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

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**Abstract.** Forest soils, as crucial sinks for atmospheric methane in terrestrial ecosystems, are significantly impacted by changes in ecosystem dynamics due to deforestation and agricultural practices. This study investigated the methane oxidation potential of rubber plantation soils in Thailand, focusing on the effect of fertilization. The methane oxidation activity of the top soils (0-10 cm) in the dry season was extremely low and slightly increased in the wet season, with lower activity for higher fertilization levels. The methane oxidation potential of the topsoil was too low to explain the in-situ methane uptake. Soils below 10 cm depth in unfertilized rubber plantations showed higher activity than the surface soils, and methane oxidation was detected at least down to 60 cm depth. In contrast, soils under the high-fertilization treatment exhibited similarly low activity of methane oxidation up to 60 cm depth as surface soils both in dry and wet seasons, indicating that fertilization of Para rubber plantation negatively impacts the methane oxidation potential of the soils over the deep profile without recovery in the dry (off-harvesting) season with no fertilization. Methane uptake per area estimated by integrating the methane oxidation potentials of soil layers was comparable to the field flux data, suggesting that methane oxidation in the soil predominantly occurs in depths below the surface layer. These findings have significant implications for understanding the environmental impacts of tropical forest land uses on methane dynamics and underscore the importance of understanding methane oxidation processes in soils.

## 1 Introduction

Methane is the most important anthropogenically enhanced greenhouse gas in the atmosphere after $CO_2$ (Forster et al., 2021). It is thus important to fully quantify and characterize all sources and sinks to include the role of terrestrial ecosystems in mediating atmospheric exchange.  Unsaturated aerobic soils are important sinks of atmospheric methane via oxidation by methane-oxidizing bacteria with a global estimation of 11–49 Tg $CH_4$ $yr^{-1}$ (Saunois et al., 2020). The global mean methane uptake rate in forest soils is reported to be $3.95 \pm 1.78$ kg $CH_4$ $ha^{-1}$ $yr^{-1}$, with a total sink of $14.98 \pm 6.75$ Tg $CH_4$ $yr^{-1}$ in 1999–2020, thus playing an essential role in the terrestrial methane sink (Feng et al., 2023). Temperate and tropical forest soils are the predominant sinks, contributing 84% of total methane sink in forest ecosystems (Feng et al., 2023).

Forest conversion is suspected of weakening the sink of atmospheric methane (Verchot et al., 2000). Rubber plantations in tropical regions have been expanding worldwide, particularly in Asia, where lowered ecosystem functions compared to forests have been demonstrated (Singh et al., 2021). Deforestation and following agricultural use of a tropical forest, such as Para rubber and oil palm plantations, tend to decrease the methane sink of soils (Lang et al., 2019; Lang et al., 2020; Aini et al., 2020; Lang et al., 2017; Zhou et al., 2021). The large-scale expansion of rubber plantations in Southeast Asia has decreased methane uptake by soil. However, a mechanistic understanding of the associated processes within the soil profile is still missing (Lang et al., 2020).

Monitoring the surface methane flux has been used to study the methane uptake of soils in tropical forests and the conversion effects of land use on it. Rubber monocultures showed lower rates of methane uptake than natural forests (Werner et al., 2006), which could turn the rubber soils into methane emitters during a certain period of the rainy season (Lang et al., 2019). The water-filled pore space, which is increased by rubber plantation and with rubber age, is correlated with the methane flux, suggesting that soil compaction by agricultural machinery may suppress methanotrophy and promote methanogenesis by reducing gas exchange (Lang et al., 2019). Methane uptake rates in Indonesian tropical forests  under deforestation and rubber plantation are negatively affected by clay content that controls the soil pore space (Ishizuka et al., 2002).

Mineral nitrogen (ammonia and nitrate) is one of the most critical factors controlling aerobic methane oxidation in soil (Bodelier and Laanbroek, 2004; Bodelier, 2011). Meta-analyses demonstrate that adding mineral nitrogen increases methane emissions and significantly reduces methane uptake in most soil ecosystems (Liu and Greaver, 2009). However, the effect of nitrogen on methane uptake by soils can vary depending on different factors, such as the enrichment level of nitrogen, ecosystem, biome, and duration of fertilization (Aronson and Helliker, 2010). Low rates of nitrogen addition in forest and tree plantation systems occasionally stimulate methane oxidation (Geng et al., 2017; Koehler et al., 2012); the first study to demonstrate the role of soil fertility on methane uptake in a tropical landscape reported nitrogen limitation of soil methane uptake in the converted land-use types including rubber plantation (Hassler et al., 2015). Another study demonstrated no significant contribution of ammonium nitrogen to predicting methane flux (Lang et al., 2019). Determining the effects of fertilizer application in rubber plantations on soil methane oxidation processes is essential to exploring soil management to compromise between natural rubber production and the maintenance of soil ecosystem function as a methane sink.

