**Response to reviewers' comments**

Dear Reviewer:

We sincerely thank you for taking the time to review our manuscript titled "***Dissolved organic matter fosters core mercury-methylating microbiome for methylmercury production in paddy soils***". Your constructive feedback has been invaluable in improving the quality and clarity of our work. In response to your comments, we have carefully revised the manuscript and highlighted all changes in yellow for your convenience. Additionally, we have provided a detailed, point-by-point response to address each of your suggestions and concerns. We deeply appreciate your thoughtful review and believe that the revisions have significantly enhanced the manuscript. We hope that the revised version meets your expectations and satisfactorily addresses all the points raised during the review process.

With great gratitude,

Dr. Bo Meng, on behalf of all co-authors

# **Comment 1:** In the abstract, DOM was crucial in determining core Hg-methylating microbiome composition (65%), what does the 65% mean, please explain it in the text.

**Response: Thank you for your insightful question. The "65%" mentioned in the abstract refers to the contribution of DOM to the composition of the core Hg-methylating microbiome. This value was calculated using structural equation modeling (SEM) results based on the method described by Tao et al. (2015). Specifically, in SEM, the contribution of a variable to its target variable was calculated as the square of its path coefficient divided by the sum of the squares of all path coefficients pointing to the same target variable. Using this formula, we calculated the contribution of DOM to the core Hg-methylating microbiome, and the result showed that DOM contributed 65%.**

**To ensure clarity, we have revised the Materials and Methods section to provide a clearer explanation of the contribution calculation method in the revised manuscript as below:**

*"We further calculated the contribution of ecological parameters, including DOM, to the core Hg-methylating microbiome, and the contribution of the core Hg-methylating microbiome to MeHg production, following the approach described by Tao et al. This calculation was performed by determining the proportion of the squared path coefficient of each parameter relative to the sum of the squared path coefficients of all parameters influencing the same target variable (Tao et al., 2015)."* (Mian text, Lines 181-185).

# **Comment 2:** In the core Hg-methylating microbiome, how much does the Geobacter account? Why the authors use the Geobacter for pure incubation experiment? why not use the soil for incubation experiment? What criteria were used to select the Geobacter?

**Response: Thank you for these excellent questions. Below, we provide detailed responses to each point:**

1. **How much does the *Geobacter* account in the core Hg-methylating microbiome?**

**In the core Hg-methylating microbiome, *Geobacter* accounts for an average of 44.5% (36.2%, 40.5%, and 56.8% in NMS, MMS, and HMS soils, respectively) of the community, based on our analysis (Fig. 2c). This highlights its significant presence and potential role in Hg methylation within the studied system.**

**To explicitly include the microbial abundances of the three most abundant genera in each soil type, we have incorporated the specific values into the revised manuscript as follow:**

*"Although most microorganisms are not annotated, the three genera with the highest abundance in each soil type are as follows: In NMS, Geobacter (36.2%), Syntrophus (1.7%), and Desulfomonas (0.4%) dominate; in MMS, Geobacter (40.5%), Granulicella (2.9%), and Olavius (2.9%) are the most abundant; and in HMS, Geobacter (56.8%), Methanoregula (0.6%), and Granulicella (2.3%) prevail (Fig. 2c)."* (Mian text, Lines 227-231).

1. **Why did the authors use *Geobacter* for pure incubation experiments? Why not use soil for incubation experiments?**

**The primary reason for selecting *Geobacter* for pure incubation experiments is its well-documented and dominant role in Hg methylation in various environments, as supported by prior studies (Bravo et al., 2018; Zhong et al., 2024). By using *Geobacter*, we aimed to isolate and clearly demonstrate the direct effects of DOM on Hg-methylation rates and microbial activity, without the interference of complex soil matrix effects.**

**While soil incubation experiments could provide additional context for ecosystem-level processes, they would introduce confounding factors, such as competing microbial communities and varying physicochemical properties of the soil. Our approach using a pure culture system allows for a controlled study to elucidate specific microbial and biochemical mechanisms.**

**To explicitly clarify the rationale for using *Geobacter sulfurreducens* PCA in the incubation experiments, we have added the text in the Methods section as follows:**

*"Geobacter was selected for these pure incubation experiments due to its dominant role in mercury methylation and its ability to isolate the effects of DOM on methylation rates without the interference of soil matrix complexity."* (Mian text, Lines 160-161).

