Response to comments from reviewers 2

GENERAL COMMENTS

The study by Zhao et al. addresses Hg contamination and mthylation in paddy fields in the province of Guizhou, China. This is a topic of high importance for human and ecosystem health in paddy field areas. The study is relevant for Biogeosciences and falls within the Aims and scope. The methods are well explained. The results are well presented, although the clarity of the Tables should be improved. The discussion is generally good, but could benefit from more links to existing literature. I have two major comments that should be addressed to improve the paper:

We are again most thankful to the anonymous reviewer for dedicating their time to provide comments and criticism. The reviewer raises many important issues. We have considered these and made appropriate changes to the text which have definitely improves this manuscript. Revisions are shown in red color in the revised manuscript, with our point-by-point response presented as follows.

(1) the Hg balance model (eq. 3 and 4). I don't really see the added value of this model. Moreover, some assumptions are very strong (eg rice transpiration amount extremely low), and the athors compare the input of 'fresh' Hg (irrigation and deposition) of 1 year, to the 'old' Hg pool accumulated over the years. Therefore the Hg balance results entirely depend on the number of years during which Hg has accumulated in the surface layer (X years of paddy field irrigation, etc.). I recommend to completely revise the model or simply drop it (unless you can clearly demonstrate what it brings to the discussion and how it supports your conclusions)

Yes, the authors definitely agree with the reviewer's comments concerning the Hg balance model. Based on the reviewer's comments herein jointly with the suggestion from reviewer #1, we simplified the model in the revised manuscript (see detail in section 3.4).

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).

(2) the authors should pay more attention and discuss in more details to the biochemical processes affecting Hg methylation. What if the Wukeng soil had had a low pH more favorable to methylation? Would the conlusions of historical vs. artisanal Hg mining still hold? The important pH difference between the two sites prevents any conclusion regarding the impact on methylation of the "type" of Hg available (old at Wukeng vs fresh at Gouxi). If the redox and pH conditions are not good for methylation, it will not occur (whatever the 'type' of Hg present in the soil). I strongly recommend to discuss this (with additional literature references), and reformulate the conclusions taking this into account.

The authors agree with the reviewer's comments herein. Based on the reviewer's comments shown above we re-organized the discussion in section 3.3, section 3.5 and section 3.6 in the revised manuscript as follows:

1) Section 3.3.2:

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). (page 13 lines 24-27 and page 14 lines 1-8).

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. Therefore, 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. (page 14 lines 9-23)

2) Section 3.5

Both the irrigation water (pH=11±0.45) and paddy water (pH=8.6±1.3) were alkaline during the rice growing season (Table 5). We suggest that the alkaline conditions of the irrigation at Wukeng could restrain Hg methylation and/or stimulate MeHg demethylation (Ullrich et al., 2001). 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. The $MeHg_{unf}$ concentration in paddy water at Gouxi was significantly higher than the concentration in either the precipitation or the irrigation water and this implies active Hg methylation within the Gouxi rice paddy. Both

paddy water and irrigation water at Gouxi exhibit a pH that can be considered optimal for Hg methylation (Table 4), favouring net methylation in the paddy fields (Ullrich et al., 2001). (page 17 lines 7-17)

As concluded in a companion paper (Zhao et al., 2016), absolute pH and organic matter might not be the most important factors regulating Hg methylation activity in rice paddy soil. Therefore, we believe that restricted supply of newly deposited Hg to depths below the soil-water interface is a plausible explanation for the sharply reducing concentration of MeHg with depth at Gouxi; newly deposited Hg is constrained to surface soil and cannot be transferred to lower depth. Therefore, a direct positive relationship between HgT_f and $MeHg_f$ concentrations in soil pore water was observed at Gouxi during the rice growing season (see section 3.3.1). However, stimulated MeHg production due to favorable geochemical conditions (e.g. sulfate) at Gouxi cannot be excluded, due to the fact that sulfate stimulating the activity of SRB were a potentially important metabolic pathway for Hg methylation in rice paddy soil in Hg mining area (Zhao et al., 2016). (page 18 lines 4-13)