In this study, we measured the potential rates of soil methane oxidation using a microcosm incubation experiment with the hypothesis that land use change and fertilization management would influence methane oxidation in tropical forest soil, 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. We tested the hypothesis that the influence of top-dressing fertilizers on soil methane oxidation reaches deeper layers of the soil profile.

## 2 Materials and Methods

### 2.1 Study site

The study mainly targeted a Para rubber (*Hevea brasiliensis*) plantation site in Sithiporn Kridakorn Research Station, Kasetsart University (SKRS, N 10°59.3', E 99°29.3'), Prachuap Khirikhan province, located in southern Thailand. The region's climate was tropical monsoon with a mean annual precipitation of 1700 mm (2010-2023), distributed between a wet season extending from May to November and a dry season from December to April. October and November were the wettest months, with over 250 mm of rain per month on average. The main features of the soil in the SKRS were deep soil with a sandy-loam texture, low water retention capacity, poor organic matter content, and low cation exchange capacity due to the dune origin. The soil was classified as Rhodic Kandiudults.

The rubber plantation of the SKRS site was set up in 2007, and four different levels of fertilization have been applied on randomized four replicate blocks (A–D) since 2014 at the beginning of tapping: Tr1, no; Tr2, low; Tr3, intermediate; Tr4, high fertilizer application rates (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). A chemical fertilizer composed of nitrogen (40% nitrate and 60% ammonium), phosphorus, and potassium (YaraMila™, Yara International ASA, Oslo, Norway) was 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 Tr2, while a second application was made late in the rainy season (October) for Tr3 and Tr4. A secondary forest and oil palm plantation adjoining the rubber plantation

**Table 1** Sampling sites of the Sithiporn Kridakorn Research Station

| Vegetation | Treatment | Site replication | Fertilization (N/P/K, kg ha$^{-1}$) | | Soil sampling depth | | |
|---|---|---|---|---|---|---|---|
| | | | Early rainy season | Late rainy season | Feb 2023 (dry season) | Aug 2023 (rainy season) | Feb 2024 (dry season) |
| Para rubber | Tr1 | 4 (Blocks A-D) | 0 | 0 | 0-10 cm (Block B) | 0-10 cm (Blocks A-D)<br>0-55 cm (Block B, 8 layers) | 0-50 cm (Blocks A-D, 5 layers) |
| | Tr2 | 4 (Blocks A-D) | 37/22/50 | 0 | 0-10 cm (Block B) | 0-10cm (Blocks A-D) | 0-50 cm (Blocks A-D, 5 layers) |
| | Tr3 | 4 (Blocks A-D) | 53/18/35 | 37/22/50 | 0-10 cm (Block B) | 0-10cm (Blocks A-D) | 0-50 cm (Blocks A-D, 5 layers) |
| | Tr4 | 4 (Blocks A-D) | 89/30/59 | 64/38/85 | 0-10 cm (Block B) | 0-10cm (Blocks A-D)<br>0-60cm (Block B, 5 layers) | 0-50 cm (Blocks A-D, 5 layers) |
| Palm | Litter | 3 | NA | NA | - | 0-10 cm | - |
| | No Litter | 3 | NA | NA | - | 0-10 cm | - |
| Forest | - | 4 | 0 | 0 | - | 0-10 cm | - |

in SKRS were also studied for comparison. The bulk density of the soils ranged from 1. 4 g $cm^{-3}$ (forest and palm) to 1.5 g $cm^{-3}$ (Para rubber); the surface of the palm soil covered with palm leaf detritus had a low density (0.94 g $cm^{-3}$).

