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科研費 KAKENHI

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研究課題名（和文）Fertilization effects on atmospheric methane uptake by a rubber tree plantation in Thailand: a direct microbial inhibition or an indirect stimulation by higher tree water use?

研究課題名（英文）Fertilization effects on atmospheric methane uptake by a rubber tree plantation in Thailand: a direct microbial inhibition or an indirect stimulation by higher tree water use?

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研究成果の概要（和文）：この国際的な共同研究プロジェクトの目的は、ゴム農園における施肥が、気候緩和に貢献する土壌による大気中メタンの吸収に与える影響について、深い洞察を得ることである。施肥とメタン吸収には二通りの相互作用が予想される。我々の最初の仮説は、施肥により樹木の蒸散が増加し、土壌へのメタンの拡散が増加することで、メタン酸化が促進されるというものであったが、これは棄却された。我々の次の仮説は、施肥によって、特に無機態窒素の濃度が高くなった際に、メタン酸化菌の活動を抑制するというものであり、これが確認された。さらに、我々は施肥がメタン生成を促進することを示した。

研究成果の学術的意義や社会的意義
Fertilization inhibited methanotrophic activity and stimulated methanogenesis, decreasing the strength of the CH4 sink of a rubber plantation, and
turns the plantation from a sink to a source of CH4 at the high rate of fertilizer application. The loss of soil CH4 uptake should be evaluated

研究成果の概要（英文）：The objective of this joint international project was to have a deep insight into the effect of fertilization on atmospheric CH4 uptake by the soil of rubber plantations because of its positive role in mitigating climate change. Two types of interaction between fertilization and CH4 uptake are expected. Our first hypothesis that fertilization increases the diffusion of CH4 in the soil due to a higher rate of transpiration of trees in fertilized plots, promoting its oxidation, was rejected. Our second hypothesis was that fertilization increases inorganic nitrogen and available phosphorus in the soil, which inhibits methanotrophic activity when concentrations become high, was confirmed. In addition, we showed that fertilization also enhanced methanogenesis.

研究分野：Forest Science

キーワード：Soil methane　Rubber plantation　Southeast Asia　Fertilization　Nitrogen cycling　Microbial ecology

１．研究開始当初の背景 [Background at the start of the research]

Methane (CH_4) is the second most important anthropogenic greenhouse gas and contributes for one third to the anthropogenic radiative forcing (IPCC 2021). Soils are the largest biological sink for atmospheric CH_4, with a global uptake of about 25 – 45 Tg per year (Saunois et al. 2020). Upland tropical forest soils massively contributed to this global sink, providing a valuable ecosystem service. South-East Asia has seen huge changes in land use in previous decades, the expansion of rubber cultivation being one of the main, along with that of oil palm. Rubber plantations covers more than 142,000 km^2 of land in Southeast Asia (Wang et al. 2023), an area expected to quadruple over the next 30 years, driven by the continuous increase in the global demand of natural rubber for the tire industry (Fox et al. 2014). Beyond the comparisons of different land-uses, little is known about the effect of management practices, particularly fertilization, on the methane budget. A large survey of rubber smallholders in Thailand, who own 90% of the rubber plantations, found that almost all rubber plantations were fertilized, two thirds of them intensively or very intensively, even if the benefit has not really been proven (Chambon et al. 2018).

The net rate of CH_4 uptake, defined as the difference between CH_4 production and oxidation rates (Le Mer and Roger 2001), depends mainly on the air-filled porosity of the soil (AFP), which depends on its water content (SWC) and its total porosity (Kruse et al. 1996; Epron et al. 2016). A high AFP enhances gas diffusion in soil, and therefore CH_4 microbial oxidation. Although fertilization is a common practice in rubber plantations, its consequences on soil CH_4 uptake has not yet been documented. Fertilization can increase tree growth and thereby tree water use if the transpiration efficiency - the ratio of dry biomass accumulation per unit water transpired -does not increase significantly. A higher rate of evapotranspiration could result in a lower SWC, especially in the upper layer of the soil, and therefore in greater AFP. This would improve the diffusion of atmospheric CH_4 and O_2 into the pores of the topsoil where CH_4 is oxidized by methanotrophs, unless SWC becomes too low and limits microbial activity (Borken et al. 2006; von Fischer et al. 2009; Bras et al. 2022).

