**Response to reviewers' comments**

Dear aoxue Yin:

Thanks for taking the time to review our manuscript "***Dissolved organic matter fosters core mercury-methylating microbiome for methylmercury production in paddy soils***". We deeply appreciate your insightful and constructive feedback, which has significantly improved the clarity and overall quality of our work. We have carefully addressed all your comments and made the necessary revisions to the manuscript. For your convenience, the changes have been highlighted in yellow. Additionally, we provided a detailed point-by-point response to each of your comments below. We hope that the revised version meets your expectations and adequately addresses all the concerns raised in the review process.

With sincere appreciation,

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

# **General comments**

This manuscript presents an insightful study investigating the relationship between dissolved organic matter (DOM) and methylmercury (MeHg) production in paddy soils, particularly focusing on the core microbiome responsible for mercury methylation. The study employs advanced genomic and metagenomic techniques to identify key microbial taxa involved in mercury methylation and explores the impact of DOM on the activity of these microorganisms. The authors highlight that DOM plays a pivotal role in shaping the composition and functional activity of the Hg-methylating core microbiome, which in turn regulates MeHg production. The conclusions are supported by extensive field sampling, genome-resolved metagenomic analysis, and controlled incubation experiments.

**Response: Thank you for your valuable feedback on our manuscript. We appreciate your positive comments regarding the investigation of the relationship between dissolved organic matter (DOM) and methylmercury (MeHg) production in paddy soils, especially focusing on the core microbiome responsible for mercury methylation.**

**In response to your comments, we have carefully revised the manuscript. We hope the changes address all concerns and improve the overall quality of the work.**

# **Comment 1:** The discussion section could further expand on how the findings integrate with broader environmental mercury cycling processes. A more detailed comparison with existing literature on Hg-methylating microbes in non-paddy soils would enhance the paper’s contribution to microbial ecology.

**Response: Thank you for your valuable comment. We have expanded the discussion section to better integrate our findings with broader environmental mercury cycling processes, as you suggested. Specifically, we highlight how dissolved organic matter (DOM) impacts mercury methylation not only in paddy soils but also in other soil environments, comparing our findings with existing literature on Hg-methylating microbes in non-paddy soils. To enhance clarity, the corresponding sentences were added in the revised manuscript as below:**

*"In addition to paddy soils, DOM's influence on microbial Hg methylation has been observed in other ecosystems, such as wetlands and sediments, where DOM enhances Hg bioavailability and shapes microbial community structures to promote methylmercury (MeHg) production. For instance, in wetlands, DOM-bound Hg has been found to change the community assembly of mercury for methylating microbes (Fagervold et al., 2014), similar to what we observed in paddy soils."* (Main text, Lines 293-297).

# **Comment 2:** The manuscript discusses DOM composition but lacks specific details on the types of organic compounds most relevant to stimulating Hg-methylating activity. Including additional chemical analyses of DOM could strengthen the conclusions.

**Response: Thank you for your valuable comment. While we did not conduct additional chemical analyses, we did examine the composition of dissolved organic matter (DOM) across paddy soils with varying levels of Hg contamination (NMS, MMS, and HMS). Our findings revealed that low-molecular-weight organic acids such as oxalic acid, formic acid, tartaric acid, and acetate were highly abundant in these soils, with their concentrations being highest in NMS soils, followed by MMS, and lowest in HMS. These compounds are known to serve as key carbon sources for Hg-methylating microorganisms, particularly in enhancing their activity.**

**Although we do not have direct data on the influence of aromatic compounds and humic substances on Hg methylation, it is possible to hypothesize that their more complex structures may make them less bioavailable or more resistant to microbial degradation, potentially leading to a weaker stimulation of Hg-methylating activity compared to low-molecular-weight organic acids. This hypothesis warrants further investigation in future studies. To clarify, we have added the corresponding sentences to the revised manuscript as below:**

*"While we did not conduct additional chemical analyses, we examined the DOM composition across paddy soils with different Hg contamination levels (NMS, MMS, HMS). Our analysis found that low-molecular-weight organic acids, including oxalic acid, formic acid, tartaric acid, and acetate, were highly abundant, particularly in NMS soils, with decreasing concentrations in MMS and HMS. These organic acids serve as key carbon sources for Hg-methylating microorganisms. Although we do not have direct data on the effects of aromatic compounds and humic substances on Hg methylation, it is possible to hypothesize that their more complex structures might reduce their bioavailability or slow microbial degradation, potentially resulting in a weaker effect on Hg-methylation compared to low-molecular-weight organic acids. This hypothesis could be explored in future studies, offering a pathway for a more detailed understanding of how DOM composition influences Hg methylation."* (Main text, Lines 328-336).

# **Comment 3:** The authors hypothesize that DOM stimulates microbial activity through metabolic pathways but do not delve deeply into the mechanistic underpinnings. Further discussion on the microbial metabolic pathways involved, possibly supported by metabolomic data, would improve the mechanistic understanding.