A rubber plantation in Chachoengsao Rubber Research Center (CRRC, N 13°33.9’, E101°27.3’), Chachoengsao province, located in central Thailand, was also studied to compare the effect of fertilization on soil methane oxidation with SKRS. The annual precipitation was 1400 mm on average for 2022-2023. The soil was classified as clayey-skeletal, kaolinitic, and isohyperthermic Typic Kandiustults. Some physicochemical properties of the CRRC soils have been reported before (Kanpanon et al., 2015; Satakhun et al., 2013). The plantation received chemical fertilizer twice yearly at 500 g per tree (N: P: K = 30: 5: 18) in the middle of the inter-row, similar to Tr3 in SKRS.

**2.2 Sample collection**

Soil samples were collected from the 0-10 cm layer in the middle of the inter-row of the rubber plantation in February 2023 and 2024 (dry season) and August 2023 (wet season). Each month's average temperature was 25.4, 27.2, and 26.5°C, respectively. In February 2023, triplicate soil samples were collected from one of the replicate blocks (block B). In August 2023 and February 2024, a soil sample was collected from each block, giving four replicate samples per treatment. Soil samples with up to 60 cm depths were also collected at the SKRS rubber plantation site from Tr1 and Tr4 of block B in August 2023, and all treatments of all replicate blocks in February 2024. Topsoil (0-10 cm) in the forest and palm plantation in SKRS and CRRC rubber plantation were collected in August 2023. Four replicate sites of the forest were randomly selected, and in the palm plantation, the two contrasting locations, i.e., with and without palm leaf detritus cover, were selected in triplicate. In CRRC, no fertilization experiment was conducted; thus, we selected locations in the middle of the inter-row for fertilized soils and those in the middle of the row for unfertilized soils per this site's fertilizer application method. The soil samples were sieved (< 2 mm) on-site and stored at room temperature to measure methane oxidation potential within a month. The sieved soil samples for chemical analysis were stored at 4°C.

**2.3 Methane oxidation potential**

The potential methane oxidation rates (PMORs) of the sieved soils were determined by a microcosm incubation experiment. Ten grams of sieved soils were put into 50-ml or 100-ml GC vials (Nichiden-Rika Grass, Kobe, Japan). The vials were capped with butyl rubber stoppers and open-top screw caps and injected with 0.25 or 0.5 ml of 1% methane to give an initial concentration of 50 ppmv in the headspace containing atmospheric air. The samples were incubated in the dark at 25°C. Gas samples (0.25 ml) were periodically sampled from the headspace, and methane concentrations were measured with a gas chromatograph with a flame ionization detector (GC-2014, Shimadzu, Kyoto, Japan). The PMORs, expressed as ng$CH_4$ $g^{-1}$ dry soil $h^{-1}$, were calculated from the linear regression of the methane concentration decreasing with incubation time, the volume of the GC vials, and the moisture content of the incubated soils by the thermogravimetric method. The PMORs of top soils (0-10 cm) were also expressed as nmol $m^{-2}$ $s^{-1}$ to compare with the in-situ soil methane flux, assuming the bulk density of the soil in situ as 1.5 g $cm^{-3}$ according to our pilot survey. The potential methane oxidation rate per area ($PMOR_{area}$, nmol $m^{-2}$ $s^{-1}$) was estimated by adding up the methane oxidation rates of different layers.

$$PMOR_{area} = \sum_{l=1}^{n}(PMOR \times BD \times Th) \times 10{,}000 \div 3600 \div 16$$

where $l$ is the soil layer, *PMOR* (ng$CH_4$ $g^{-1}$ dry soil $h^{-1}$) is the potential methane oxidation rate, *BD* is the bulk density (1.5 g $cm^{-3}$), *Th* (cm) is the thickness of the soil layer, 10,000 converted $cm^{-2}$ to $m^{-2}$, and 3600 converted $h^{-1}$ to $s^{-1}$, and 16 is the molar mass of methane.

2.4 Soil methane flux

Soil methane fluxes of the SKRS rubber plantation were measured a few days before or after soil sampling on 24 (6 replicates ×4 blocks) PVC collars (20 cm in diameter and 13 cm in height) in each fertilization treatment. Collars were covered with a soil chamber (Li 8100-103, Li-Cor; Lincoln, USA). Methane flux was calculated from the rate of change in the methane concentration measured using a trace gas analyzer (Li 7810, Li-Cor) as described in (Epron et al., 2023).

**2.5 Soil chemical analysis**

Soil pH was measured in distilled water (soil-water ratio of 1:1). Soil electrical conductivity was measured using an EC meter after mixing soil with fivefold distilled water. Soil organic carbon was measured using the wet oxidation method (Walkley and Black, 1934), and soil total nitrogen was measured using the Kjeldahl method (Bremner, 1996).