1. **What criteria were used to select *Geobacter*?**

***Geobacter* was chosen based on several criteria: (i) Its established status as a key Hg-methylating microorganism in diverse environments, including paddy soils (Zhong et al., 2024); (ii) Its high abundance within the core Hg-methylating microbiome observed in our study (Fig. 2c); (iii) Its metabolic versatility, which makes it an ideal model organism for studying mercury methylation pathways under controlled conditions (Schaefer and Morel, 2009). These factors collectively make *Geobacter* an excellent candidate for exploring DOM-microbe interactions and their implications for methylmercury production.**

**We hope this explanation clarifies our rationale and methodology.**

# **Comment 3:** Why wasn’t Hg speciation measured or presented to confirm the hypothesis?

**Response: Thank you for your insightful suggestion. While Hg speciation measurements would undoubtedly provide additional insights into the mechanisms of Hg methylation, the primary focus of this study was to investigate the role of DOM and microbial dynamics, particularly the core Hg-methylating microbiome, in driving MeHg production in paddy soils. This decision was made to prioritize a detailed examination of how DOM influences microbial Hg methylation through changes in community composition and activity.**

**Additionally, indirect evidence from random forest analysis and SEM highlighted the dominant influence of DOM on the core Hg-methylating microbiome compared to abiotic factors such as redox conditions and Hg bioavailability (Fig. S4 and Fig. 4). These results support the robustness of our conclusions regarding DOM's central role in Hg methylation.**

**While Hg speciation measurements were beyond the scope of this study, we acknowledge their importance and propose that future research combining direct Hg speciation analyses with DOM compositional studies could further elucidate how Hg speciation interacts with DOM and microbial communities to influence Hg methylation.**

**To better understand, the corresponding sentences are added in the revised manuscript as below:**

*"Future research could integrate direct Hg speciation measurements with detailed DOM compositional analyses to better understand how specific DOM components and Hg species interact to influence microbial Hg methylation."* (Mian text, Lines 334-336).

# **Comment 4:** The discussion would improve by explaining how specific components of DOM influence the distribution of Hg-methylating gene communities?

**Response: Thank you for this insightful comment. We agree that explaining how specific components of DOM influence the distribution of Hg-methylating gene communities would improve the discussion. To address this, we have revised the manuscript to elaborate on the mechanisms by which different DOM components, particularly low-molecular-weight organic acids, shape core Hg-methylating microbiomes.**

**Our study identified several key low-molecular-weight organic acids, such as oxalic acid, formic acid, and acetate, in paddy soils. These compounds play a pivotal role in stimulating core Hg-methylating microorganisms by providing essential carbon sources that fuel critical metabolic pathways. Specifically, *Geobacter sulfurreducens* and *Desulfovibrio desulfuricans* utilize acetate and fumarate in the TCA cycle (Hu et al., 2013; Liu et al., 2018), facilitating anaerobic respiration and electron transport processes that enhance Hg methylation rates. Similarly, methanogenic archaea like *Methanoregula* and *Methanosarcina* use formate and acetate through methanogenesis (Sakai et al., 2010; Schöne et al., 2022), further contributing to Hg methylation. These mechanisms suggest that low-molecular-weight organic acids selectively promote the growth and activity of Hg-methylating microorganisms, thereby influencing the distribution of Hg-methylating gene communities.**

**To better understand, the corresponding sentences are re-organized in the revised manuscript as follow:**

*"These findings demonstrate that DOM composition strongly influences microbial Hg methylation by stimulating key metabolic pathways. For instance, Geobacter sulfurreducens and Desulfovibrio desulfuricans use acetate and fumarate in the TCA cycle, supporting anaerobic respiration and electron transport that enhance Hg methylation (Hu et al., 2013; Liu et al., 2018b). Similarly, methanogenic archaea such as Methanoregula and Methanosarcina utilize formate and acetate through methanogenesis, further contributing to Hg methylation (Sakai et al., 2010; Schöne et al., 2022). Although metabolomic data were not included in this study, future research incorporating such analyses could provide valuable insights into how specific DOM components influence microbial metabolism and Hg methylation, revealing key metabolites and pathways such as acetate fermentation, methanogenesis, and electron transfer processes. This highlights how specific DOM components shape the core Hg-methylating microbiome and influence its role in MeHg production."* (Mian text, Lines 321-329).

# **Comment 5:** The conclusion needs to be strengthened. It currently restates results that are largely expected. Highlight the novel findings and their significance more clearly. Meanwhile, the environmental implication of this study can be discussed to demonstrate the impact of this study.

**Response: Following the reviewer's suggestion, we have revised the conclusion to better emphasize the novel findings of this study and their broader significance. Specifically, we highlighted the role of DOM in influencing the core Hg-methylating microbiome and its direct impact on MeHg production. Additionally, we briefly discussed the environmental implications of our findings, focusing on the potential effects of human activities and climate change on DOM dynamics and Hg methylation processes.**

**To better understand, the corresponding sentences are re-organized in the revised manuscript as follow:**

*"This study provides novel evidence that DOM significantly influences MeHg production by altering the composition and stimulating the activity of the core Hg-methylating microbiome. While DOM regulates the composition of other members of the Hg-methylating microbiome, its impact on MeHg production is primarily mediated through the core Hg-methylating microbiome. Using metagenomic binning and pure incubation experiments, we demonstrated that low-molecular-weight DOM directly promotes MeHg production by enhancing the metabolic activity of core Hg-methylating microorganisms. These findings underscore the central role of the core Hg-methylating microbiome in Hg cycling and highlight DOM as a critical driver of microbial Hg methylation. As human activities and climate change continue to alter DOM composition and concentration, their influence on Hg methylation dynamics warrants further investigation to better predict and mitigate Hg-related environmental and health risks."* (Mian text, Lines 351-359).

# **Reference**

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