Fertilizers can also have direct but opposite effects on soil CH_4 uptake. It can alleviate N or P limitation on methane oxidizing bacteria (MOB) in soils of tropical forests depending on their nutrient status because MOB as other microorganisms need N and P to sustain their growth and activity (Bodelier and Laanbroek 2004; Veldkamp et al. 2013; Martinson et al. 2021). On the other hand, soil CH_4 oxidation can be reduced by excessive level of nitrogen input (Steudler et al. 1989; Zhang et al. 2020; Lee et al. 2023 Jan 31). Several mechanisms have been suggested to explain this inhibition. Ammonia-oxidizing bacteria, which can oxidize CH_4 instead of NH_4^+ when NH_4^+ availability is low, because of the similarity between the two enzymes ammonia monooxygenase and methane monooxygenase, shift their activity to NH_4^+ oxidation when N limitation is alleviated (Bédard and Knowles 1989). Similar substrate competition can affect soil CH_4 oxidation when NH4+ competes with CH_4 for the active site of the methane monooxygenase, which however do not provide C to sustain the growth of methanotrophic bacteria and produce nitrite which is toxic for them (Schnell and King 1994). In addition, cations in fertilizers, such as potassium, are capable to compete with NH_4^+ for the exchange sites on the clay-humus complexes in the soil, releasing NH_4^+. Nitrate, probably after its reduction to nitrite, has also been suspected of being a potent inhibitor of CH_4 oxidation in some soils (Wang and Ineson 2003; Reay and Nedwell 2004; Mochizuki et al. 2012). On the opposite, phosphate was found to mitigate the inhibitory effect of N on CH_4 oxidation in some sites while it has been suspected to stimulate methanogenesis, reducing the net uptake of atmospheric CH_4 (Zhang et al. 2011; Zheng et al. 2016).

２．研究の目的 [Research objectives]

The objective of this joint international project waswa to have a deep insight into the effect of fertilization on atmospheric CH_4 uptake by the soil of rubber plantations because of its positive role in mitigating climate change. Two types of interaction between fertilization and CH_4 uptake are expected. Our first hypothesis was that fertilization increases the diffusion of CH_4 in the soil due to a higher rate of transpiration of trees in fertilized plots, promoting its oxidation. Our second hypothesis was that fertilization, and especially inorganic nitrogen, stimulates the development of methanotrophic microorganisms when they are very limiting, but inhibits methanotrophic activity when concentrations become high.

３．研究の方法 [Research methods]

The experimental rubber plantation is located at the Sithiporn Kridakorn Research Station of Kasetsart University, Prachuap Khirikhan province, Thailand (10°59’13”N, 99°29’22”E, 10 m a.s.l.) at the transition between two climate groups, the tropical rain forest and the Tropical Monsoon Climate. The rubber plantation (9 ha) was set up in 2007 at a density of 500 trees ha^{-1}. A complete randomized block design was established at this time with four blocks with four fertilizer treatments (N/P/K): T1 (none), T2 (35/20/48 kg ha^{-1} $year^{-1}$), T3 (70/40/95 kg ha^{-1} $year^{-1}$), T4 (119/68/162 kg ha^{-1} $year^{-1}$). T3 falls within the range of the recommended rates of fertilizer application for mature rubber plantations in Thailand by Thai public institutions, recommendations exceeded by 40% of rubber farmers (Chambon et al. 2018). The fertilizer was applied only in the early rainy season for T2 while a second application was made late in the rainy season for T3 and T4. The fertilizer was applied evenly to only half of the area between two planting rows.

Soil CH_4 fluxes ($F_{S\text{-}CH_4}$) were measured over one and half year at approximately two weeks intervals (39 measurement dates between Sep 6, 2022 and Feb,19 2024). 96 PVC collars were distributed in four blocks and four fertilizer. Collars were covered with a 20 cm soil chamber (Li 8100-103) and the changes in the CH_4 concentrations in the closed chamber were recorded for 3 min using a cavity-enhanced absorption spectroscopy gas analyzer (Li 7810, Li-Cor; Lincoln, USA). The slopes of the linear variations in CH_4 concentration over time, calculated after discarding the first 60 s of gas concentration measurements were used to calculate CH_4 flux. A positive CH_4 flux indicates a net flux to the atmosphere while a negative flux indicates a net flux from the atmosphere.