**2.6 Statistics**

Linear regression analysis was performed using Origin 2024 (OriginLab Corporation, Northampton, USA). Differences in PMORs and soil methane fluxes between treatments were tested using a one-way variance analysis and the Kruskal-Wallis test with the post hoc Tukey's HSD and Dunn-Bonferroni tests, respectively (SPSS for Windows, version 22.0). Principal component analysis was conducted using Primer-e 7 (Plymouth, UK) to detect relationships among the different measures of the topsoil of the Para rubber plantation.

**3 Results and discussion**

**3.1 Methane oxidation potential of the surface soils**

We first focused on the methane oxidation potentials of the 0-10 cm layer of soil, based on previous findings that atmospheric methane uptake in soils is highest in the surface layer (Lang et al., 2020). The soil sampled in the dry season (February 2023) showed minor methane oxidation; only a slight decrease in methane concentration was observed with soils receiving no fertilization (Tr1, Figure 1a). In the wet season (August 2023), a slight but detectable decrease in methane with time was observed in all treatments compared to the dry season, and Tr1 showed a more significant decrease in methane than the other treatments (Figure 1b).

The soils collected in August 2023 from sites other than the SKRS rubber plantation showed methane oxidation activities with more considerable variations (Supplementary Figure S1). The forest soils exhibited a significant spatial variation in methane

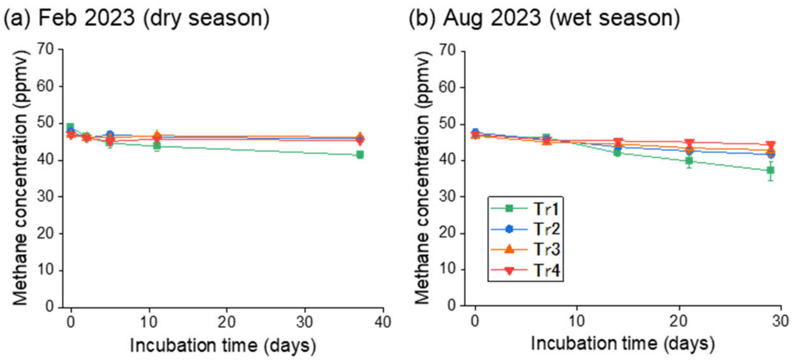


**Figure 1:** Methane oxidation of surface (0-10 cm) soils of the Para rubber plantation in (a) February and (b) August 2023. The February data represent the average and standard error (n=3) of block B, and the August data represent the average and standard error (n=4) of the four blocks (blocks A to D).

oxidation. Palm soils showed linear methane consumption, and no significant effect of the litter cover was observed. Methane consumption of CRRC rubber plantation soils sampled in the planting rows was higher than that of those sampled in the middle of the inter-rows where fertilizer was spread.

The calculated PMORs for the topsoil in August 2023 ranged between 0.02 and 1.23 $ngCH_4\ g^{-1}$ dry soil $hr^{-1}$ (Figure 2). The PMORs of Para rubber soils in SKRS ranged from 0.02 to 0.17 $ngCH_4\ g^{-1}$ dry soil $hr^{-1}$ with higher rates in Tr1 (no fertilization) than in Tr4 (highest fertilization) ($p < 0.05$), suggesting that fertilization with a critical level lowered soil methane oxidation, which was consistent with previous studies on forest soils (Liu et al., 2024; Aronson and Helliker, 2010). The PMORs of the SKRS rubber soils were among the lowest values reported for European forest soils (Täumer et al., 2021). The rubber soils in

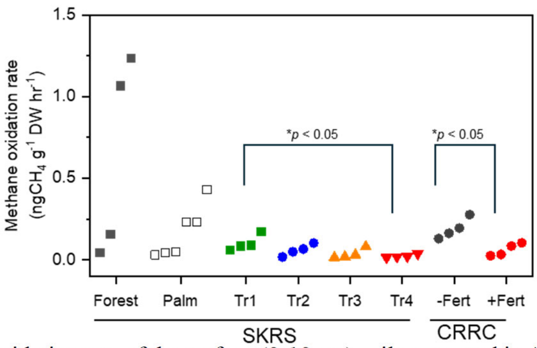


**Figure 2:** Potential methane oxidation rate of the surface (0-10 cm) soils corrected in August 2023 (wet season). The unfertilized soils (Tr1 in SKRS and –Fert in CRRC) have higher rates than the fertilized soils (Tr4 and +Fert, respectively).