**Response: Thank you for your insightful comment. We fully acknowledge the importance of understanding the microbial metabolic pathways involved in DOM-stimulated Hg methylation, as it is crucial for elucidating the underlying mechanisms of this process. Although our current study primarily focused on identifying core Hg-methylating microorganisms and analyzing DOM composition, we agree that a deeper exploration of the metabolic pathways involved would significantly enhance the mechanistic understanding of how DOM influences these microorganisms.**

**In our study, we observed that different DOM compounds, particularly low-molecular-weight organic acids such as oxalic acid, formic acid, and acetate, were utilized by key Hg-methylating microorganisms (e.g., *Geobacter* and *Desulfovibrio* species). These microorganisms are known to employ key metabolic pathways such as the TCA cycle (tricarboxylic acid cycle) and acetate metabolism, which could be directly linked to the utilization of DOM components. For instance, *Geobacter sulfurreducens* and *Desulfovibrio desulfuricans* preferentially use acetate, which feeds into the TCA cycle, providing electrons for anaerobic respiration and potentially influencing Hg methylation rates (Hu et al., 2013; Liu et al., 2018b). Additionally, *Methanoregula* and *Methanosarcina* species involved in methanogenesis could be utilizing formate and acetate through the methanogenesis pathway, further contributing to Hg methylation (Sakai et al., 2010; Schöne et al., 2022).**

**While we did not include metabolomic data in this study, we recognize the need for future research in this area. Incorporating metabolomic profiling would be highly beneficial for identifying key metabolites and providing more detailed insights into the specific metabolic pathways influenced by DOM, such as acetate fermentation, methanogenesis, and electron transfer processes involved in Hg methylation. To enhance clarity, the corresponding sentences were added in the revised manuscript as below:**

*"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."* (Main text, Lines 318-320).

# **Minor Comments**

# **Comment 4:** The introduction is well-written but could benefit from a clearer explanation of the broader ecological importance of Hg methylation in paddy fields versus other environments.

**Response: Thank you for your constructive comment. We appreciate your suggestion to provide a clearer explanation of the broader ecological significance of Hg methylation in paddy fields compared to other environments. We have expanded the introduction to address this point:**

*"Compared to other environments such as wetlands and aquatic sediments, paddy fields present unique ecological conditions that make them significant hotspots for Hg methylation. The frequent flooding and draining cycles, high organic matter content, and dynamic redox conditions in paddy soils create an environment that supports high levels of microbial activity, particularly Hg-methylating microorganisms (Yin et al., 2013). These conditions not only enhance MeHg production but also increase the likelihood of MeHg entering the food web through rice consumption, posing significant health risks (Zhang et al., 2010). Understanding Hg methylation in paddy fields is therefore crucial, as rice is a critical exposure route for MeHg in humans."* (Main text, Lines 43-49).

# **Comment 5:** Line16 suggest “remains”

**Response: Thank you for your suggestion. We have revised the sentence as recommended.**

# **Comment 6:** Line 64 “…other factors also play roles in MeHg production…”

**Response: Thank you for your comment. We have revised the sentence as suggested.**

# **Comment 7:** Line 62, Line 237, Line 299, Lne 321 suggest unify the description of "low-molecular-weight DOMs" throughout the maus.

**Response: Thank you for your comment. We have unified the description of "low-molecular-weight DOMs" throughout the manuscript for consistency.**

# **Comment 8:** Line 255 Whether the results support the argument that "MeHg concentration was strongly linked to hgcA gene abundance even compared to abiotic factors", it seems to be no description of the relevant results

**Response: Thank you for your comment. Our results demonstrated that the contribution of *hgcA* gene abundance to MeHg concentration was stronger compared to the contributions of Hg bioavailability and redox conditions. Specifically, alteration of the core Hg-methylating microbiome composition, which is closely linked to *hgcA* gene abundance, significantly regulated soil MeHg concentration (λ = 0.84, p < 0.001) (Fig. 4). In comparison, the contributions of Hg bioavailability (10%) and redox conditions (25%) were much lower than that of DOM (65%) (Fig. 4), further supporting the link between *hgcA* gene abundance and MeHg concentration, even in the presence of varying abiotic factors. To enhance clarity, the corresponding sentences were re-organized in the revised manuscript as below:**

*"Additionally, SEM result showed that the core Hg-methylating microbiome composition, which is closely linked to hgcA gene abundance, significantly regulated soil MeHg concentration (λ = 0.84, p < 0.001) (Fig. 4). In comparison, the contributions of Hg bioavailability and redox conditions to the core Hg-methylating microbiome composition are 10% and 25%, respectively, which are much lower than that of DOM (65%) (Fig. 4)."* (Main text, Lines 242-246).