Soil temperature at a depth of 10 cm and the volumetric soil water content from the 0 to 6 cm layer were measured near the PVC collars simultaneously with the flux measurements. Xylem sap flux density was measured from Feb 20, 2023 to Feb 24, 2024 on four trees in each treatment selected to cover the diameter variation. The monthly averages of the cumulative daily volume of sap flow (cm^3 day^{-1}) were calculated for each tree then average for each fertilization treatments.

The soil CH_4 concentrations were measured at two soil depths (10 and 40 cm) close to 24 soil collars (six in each fertilization treatments) using two stainless steel pipes inserted vertically in the soil. An air sample (0.5 ml) was drawn from each tube through a septum into a syringe and injected through the septum in the gas analyser.

Potential CH_4 oxidation of soils collected at different depths by incubating 10 g of soil in 100 mL vials filled with 50 ppm of CH_4.Microbial communities were characterized by sequencing the amplicon of the 16 rRNA gene.

Soil N availability was assessed using ion exchange resin bags filled with 15 ml of mixed ion exchange resin beads (AmberLite MB20, Sigma-Aldrich). and buried in the mineral soil 5 cm below the litter layer in each of the four blocks and four fertilizer treatments on four occasions for three to four months. After collection, the resin bags were extracted with 2 M NaCl and analysed for NO_3^- and NH_4^+ by colorimetry.

Gross nitrogen mineralization and dissimilatory nitrate reduction to ammonium was estimated using the ^{15}N pool dilution method on intact soil cores in the field by injecting either ^{15}N labelled nitrate and ammonium and tracing ^{15}N in nitrate, ammonium and extractable organic N by isotope ratio mass spectrometry (IRMS).

4．研究成果［Research results］

Fertilization reduces soil CH_4 uptake during the dry season proportionally to the amount of fertilizer added and turns soil of the heavily fertilized treatment (T4) from a sink into a source of CH_4 during the rainy season (Figure 1). In contrast to our hypothesis, fertilization did not increase the air-filled porosity which was on the opposite slightly lower in the fertilized treatments than in

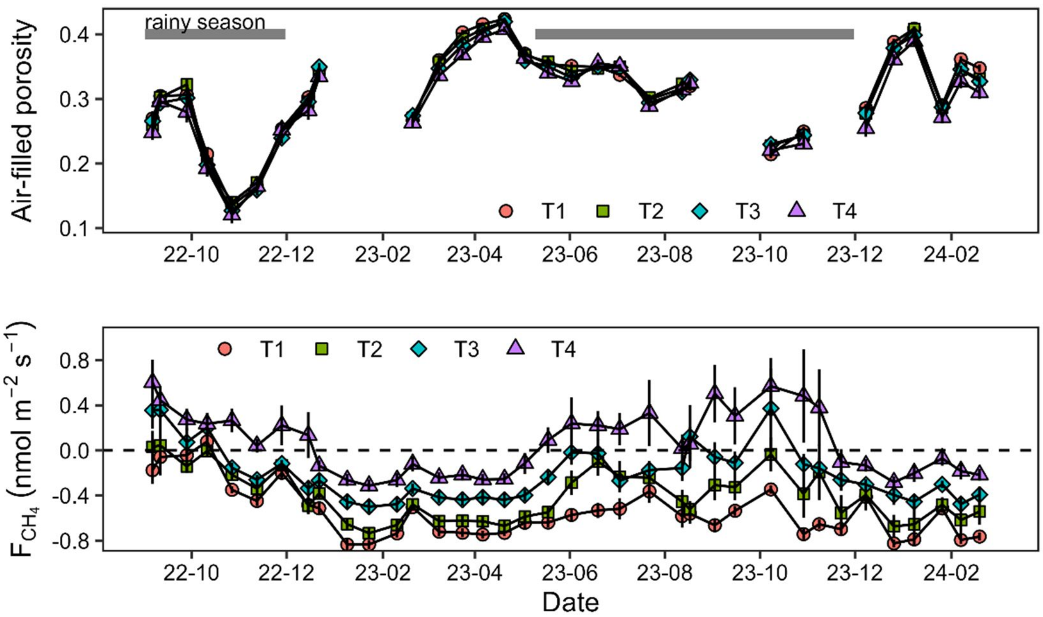


Figure 1. Seasonal evolution of air-filled porosity and atmospheric soil CH4 uptake in four fertilization treatments (T1: none;T2: low;T3: moderate;T4: high)

the control, consistent with a tendency of a lower rate of transpiration of trees in fertilized plots during the dry season (~13 L d^{-1} compared to 16 L d^{-1} in the control in February for example). Therefore, the change in tree water use with fertilization did not increase the diffusion of CH_4 in the soil and did not promote its oxidation.