CRRC from no fertilized sites (planting rows) also showed higher methane oxidation rates than those from the fertilized sites (inter-rows, $p < 0.05$). The forest soil showed the most considerable spatial variation; two of the four recorded the highest PMORs in this study, which were in the higher range observed in European forest soils (Täumer et al., 2021). The palm soils also showed considerable variation, with no effect of litter cover. The differences in PMORs between the different land uses (forest, rubber, oil palm) were not significant due to the considerable spatial variation in the forest and palm soils, requiring detailed investigation with increasing sample numbers to obtain the conclusive results of the impact of vegetation change on a soil methane oxidation potential.

There was no clear relationship between the PMORs and soil parameters measured in this study, i.e., pH, EC, organic carbon, total nitrogen, and water content (Supplementary Figure S2). 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). 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 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 inhibit soil methane oxidation (Feng et al., 2020), but soil water contents measured in this study may not have such an inhibitory effect.

The in-situ methane flux in the SKRS rubber plantation soil was negative in the dry season (February 2023 and 2024), indicating that methane oxidation predominates methane production, and the soil functioned as a net methane sink. Medium and high fertilization (Tr3 and Tr4) suppressed the in-situ soil methane uptake compared to no fertilization (Tr1, Table 2), which was consistent with the results of PMORs. In the wet season (August 2023), the methane fluxes in Tr1 and Tr2 were comparable to those in the dry season, while Tr3 and Tr4 showed positive methane fluxes on average, indicating that methane production in the soil exceeded methane oxidation. The seasonal shift of methane flux from sink to source in a rubber plantation was reported before (Lang et al., 2019). 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.

**Table 2** In-situ methane flux and PMOR of surface soil (0-10 cm) in SKRS

| | Treatment | Methane flux ($nmol\ m^{-2}\ s^{-1}$) | n | PMOR ($nmol\ m^{-2}\ s^{-1}$) | n |
|---|---|---|---|---|---|
| Feb 2023 | Tr1 | -0.503 ± 0.029 $^{a}$ | 24 | 0.145 ± 0.014 $^{a}$ | 3 |
| (dry season) | Tr2 | -0.479 ± 0.044 $^{ab}$ | 24 | 0.039 ± 0.002 $^{b}$ | 3 |
| | Tr3 | -0.338 ± 0.046 $^{b}$ | 24 | 0.015 ± 0.008 $^{b}$ | 3 |
| | Tr4 | -0.121 ± 0.065 $^{c}$ | 24 | 0.031 ± 0.001 $^{b}$ | 3 |
| Aug 2023 | Tr1 | -0.562 ± 0.090 $^{a}$ | 24 | 0.263 ± 0.065 $^{a}$ | 4 |
| (wet season) | Tr2 | -0.522 ± 0.074 $^{a}$ | 24 | 0.156 ± 0.046 $^{ab}$ | 4 |
| | Tr3 | 0.122 ± 0.281 $^{b}$ | 24 | 0.096 ± 0.040 $^{ab}$ | 4 |
| | Tr4 | 0.058 ± 0.151 $^{b}$ | 24 | 0.063 ± 0.013 $^{b}$ | 4 |
| Feb 2024 | Tr1 | -0.764 ± 0.037 $^{a}$ | 24 | 0.037 ± 0.007 $^{a}$ | 4 |
| (dry season) | Tr2 | -0.542 ± 0.117 $^{a}$ | 24 | 0.029 ± 0.006 $^{ab}$ | 4 |
| | Tr3 | -0.393 ± 0.071 $^{b}$ | 24 | 0.013 ± 0.002 $^{b}$ | 4 |
| | Tr4 | -0.216 ± 0.051 $^{b}$ | 24 | 0.015 ± 0.005 $^{ab}$ | 4 |

The values are means±standard errors. Values of different letters are significantly different among the treatments in respective seasons ($P$ <0.05)

### 3.2 Methane oxidation potential of the soils collected from deep layers

Then, we tested the hypothesis that the deeper soils could contribute to methane consumption. The soils collected from the layers deeper than 10 cm in the rubber plantation in the dry season (February 2024) showed active methane oxidation compared to the topsoil (0-10 cm) in Tr1 (no fertilization) (Figure 3 and Supplementary Figure S4); one exception was in the block D

