Thank you so much for your positive and constructive feedback on our manuscript. We revised the manuscript based on your comments. Especially, we added more information on the methods and clarified the difference between in-situ methane fluxes and estimated oxidation potentials. We hope that the revised manuscript is suitable for publication.

We reply to your valuable comments as follows. The MS word file is also attached for easier visibility.

>Major concerns

The field fluxes measurements and microcosm experiment lack important information such as soil moisture and physical properties, which are highly relevant to methane processes in the soil. Considering the sandy-loam texture at the SKRS site, low methane oxidation potential in topsoil in the dry season might be due to low moisture and temperature. Did the authors measure and adjust moisture for soils from different sampling layers before incubation?

→The average air temperature at the SKRS site was 25.4, 27.2, and 26.5 °C in February 2023, August 2023, and February 2024, respectively. This was comparable with the temperature set for the incubation (25°C), and would thus not limit the methane oxidation potential. We added air temperatures in the materials and method. We measured the methane oxidation potential without adjusting the soil moisture. Soil moisture ranged from 5.1 to 12.7 % for the top layer (0-10 cm) and was not correlated with potential methane oxidation rates. We added the relationship between potential methane oxidation rate and soil water content in Supplementary Figure S2. The potential methane oxidation rate of the deeper layer soils was also not correlated with soil water content. We added a few sentences to the discussion on the potential inhibition of methanotrophic activities by water stress as described below. However, a low methane oxidation potential in the topsoil was also observed in the rainy season.

"Water balance is an important factor in regulating the methane dynamics in forest soils (Feng et al., 2020; Bras et al., 2022), but no correlation between the soil water content and PMOR was observed in this study. Either drought stress under low soil water content or limited oxygen under high soil water content can have an inhibitory

effect on soil methane oxidation (Feng et al., 2020), but the soil water contents measured in this study may have no such inhibitory effect."

Bras, N., Plain, C., and Epron, D.: Potential soil methane oxidation in naturally regenerated oak-dominated temperate deciduous forest stands responds to soil water status regardless of their age—an intact core incubation study, Annals of Forest Science, 79, 10.1186/s13595-022-01145-9, 2022.

>It is worthy of adding more discussions on the gaps between in-situ methane fluxes and estimated PMORs, and possible reasons why the surface soil layer had lower PMORs than the subsoil layers, as well as how fertilization suppressed methane oxidation. Alternatively, adding an outlook after the conclusion about what needs to be done in future to address these questions.

→Thank you for your helpful feedback.

The discrepancies between in-situ methane fluxes and estimated PMORs can be related to the fact that the vertical gradients in methane and oxygen concentration that exist in-situ in undisturbed soil profiles are not reproduced in the ex-situ incubation in which the soil from each layer is exposed to the same concentrations. PMORs measured on subsoil samples may, therefore, overestimate the actual oxidation occurring in situ deep in the soil profile. Furthermore, the incubations were carried out at a much higher methane concentration than expected in the soil profile, although an alternative source of methane may exist in deeper layers if methanogenesis occurs there. In this case, methanogenesis in the deeper layers may sustain methane oxidation in the upper soil layers by supplying the substrate (methane). We discussed these fundamental differences between the two approaches in the revised manuscript.

"*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 can be related to the fact that the vertical gradients of methane and oxygen concentration that exist in situ in undisturbed soil profiles are not reproduced in the ex-situ incubation in which soil of each layer is 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 oxidation rate.*"

Bender, M. and Conrad, R.: Methane oxidation activity in various soils and freshwater sediments: Occurrence, characteristics, vertical profiles, and distribution on grain size fractions, Journal of Geophysical Research-Atmospheres, 99, 16531-16540, 1994.

The possible reasons why the surface soil layer had lower PMORs than the subsoil have been added to the discussion. Please see our answer to your specific comments about lines 161-163

We also added more discussion on how fertilization can suppress methane oxidation in the revised manuscript.

"Ammonium competitively suppresses methane monooxygenase due to the similarity with ammonia monooxygenase. Nitrate is also reported to strongly inhibit the 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."

Benstead, J. and King, G. M.: The effect of soil acidification on atmospheric methane uptake by a Maine forest soil1, Fems Microbiol Ecol, 34, 207-212, 10.1111/j.1574-6941.2001.tb00771.x, 2001.