Vertical profiles of CH_4 concentration (Figure 2) showed lower soil CH_4 concentration gradient in fertilized plots during the early rainy season (Aug 2023) and the dry season (Feb 2024) and CH_4 production in the top soil but not in the deep soil, during the rainy season in Oct 2023 when the soil was very wet.

Incubation experiment revealed that higher potential rate of CH_4 oxidation was observed below 10 cm than above and that the estimated CH_4 flux from cumulative potential rate of CH_4 oxidation over the soil profile is comparable to the flux *in situ*. Deeper soil is the actual sink of atmospheric CH_4 where a negative effect of fertilization was observed.

Fertilization reduced the amount of DNA in soil samples and the diversity of the microbial community. The microbial community shifts with soil depth. The molecular analysis of the microbial community is still on going for the last sampling in Feb 2024.

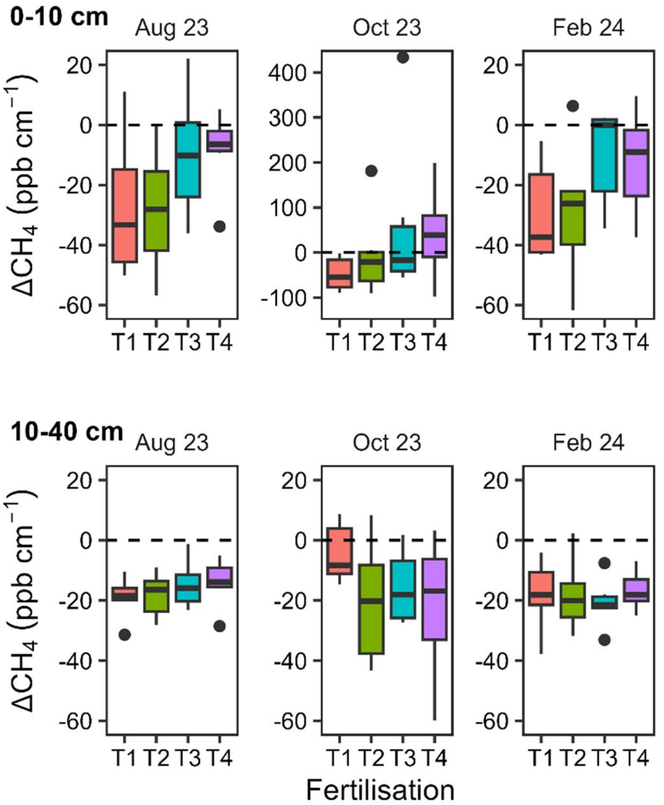


Figure 2. Soil CH_4 concentrations measured at 0, 10 and 40 cm depth were used to calculated concentration gradient in the top soil (0 - 10 cm) and below (10 - 40 cm)

Higher concentrations in mineral nitrogen (NO_3^- and NH_4^+) were captured by the resin bags buried in the fertilized plots (Figure 3) which could have inhibited methanotrophy and enhanced methanogenesis. We are currently measuring phosphate concentration as well. The results were consistent with the total dissolved nitrogen hat we measured in soil solutions collected using lysimeters installed in T1 and T3 by another team in the frame of another project. Higher DOC concentrations were also measured in the soil solutions collected in T3 compared to T1, providing substrates for methanogenesis.