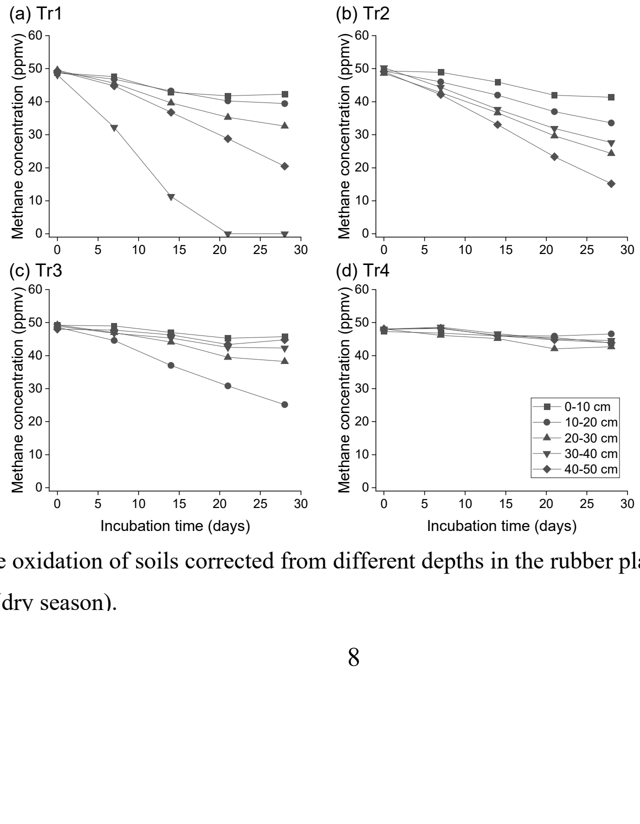


**Figure 3:** Methane oxidation of soils corrected from different depths in the rubber plantation (block B) in February 2024 (dry season).

where the methane consumption of the topsoil of Tr1 was comparable to the deeper layer soils (Figure S4). Active methane consumption in the deeper soils was also observed in Tr 2 except for one replicate block (block A, Figure. S4). Methane oxidation was less active throughout the soil profiles in Tr3 and Tr4 than in Tr1 and Tr2, except for Tr3 in Block B, which showed more methane oxidation in the 10-20 cm layer (Figure 3). The active methane oxidation in the deeper layers of Tr1 and low methane oxidation throughout the soil profiles in Tr4 were also observed in the wet season (Supplementary Figure S5).

We measured PMORs on 22 samples of deeper layers (> 10 cm) from Tr1. Among them, 20 samples showed up to 30 times higher PMORs than the topsoil (up to 10 cm) at the same site and sampling time (Figure 4 and Supplementary Figure S6). On the other hand, only three samples from deeper layers of Tr4, out of 20 samples in total, showed slightly higher PMORs than

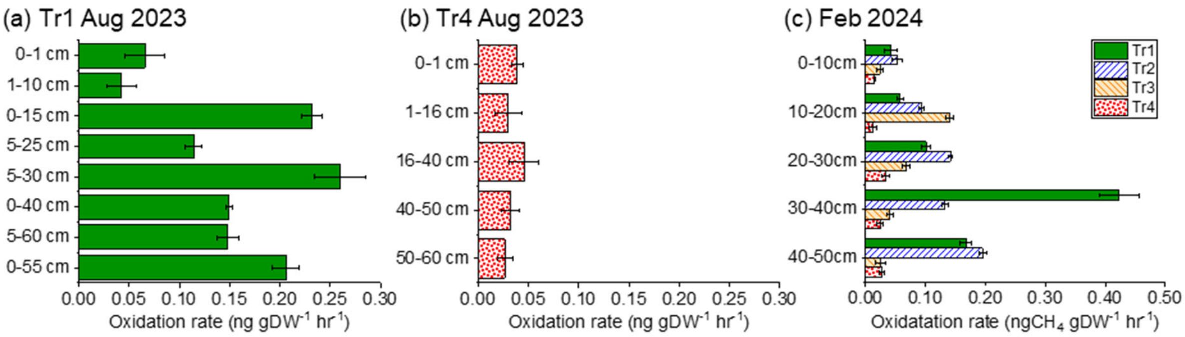


**Figure 4:** Depth profile of potential methane oxidation rate of the SKRS rubber plantation soils (Block B). The error bars are based on the error of the regression slope. August is in wet season and February in dry season.

the topsoil, and the rest of the samples showed PMORs comparable to or lower than the topsoil. Deep soil layers had higher PMOR than the topsoil for 75% of Tr2 samples (12 out of 16) and 50% of Tr3 samples (8 out of 16).