Lim, J., Wehmeyer, H., Heffner, T., Aeppli, M., Gu, W., Kim, P. J., Horn, M., and Ho, A.: Resilience of aerobic methanotrophs in soils; spotlight on the methane sink under agriculture, Fems Microbiol Ecol, 10.1093/femsec/fiae008, 2024.

Mochizuki, Y., Koba, K., and Yoh, M.: Strong inhibitory effect of nitrate on atmospheric methane oxidation in forest soils, Soil Biology and Biochemistry, 50, 164-166, https://doi.org/10.1016/j.soilbio.2012.03.013, 2012.

>Specific comments

>line 28: change off-season, use consistent terms for the seasons

→Changed to dry (off-harvesting) season

>line 23: delete the first potential

→Thank you for your notice. The first one was deleted.

>lines 28-30: Although the integrated potential numbers (Figure 5) might match with in-site measured methane fluxes, considering very different methane and oxygen concentrations in deeper soil layers under the field condition and incubation setting, be cautious to conclude that methane oxidation in the soil predominantly occurs in depths below the surface layer based on only one site. I suggest the authors present the integrated numbers as an additional column in Table 2. A clearer distinction between potential and in-situ rate should be made throughout the texts.

→We divided our study into two parts: 1) low methane oxidation potential in the topsoil layer under different land uses and fertilization levels for a para rubber plantation and 2) the depth profile of methane oxidation potential of para rubber soils influenced by fertilization. We want to keep the structure to highlight our two findings. We double-check the texts to ensure a clear distinction between potential oxidation rates and net in-situ fluxes.

We agree that we cannot conclude that soil methane oxidation predominantly occurs at depths below the surface layer based on ex-situ incubation alone because, as mentioned above, we are aware that PMORs measured on subsoil samples may overestimate the actual oxidation occurring in-situ deep in the soil profile.

>lines 64-71: could you formulate them into hypotheses? Line 165 mentioned the hypothesis.

→We added two hypotheses in the last section of the introduction:

"In this study, we measured potential soil methane oxidation rates using a microcosm incubation experiment to test the hypothesis that land-use change and fertilization management influence soil methane oxidation in tropical tree plantations focusing on a para rubber plantation. While most studies assume that methane oxidation in forest soils occurs primarily in the surface soil, we also targeted the deeper soil layers and tested the hypothesis that the effects of topdressing fertilizers on soil methane oxidation

extend to deeper layers of the soil profile."

>lines 75-78: the duration of wet season and dry season is unclear, please specify the start and end of each season.

→We revised the sentence, clarifying the duration of the wet season and dry seasons.

>lines 81-83: how long have been the fertilizer treatments set up in the rubber plantation at SKRS? What are the fertilizer forms especially N applied in the treatments? If the fertilization treatments have been carried out for a long time, a gradient of soil properties might be already established between treatments.

→We added information on the setting up of the plantation and the fertilizers applied.

"The rubber plantation of the SKRS site was set up in 2007 and has been applied with four different levels of fertilizer treatment with randomized four replicate blocks (A–D) since 2014 at the beginning of tapping: Tr1, no; Tr2, low; Tr3, intermediate; Tr4, high (Table 1). Tr3 falls within the range of the recommended fertilizer application rates for mature rubber plantations in Thailand by Thai public institutions; recommendations exceeded by 40% of rubber farmers (Chambon et al., 2018). Chemical fertilizers of nitrogen (40% nitrate and 60% ammonium), phosphorus, and potassium (YaraMilaTM, Yara International ASA, Oslo, Norway) are top-dressed in the wet season, evenly to half of the area between the planting rows. The fertilizer was applied only in the early rainy season (May) for T2 while a second application was made late in the rainy season (October) for T3 and T4."

>lines 95-99: it is interesting to see how field sampling progressively changed over time, at the same time, it limited what statistical analysis could test, e.g. seasonal effect, land use effect, interactions, etc.

→Thank you for the comments. We first focused on the topsoil layer, assuming it should be the most active part of methane oxidation, as reported in different

studies. However, our initial experiment in February 2023 indicated that the surface soils had only limited methane oxidation potential compared to in-situ methane fluxes. We, therefore, adapted the sampling strategy over time. Since samples collected from deeper soil layers in August 2023 showed higher potential methane oxidation, we intensified this sampling along the vertical profile in February 2024, our last field campaign in this overseas project. Despite some limitations, we believe that we report new findings in this short paper, but we added a sentence on the necessity for a systematic study in the conclusion of the revised manuscript.