3) Section 3.6

We cannot distinguish between newly deposited Hg and old Hg stored in paddy soil over decades; this hypothesis needs further testing to obtain such information regarding Hg dynamics. In comparing across different typical Hg contaminated rice paddy fields, the crucial question regards assessing the pool of Hg available for methylation, which is crucial to estimating realistic and accurate methylation rates. To this point, detailed work is urgent to further ascertain the relative importance of newly deposited Hg versus in situ Hg in contribution to the Hg that is methylated and bioavailability in rice paddy ecosystems. (page 19 lines 14-21)

Because MeHg can be demethylated to IHg biotically and abiotically in soil or paddy water, rapid cycling occurs between the IHg and MeHg pools. Current data were limited to only the rice growing season, not the entire year or a long period of time, and therefore our results represented an initial rather than a long-term influence of newly deposited Hg on the MeHg production. Longer-term responses and a more complex landscape are needed in rice paddy ecosystems. The overall contributions of old versus newly deposited Hg to methylation in paddy soil and the overall activity time to change in atmospheric Hg deposition could likely

depend on the balance of Hg deposition and the rate of deposited Hg bound to soil complexes. This hypothesis does not imply that MeHg concentrations in rice paddies will necessarily recover quickly after implementation of Hg emission controls. The response of MeHg levels in rice paddies to reductions in Hg emissions will depend on how long previously deposited Hg is stored in paddy soil and its availability to SRB. This issue is poorly understood, but previous declines in Hg loading suggest that MeHg levels in soil from abandoned Hg mining areas begin to respond within a few years of Hg reductions. (page 19 lines 22-28 and page 20 lines 1-6)

SPECIFIC COMMENTS and TECHNICAL COMMENTS

(1) mention somewhere in the abstract the location (China, and at least the province)

Yes, the detailed location "Guizhou province China" was added in the revised manuscript (see page 1 lines 18-19)

(2) use "inorganic Hg" (or define the abbreviation earlier)

Yes, we defined IHg as inorganic Hg in the revised manuscript. (page2 line 1)

(3) sentence is unclear "in situ production of MeHg is dependent on elevated IHg in the atmosphere and on the deposition of"... ?

Yes, we re-organized this sentence in the revised manuscript as "We propose that the in situ production of MeHg is dependent on elevated Hg in the atmosphere and on the newly deposited Hg into a low pH anoxic geochemical system"(see page 2 lines 3-4).

(4) put the references chronologically.

Yes we re-organized references as (Horvat et al., 2003; Qiu et al., 2008; Meng et al., 2014) in the revised manuscript (page 2 lines 14-15).

(5) to support the assumed "consensus", more than one author should be provided.

Yes, two more references (Qiu et al., 2012; Yin et al., 2013) were added in the revised manuscript (page 3 line 10)

(6) large scale / small-scale: be consistent with the "-"

Yes, "large scale" was changed to "large-scale" in the revised manuscript (see page 3 line 13).

(7) these are all the possible compartments where Hg can be found... this sounds like an "empty" argument or too generic

The authors agree with the reviewer's comments herein. Therefore, we re-organized this sentence in the revised manuscript as follows: "Studies on MeHg and rice emphasize that factors that control the biochemical cycling of Hg within rice paddy ecosystems are very complex including the concentration and distribution of Hg in ambient air, wet/dry deposition, irrigation water, and solid and liquid phases of soil". (see page 3 lines 23-24)

(8) confusing. What is "assessing the status of Hg species" ?consider rephrasing ("we analyzed Hg speciation in ...")

The authors agree with the reviewer's comments herein. We re-worked this sentence in the revised manuscript as follows: "To expand our knowledge of the biochemical processes that affect Hg methylation we analyzed Hg speciation in different compartments of the rice paddy ecosystem." (see page 4 lines 5-6).