The estimated potential methane oxidation rates per area of the SKRS rubber plantation soil tended to decrease along the fertilization level (Figure 5), and Tr1 and Tr2 showed higher potential methane oxidation rates than Tr3 and Tr4 ($p < 0.05$). Our results demonstrated that 1) unlike the previous studies, the deeper layer soils in a rubber plantation could contribute to soil methane oxidation, and 2) the top dressing of fertilizers suppressed the soil methane oxidation potentials throughout the soil profile down to at least 60 cm.

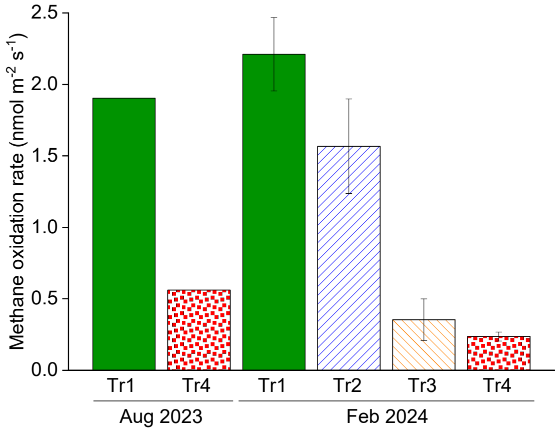


**Figure 5:** Estimated methane oxidation potential per area of SKRS rubber plantation under different fertilization. Data in August 2023 (wet season) were obtained from block B only, and those in February 2024 (dry season) were the average and standard error of the four replicate blocks (blocks A to D).

In the SKRS rubber plantation, Tr3 and Tr4 received more fertilizer than Tr2. In addition, Tr2 was fertilized only once a year at the beginning of the wet season, while another fertilizer application was conducted in Tr3 and Tr4 in the late wet season. 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 its 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.

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. Methane oxidation in the soil is insensitive to a wide range of oxygen levels (2-20%) but suppressed at extremely low oxygen levels (<2 %) (Walkiewicz and Brzezińska, 2019; Walkiewicz et al., 2018; Bender and Conrad, 1994). Methane concentration in the soil of the study site is often lower than the atmospheric level and much less compared to that in the ex-situ incubation, even in the hotspots of methane accumulation during the wet season (3.76 ppm) (Epron et al., 2025). PMORs measured on subsoil samples may 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.

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.

## 4 Conclusion

In this study, we adapted the sampling strategy over time since the topsoil we collected in our first sampling showed a low methane oxidation potential, unlike previous studies. We, therefore, subsequently targeted the deeper soil layers. 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. Nevertheless, our results provide a new insight into the impact of tropical agricultural land use on the ecological function of soils in the cycle of a potent greenhouse gas. Even a recommended fertilizer application rate in a rubber plantation could hurt soil methane oxidation potential spatially and temporarily, altering the methane cycle of tropical soils. Harmonious land use of tropical soils for rubber plantations in the future should consider the risk of reduced methane uptake due to fertilization. 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.

## Data availability

The data generated in this study are available from the corresponding authors upon reasonable request.

## Supplement

The supplement related to this article is available online.

## Author contributions

JM: conceptualization (lead); investigation (lead); methodology (lead); supervision (lead); formal analysis (lead); writing (original draft) (lead) and writing (review and editing) (equal). KS: investigation (equal); methodology (equal); writing (review and editing) (equal). CD: investigation (equal); methodology (equal); writing (review and editing) (equal). OD: investigation (supporting); writing (review and editing) (equal). RC: resources (equal); investigation (supporting); writing (review and editing) (equal). WR: investigation (supporting); writing (review and editing) (equal). WA: investigation (supporting); writing (review and editing) (equal). MS: investigation (supporting); writing (review and editing) (equal). PK: investigation (supporting); writing (review and editing) (equal). DE: conceptualization (supporting); investigation (equal); writing (review and editing) (equal).

**Competing interests**

The contact author has declared that none of the authors has competing interests.

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