Manuscript number: egusphere-2024-293730 Sep 2024

Title: Methane oxidation potential of soils in a rubber plantation in Thailand affected by fertilization

Response to Topic Editor

Dear Dr. Emily Solly,

Thank you very much for editing our manuscript. We appreciate your helpful comments after the manuscript was reviewed. The manuscript was revised according to the comments from you and the reviewers. Please see the changes in red in the author's track-changes file.

The referees found that your manuscript presents novel information about the oxidation of methane of soils in tropical rubber plantations, in particular providing knowledge on the overlooked subsoils and the responses to changes in fertilization regime. However, they also raised several points that still should be addressed. In particular, both referees had some concerns regarding the lack of provision of some aspects of your sampling design, the reporting of the environmental conditions during the microcosm study, and the aggregation of the soil samples. This information is highly relevant to explain methane processes in the soil.

We addressed all the points the reviewers raised and revised our manuscript. We would appreciate it if you kindly check our reply on the reviewers' comments (AC2 and AC3) in the interactive discussion, where we also clarified how we revised our manuscript.

In additional to several other specific comments, the referees also found that the discussion is currently lacking to report some potentially interesting findings, such as:

1) the observation the soil nitrogen was strongly correlated with higher methane oxidation,

We described the observation in the revised manuscript as follows:

"A weak positive correlation with total nitrogen was detected, and principal component

analysis demonstrated that the PMOR and total nitrogen had similar eigenvalues, driven by Tr1 soils, particularly in the wet season (August 2023, Supplementary Figure S3). The results may imply that soil organic nitrogen slowly supplies inorganic nitrogen at a rate that does not suppress but supports methane-oxidizing bacteria (Geng et al., 2017) in contrast to the high application of chemical fertilizers that often suppress methane oxidation (Liu and Greaver, 2009; Aronson and Helliker, 2010)." (L 168-173)

2) the differences between in-situ methane fluxes and estimated PMORs,

We described the differences as below:

"*The estimated aerial PMORs of the surface soil (0-10 cm) were much lower than the methane flux on site in the dry season; the same trend was observed in Tr1 and Tr2 in the wet season. PMORs measured in this study likely overestimate the actual oxidation as the initial methane concentration (50 ppmv) higher than the atmospheric level would accelerate methane oxidation (Bender and Conrad, 1994). Thus, the significant gap between the PMOR of topsoil and the methane uptake in situ suggests that the methane oxidation in the topsoil does not explain the in-situ methane uptake in the para rubber plantation studied.*" (L. 184-188)

"*The estimated rates ranged between 0.24 (Tr4) and 2.21 (Tr1) nmol m-2 s-1, which exceeded the in-situ fluxes. The gaps between in-situ methane fluxes and estimated PMORs per area can be related to the fact that the vertical gradients of methane and oxygen concentration that exist in situ in undisturbed soil profiles were not reproduced in the ex-situ incubation in which soil of each layer was exposed to the same concentrations. PMORs measured on subsoil samples may, therefore, overestimate the actual oxidation occurring in situ deep in the soil profile (Bender and Conrad, 1994). Another possible explanation is that the in-situ fluxes represent net methane uptake, i.e., the balance between oxidation and production, thus could be lower than the gross oxidation rate.*" (L. 221-227)

3) possible reasons why the surface soil layer had lower PMORs than the subsoil layers

Although further investigation is of course needed, we discussed some potential reasons as follows:

"*Our findings contrast with previous studies that reported higher high- and low-affinity methane oxidation in the topsoil than below, though some exceptions were noticed* *when high mineral N concentrations were measured in the topsoil (Reay et al., 2005; Xu et al., 2008). However, in our study, the discrepancy between in-situ soil methane uptake and PMOR was observed in all treatments, including T1, although the gap was less pronounced in T1 than in the three other treatments receiving fertilization. Low soil water content, especially during the dry season, can be another factor pushing methane oxidation down to the soil profile since drought stress is known to inhibit methanotrophic activity (Schnell and King, 1996; Borken et al., 2006; Bras et al., 2022). However, the discrepancy between in situ soil methane uptake and PMOR was observed in all seasons at our site. Alternatively, methane oxidation can be inhibited by several chemical compounds such as monoterpenes and ethylene that can be abundant in the upper soil layer under several types of vegetation (Amaral and Knowles, 1998; Jäckel et al., 2004; Maurer et al., 2008). While we did not assess the presence of potential inhibitors of methane oxidation in our study, this hypothesis cannot be ruled out.*" (L. 228-238)

4) the reason why fertilization suppressed methane oxidation

We discuss the possible reasons as below:

"*Fertilization, especially nitrogen fertilizer application, is often reported to inhibit soil methane oxidation (Täumer et al., 2021; Bodelier, 2011; Bodelier and Laanbroek, 2004). Ammonium competitively suppresses methane monooxygenase due to the similarity with ammonia monooxygenase. Nitrate is also reported to strongly inhibit atmospheric methane oxidation in forest soils (Mochizuki et al., 2012). Both ammonium and nitrate fertilizers are applied in the rubber plantation in this study, which likely suppressed methane oxidation. In addition to the high amount of fertilization, recurring and prolonged disturbances of methane oxidation by fertilization in Tr3 and Tr4 may outcompete the resilience of methane oxidation (Lim et al., 2024). Notably, fertilizers applied on the surface had a suppressive effect on methane oxidation in the deeper layers, at least up to 60 cm. Soil acidification is another possible cause of suppressed methane oxidation of forest soil by fertilization (Benstead and King, 2001), but there is no relationship between soil pH and potential methane oxidation rate in this study.*" (L. 212-220)

5) an indication of future research directions

We discuss it in the conclusion section:

"*A more systematic study is necessary for the future, where high-affinity methane oxidation and methane production should also be addressed. The increase in methane oxidation with depth can be related to a shift in the composition of the methanotrophic community from high- to low-affinity methanotrophs, which remains to be investigated.*" (L. 241-244)

"*As soil organic nitrogen weakly but positively correlated with soil methane oxidation potential, soil enrichment with organic nitrogen, e.g., by organic fertilizer application, may be an option to minimize or even reverse the negative impact of fertilization on methane oxidation of tropical soils, which should be a target of future research.*" (L. 248-251)