHg.

Yes, we defined IHg as "inorganic Hg" in the revised manuscript. (seepage line)

Page 3 Line 21-25: I feel these details should be in the methods since they describe your actions.

Yes, these sentences were moved to section 2.2 (sample collection and preparation) in the revised manuscript. (see page 5 line 23-26)

Page 4: Is it possible to provide coarse latitude and longitude for the Wanshan mining district in the text?

Yes, we added latitude and longitude of the Wanshan mining district in the revised manuscript (see page 4 line 13).

Page 7 Line 3: The phrase 'under argon' is in exact. Please re-phrase with details.

Yes, detailed information was added in the revised manuscript as follows: firstly, the air (oxagen) in the glove bag was furthest eliminated manually. Then, the pure argon from a portable argon tank was injected into the glove bag through a Teflon tubing which was connected to the glove bag. This information was added in the revised manuscript. (see page 7 lines 15-17)

Page 7 Line 10: Extra period at beginning of sentence.

Yes, we re-worded this sentence in the revised manuscript as follows "on each sampling times (Days 0, 20, 40, 60, and 80) a second soil core was collected and immediately placed into liquid nitrogen." (see page 7 lines 24-25)

Page 8 Line 10: correct to EPA method 1630.

Yes, we re-worked this sentence in the revised manuscript as follows: "following EPA method 1630 (U.S. EPA, 2001)" (see page 8 line 20-21)

Page 8Line 12: Please define HgT_{unf} and HgT_f explicitly at first use.

Yes we defined HgT_{unf} and HgT_f as total Hg (HgT_{unf}) and dissolved total Hg (HgT_f),respectively, in the revised manuscript (see page 6 line 14 and page 6 line 20-21).

Page 8 Line 26-Page9 Line 1: Please include "respectively" to indicate the relationship between the blank concentrations with THg and MeHg.

Yes, we added "respectively" in the the corresponding sentence in the revised manuscript (see page 9 line 12).

Page 9 Line 13-15: Please mention these are non-parametric tests for those unfamiliar with those tests.

Yes, we added the information "non-parametric tests" in the revised manuscript. (see page 9 lines 26-27)

Pages 9-15: It is conventional for this journal to include a space between numbers and symbols, particularly when expressing the mean and standard deviation.

Yes, we revised the manuscript very carefully and added "space" between numbers and symbols throughout the manuscript.

Page 15 Line 6: Was this model used for Hg and MeHg?

This model was used for THg and MeHg in the soil surface layer. We re-worked this sentence in the revised manuscript (page 16 line 12)