(9) if I'm correct you investigate methylation, not other transformations. Then replace by "Hg methylation"

Yes, we re-worked this sentence in the revised manuscript as follows "to assess the primary source and mechanism for Hg methylation within paddy soil at a Hg mining area." (see page 4 lines 7-8)

(10) can you be more precise about what you consider to be seepage and outflow? Is seepage infiltration to the subsoil ? Does outflow mean runoff ?

Yes, seepage infiltration is to the subsoil and outflow means runoff. We re-organized this sentence in the revised manuscript as follows "The paddy plots received water through

precipitation and stream water irrigation, while evaporation to air and seepage to the subsoil were the primary vectors for water loss. There was no direct runoff from either paddy." (page 5 lines 16-18)

(11) this is not the minus sign. Consider replacing all - by –

We revised the manuscript very carefully, and all the "-" was changed to "-" throughout the manuscript.

(12) can you clarify; do you mean the variability between the triplicate samples?

Yes, we re-worked this sentence in the revised manuscript as follows: "The variability between the triplicate samples were less than 7.5% for THg and MeHg analysis for both water and soil samples." (page 9 lines 14-15)

(13) I don't understand this sentence. Hg in precipitation is equal or closely linked to "wet deposition", while dry deposition is another process.

As shown in the manuscript both dry and wet atmospheric deposition were collected concurrently with the TGM measurement once every 20 days using this sampling method (see page 6 lines 11-13). Therefore, we defined the precipitation as "wet and dry deposition" in the revised manuscript (see page 10 line 9).

(14) what about the ratio for the Huaxi regional background, which is quite high for paddy water ? Any explanation ?

In comparison, the $MeHg_{unf}/HgT_{unf}$ ratios in precipitation (0.76±0.41 %), irrigation water (2.2±0.98 %), and paddy water (10±7.9 %) were relatively elevated, probably due to the lower HgT_{unf} concentrations in the corresponding samples (Meng et al., 2011). We added these sentences in the revised manuscript (see page 11 lines 13-16)

(15) was the difference significant ? K-W test ?

Yes, significant difference was observed ($p<0.01$). The detailed information concerning

statistical analysis data was added in the revised manuscript as follows: “K-W test, $p<0.01$” (see page 12 line 2).

(16) the term "mechanistic relationship" is too vague. If methylation is active, is it expected that HgT_f and $MeHg_f$ are correlated? (was it the case in your previous studies ? Meng et al 2014+ check literature). Then, if methylation is an important process at the artisanal mining site only, you can state it and try to explain why it is, and why not at the other site.

We believe that net MeHg production is principally governed by the supply of fresh deposited Hg to soil (page 19 lines 10-11). Restricted supply of newly deposited Hg to depths below the soil-water interface is a plausible explanation for the sharply reducing concentration of MeHg with depth at Gouxi; newly deposited Hg is constrained to surface soil and cannot be transferred to lower depth. Therefore, a direct positive relationship between HgT_f and $MeHg_f$ concentrations in soil pore water was observed at Gouxi during the rice growing season (page 18 lines 6-10).

The Wukeng site has received significant historic Hg deposition as a function of large scale mining, but is not currently receiving significant inputs of fresh Hg. Atmosphere-derived mercury is physically unstable and bioavailable when it first enters the rice paddy (Hintelmann et al., 2002; Schuster, 2011). Immediate reactions of this new Hg with soil constituents are governed by adsorption-desorption interactions with soil surfaces (Schuster, 1991) which favour the retention of Hg in the surface layers of the soil profile. Over time this newly deposited Hg will be transformed into more stable, less available forms (Schuster, 1991), and the net methylation potential of this Hg will consequently decrease. The relatively low MeHg concentration in soil at Wukeng is indicative of old Hg which has become tightly bound to soil complexes over time, and is unavailable for methylation (Hintelmann et al., 2002). Consequently, there is no correlation between HgT_f and $MeHg_f$ in soil pore water at Wukeng (see section 3.3.1). (see page 18 lines 14-24).