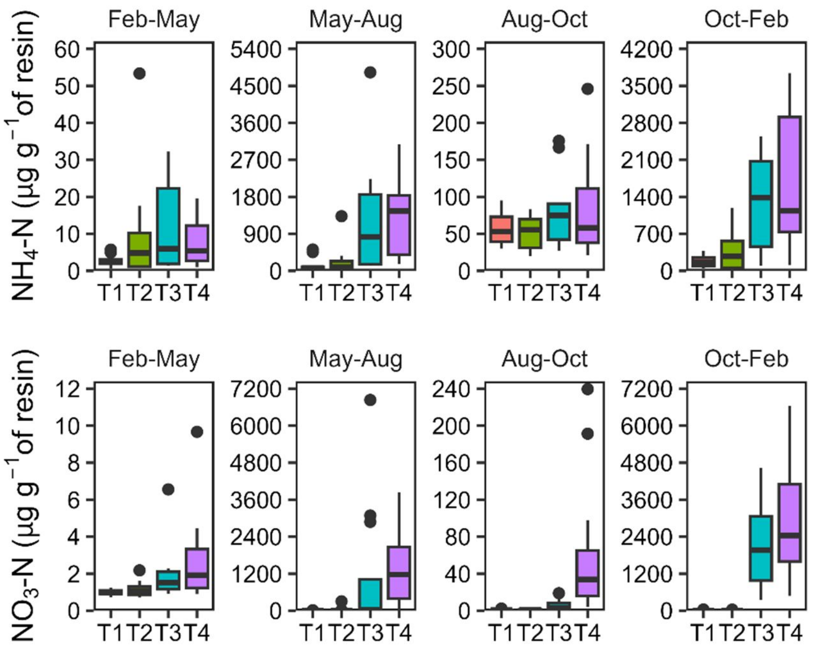


Figure 3. Accumulation of ammonium and nitrate in resin bags placed in the top soil layer for about 3 months during the dry season (Feb 2 - May 17), early in the rainy season after fertilization application before fertilization in T2, T3 and T4 (May 18 - Aug 14), during the rainy season (Aug 14 - Oct 7) and at the end of the rainy season / early dry season after fertilizer application in T3 and T4 (Oct 10 - Feb 20)

The soil samples collected after labelling soil core in the field with either ^{15}N labelled NO_3^- and NH_4^+ to estimate gross nitrogen mineralization and dissimilatory nitrate reduction to ammonium are currently ready to be analysed soon by isotope ratio mass spectrometry (IRMS).

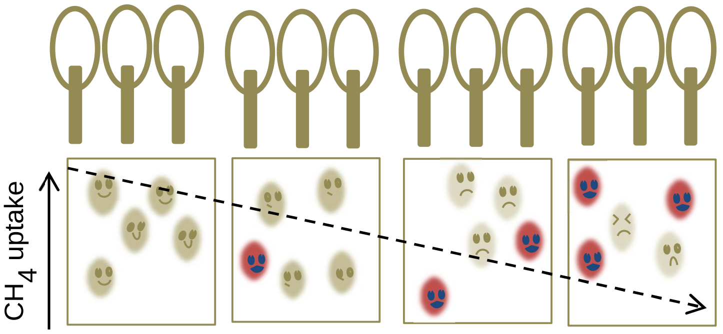
Table 1: Annual soil CH_4 budget in the four fertilization treatments from Sep 2022 to Aug 2023

Treatment	Soil CH_4 flux kg CH_4 ha^{-1} yr^{-1}
T1: none	-2.6 ± 0.2
T2: low (75/45/100 NPK)	-2.0 ± 0.2
T3: moderate (180/80/170)	-1.1 ± 0.2
T4: high (306/136/289)	+0.2 ± 0.3

In conclusion, fertilization decreased the strength of the CH_4 sink of a rubber plantation, and even turns the plantation from a sink to a source of CH_4 at the highest rate of fertilizer application (Table 1).

Only CH_4 sources (rice, vegetation burning, enteric fermentation, manure, wetland) are currently considered when reporting GHG emission in the AFOLU[1] sector. However, the loss of soil CH_4 uptake matters and the decrease in sink strength related to management such as fertilization should be evaluated and also reported.

Graphical abstract



- Fertilization did not increase the size of the tree significantly
- No increase in tree water use with fertilization that would have improved atmospheric CH_4 diffusion in the top soil layer
- Fertilization inhibited methanotrophic activity and stimulated methanogenesis

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[1] Agriculture, Forest and Other Land Use (United Nations Framework Convention on Climate Change)

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計3件（うち招待講演　0件／うち国際学会　1件）

１．発表者名 Daniel Epron et al
２．発表標題 Fertilization turns a rubber plantatio from sink to methane source
３．学会等名 European Geosciences Union Assembly（国際学会）
４．発表年 2024年

１．発表者名 Daniel Epron et al
２．発表標題 Fertilization turns a rubber plantatio from sink to methane source
３．学会等名 Japan Geoscience Union meeting
４．発表年 2024年

１．発表者名 Jun Murase et al
２．発表標題 Methane oxidation potential of soils in a rubber plantation in Thailand
３．学会等名 Japan Geoscience Union meeting
４．発表年 2024年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関