"In this study, we adapted the sampling strategy over time due to the fact that the topsoil has a low methane oxidation potential, unlike previous studies, and thus, we targeted the deeper layers in the middle of the study; a more systematic study is necessary for the future, where high-affinity methane oxidation and methane production should 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 studied. Nevertheless, our results provide a new insight into the impact of agricultural land use of tropical forests on the ecological function in a greenhouse gas cycle."

>lines 106-107: sieved fresh soil? Which samples were put into 50-ml GC vials? Considering the long incubation time (30 days) in this study, was it possible oxygen became limited during the incubation? The limitation of using high initial methane concentration in incubation should be communicated to readers, i.e. not favoring high-affinity methanotrophs that oxidize low concentrations of methane (more dominant in aerated soils). This might be one of the reasons for the low estimation of oxidation potential.

→We incubated the sieved soils at the atmospheric level of oxygen, and the soil samples with high methane oxidation potentials showed a linear decrease in methane concentration from the beginning of the incubation period even though the soils contained higher organic carbon ranging from 10-23 g kg^{-1}. Furthermore, no correlation between PMOR and soil organic carbon content was observed (Supplementary Figure S2). Thus, we consider that oxygen was not a limiting factor for the measurements of methane oxidation potential in this study.

It is true that high-affinity methanotrophs can be saturated at much lower methane concentration than 50 ppm, but to our knowledge, there is no report that

high methane concentration would inhibit their oxidation capacity. However, we agree that 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. The fact that high-affinity methanotrophs were found to be less sensitive to nitrate than low-affinity methanotrophs (Reay et al., 2005) is consistent with our observation that the effect of fertilization was more evident in the subsoil than in the topsoil. However, another study reported a negative relationship between mineral nitrogen and methane oxidation at ambient concentration and a positive relationship at elevated methane concentration (Chan and Parkin, 2001). The composition of the methanotrophic community deserves to be addressed in future research.

We added this suggestion for future research in the conclusion of the revised manuscript as you may see above.

>lines 152-155: higher total N correspond to higher PMORs? This seems contradictory to the negative fertilization effect on PMORs and in-site methane fluxes (figure 2, lines 142-144). Could the authors add the surface soil (0-10 cm) properties by treatment to Table 2 or in supplement? I do not understand the argument here either, is organic fertilizer applied in this study?

→A supplementary table will be given for soil properties. No organic fertilizer was applied; we clarified this in the revised text.

>Figure 2: what does 'corrected' mean?

→Sorry for the typo, it is "collected."

>line 157: medium is more suitable than middle

→Corrected

>line 161-163: very important observation, it is worth discussing possible reasons for the gap between PMOR and methane flux in situ.

→We assumed that the PMOR would be higher than in situ soil methane uptake, because the methane concentration used in incubation vials was much higher than the atmospheric level. Nevertheless, the PMOR in the surface soils (0-10 cm) was low compared to the in-situ methane flux. Although negative artifacts on methane oxidation in the incubation experiment cannot be completely ruled out, the results

directed us to focus on soils from the deeper layers, which was addressed in the following section. We added the following sentences:

"The estimated aerial PMORs of the surface soil (0-10 cm) were much lower than the methane fluxes measured on site in February 2023 during the dry season; the same trend was observed in Tr1 and Tr2 in August 2023 during the wet season. PMORs measured in this study likely overestimate the actual oxidation because the initial methane concentration (50 ppmv), higher than the atmospheric level, would accelerate methane oxidation (Bender and Conrad 1994). Thus, the significant discrepancy between the topsoil PMOR and the in-situ methane uptake suggests that the methane oxidation in the topsoil does not explain the in-situ methane uptake in the studied para rubber plantation."

The discrepancy between the in situ methane flux and PMOR per area including the deeper soil layers, i.e., higher PMOR per area than the in-situ flux, has also been discussed in the following section as you may see above.

>Figure 3: I think keeping one set of legends is sufficient here because of the same sampling depths.

→Done

>lines 196-199: what are the bases for this statement? The correlation in Figure S2 was total N and the authors did not mention organic fertilizers in the methods description at all.

→No organic fertilizers were added to the plot. We meant the soil organic matter. We clarified it in the text.