(17) I also believe this, but please insert references supporting this, as observed in other paddy field studies. Also, although it is implicitly stated, complete the sentence by reminding that after Day 80 the field is no more flooded, and hence methylation is probably stopped. --

edit OK I see now it is discussed a little bit further. Then, this sentence should be (re)moved, so that all interpretation is put together (not a bit here, and the rest further in the text)

The authors definitely agree with the reviewer's comments herein. We deleted this sentence in the revised manuscript. More detailed explanation was added in the revised manuscript as follows:

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. Therefore, 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 9-23).

(18) be more specific about the processes. E.g. redox conditions change when the file dis dried, therefore MeHg degradation occurs. + give references of the biochemical processes taking place.

Yes, we re-organized this sentence in the revised manuscript as follows: 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) (see page 14 lines 20-22)

(19) these references are relevant to a certain extent, but treated forest soils only. Are there references specific to paddy fields for this aspect ?

Yes, the authors agree with the reviewer's comments herein. We re-worked the model in the revised manuscript (see section 3.4 and response to comment #1). The references mentioned in this comment were removed from manuscript.

(20) not sure that "native" is the appropriate word. Native makes me think to geogenic i.e. "natural" Hg, while here it is mostly from anthropogenic sources. Consider rephrasing.

Yes, the "native Hg" was change to "old Hg" in the revised manuscript. Furthermore, "old Hg" was defined as "Mercury already present in the soil is termed 'old Hg', which can be either of geogenic and anthropogenic origin" in the revised manuscript (page 15 line 7-9).

(21) is this water accumulated in the rice ??? then 34 cm seems a lot ! but unfortunately I cannot check Lan et 2010. The amount of transpiration seems very, very low if I compare e.g. to Brunel et al (1992) WRR 28 (5):1407-1416.

Very sorry for our stupid mistake. After re-checked this data from the cited literature, we found that M_d is very low and needn't to be considered in this model. Hence, we removed M_d from equation 4. Furthermore, we re-calculated the data in the revised manuscript (page 16 lines 5-11).

(22) which? apart from runoff, what else could it be ? draining the paddy field ?

Mo is the cumulative amount of water lost by other pathways (e.g. animal activities and draining the paddy during the ripening period). We reworked this sentence in the revised manuscript. (see page 16 lines 8-9)

(23) MAJOR COMMENT.

You need to take into account that the pool of 'old' Hg is probably constituted (partly) by deposition and irrigation from previous years. The Hg balance that you implemented compares 1 year of Hg input via deposition and irrigation to Hg accumulation over XX years. Is this the purpose of the Hg balance model? What kind of useful information does this bring?

Yes, the authors definitely agree with the reviewer's comments herein. After carefully consideration, we re-worked the model in the revised manuscript (see detail in section 3.4 and the response to comment #1).

(25) what if the Wukeng soil had had a low pH more favorable to methylation ? The important difference of pH between the two sites prevents in my opinion any conclusion regarding a possible difference between old / fresh Hg available for methylation. If the redox and pH conditions are not good for methylation, it will not occur (whatever the 'type' of Hg present in the soil). I recommend to discuss this, and reformulate the conclusions taking this into account.

This comment is same to comment #2. Please go back to the detailed response to comment #2.

(26) this Table contains a lot of information but is difficult to read. One cannot easily see to which sample matrix each result belongs (I think the text in left column should be vertically aligned to the top - this might already improve the readability but please try to improve this Table).

Yes, we re-worked Table 2 based on the reviewer's comments in the revised manuscript (see page 28 lines 1-7 and page 29 lines 1-6)

(27) increase slightly axis tick label and legend font size.

Yes, we re-worked Figure 2 according the reviewer's comments in the revised manuscript (page 33 lines 1-4)