

Methane Uptakes and Emissions in Upland Tropical Forest and Agricultural Soils

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Abstract: Three land use types in Thailand; 1) the natural forests (hill evergreen forest (HEF), dry evergreen forest (DEF), moist evergreen forest (MEF) and mixed deciduous forest (MDF)), 2) a reforested forest (ARF) and 3) agricultural field (AG) were studied for their methane fluxes, oxidation potential and kinetics. Net atmospheric methane consumption was observed at all forest and reforested sites, with the monthly consumption rate ranging from $-0.6 \text{ mg CH}_4 \text{ m}^{-2} \text{ day}^{-1}$ at the reforested site to $2.4 \text{ mg CH}_4 \text{ m}^{-2} \text{ day}^{-1}$ at the hill evergreen forest site. At the agricultural site the net methane emission of $13.6 \text{ mg CH}_4 \text{ m}^{-2} \text{ day}^{-1}$ was found. At dry evergreen forest and reforested sites, a clear zonation for active methane oxidation layer was detected along the soil depths. The most active oxidation lied between 15 cm and 40 cm while in agricultural soil no clear active layer was observed. Stratification of active oxidation zones coincided with the trends of inorganic nitrogen content profile. In DEF and ARF soils, high concentration of inorganic nitrogen compounds (usually $> 100 \text{ mg NO}_3^- \text{ or NH}_4^+ \cdot \text{kg soil}^{-1}$) was detected in the top 15-cm soil while there was no clear distribution trend found in AG soil. Examining kinetic coefficients of these active layers revealed that soil at all natural forest sites had high affinity for methane (K_m of 52 ppmv) but rather low methanotrophic capacity (V_{\max} of $0.82 \text{ nmol} \cdot \text{g soil}^{-1} \cdot \text{h}^{-1}$). Soil at ARF and AG sites, on the other hand, showed low affinity for methane (K_m of 724 ppmv and 1454-2362 ppmv, respectively). However, soils at these two sites were capable of oxidizing high concentration of methane (V_{\max} about $10 \text{ nmol} \cdot \text{g soil}^{-1} \cdot \text{hr}^{-1}$). These results indicate that land use type significantly affects rates, depth distribution and kinetics of methane oxidation in tropical soils.

Keywords: Methane oxidation, depth distribution, methane oxidation kinetics, and land use.

1. Introduction

Microbial uptake in upland soils represents the major terrestrial sink of atmospheric methane. This sink consumes about 9-47 Tg (10^{12} g) of CH_4 annually, accounting for about 5% of the global methane sink [1-2]. In most cases, oxidation activity is found highest in undisturbed forest soil [3-4].

In many oxic soils, biphasic methane oxidation kinetics was observed and this is presumably attributed to the presence of different methanotrophic communities [5]. The members of the first group are methanotrophs that have high capacity (high V_{\max}) but low affinity (high K_m value) for methane. They are capable of oxidizing methane at elevated concentrations (>1000 ppmv). The members of the second group exhibit high affinity for methane and are able to consume methane at atmospheric concentration. Most of the cultured and isolated methanotrophs belong to the first group. However, the presence and role of methanotrophs belonged to the second groups have been confirmed by culture-independence techniques [6-8]. Most studies show that kinetic characteristics of methane uptake can change according to soil conditions [9]. Nutritional starving was also able to change K_m of soil enrichment culture. It is not known, however, such changes in kinetic characteristics are due to changes in methanotrophic community upon changing methane and other nutrient availability, or due to adaptive capability of methanotrophs.

Among upland soils, forest soils usually show higher methane uptake rate than cultivated soils. Changes in land use, especially cultivation of formerly undisturbed soils, usually results in loss of methane oxidation capacity [10]. This has been attributed to inhibition by nitrogen fertilizers applied during crop cultivation [11-12]. Inorganic nitrogen compounds, especially ammonium (NH_4^+), strongly inhibit methane oxidation, presumably through the competition with methane as a substrate. It has also been suggested that changes in soil water regime, soil pH, some physical properties such as water

permeability and gas diffusion rate induced by change in land use significantly affecting on methane oxidation capacity of soils.

The most remarkable change in land use in the past decades is deforestation in the tropics. It is estimated that deforestation rates in the tropics are as rapid as $2\% \text{ yr}^{-1}$ [13]. Such land use change leads to loss of methane oxidation potential and thus may have implicated the global methane budget by contribution to increasing atmospheric concentrations. However, recently reforestation in the abandoned lands has been promoted in many tropical countries. It is not known whether or not such activity could recover methanotrophic capacity of soil to the level that comparable to natural forest soils. Our previous studies show that land use pattern significantly affects methanotrophic community [11]. Here we report that such land use activity could affect the kinetics of methane oxidation. Our main objective is to assess how oxidation kinetic of tropical upland soils is affected by converting natural forest into cultivation land and *vice versa*.

2. Experimental

2.1 Study sites

Three land use types: forested, reforested and cultivation soils) were investigated in the present study. Five types of forests were included. These were; 1) a natural dry evergreen forest (DEF) located in the Sakearat Environmental Research Station. The dominant tree species were *Hopea ferrea*, *Pterocarpus marcrocarpus*, *Xylia xylocarpar*, *Dalbergia cochinchinensis*, *Lagerstroemia duppereana*, and *Shorea henryana*, 2) the reforestation site (ARF) located about 5 km away from the DEF site was planted with fast-growing, nitrogen-fixing tree species *Acacia mangium* in 1988. Soil texture at DEF site was Clay (clay content = 51%) while at ARF it was sandy clay loam (clay content = 30%), 3) hill evergreen forest (HEF), 4) mixed deciduous forest (MDF), and 5) moist evergreen forest (MEF). Both HEF and MDF sites were located in Nan province, North

of Thailand. However, the MEF was located in Trat province, East of Thailand. The HEF situated at 1500 m above MSL, with annual average minimum and maximum temperatures of 17°C and 26°C, respectively. The annual rainfall during 1995-2001 was 1308-1681 mm. The MDF site was characterized by lesser tree density compared to HEF, and populated by large and mid-sized trees. Bamboos generally grew in pockets. Teak trees were also found in the study site. During the dry season the trees lost their leaves, and forest fires generally swept across the area once a year. When rainy season starts, the forest became green and lush. The average elevation is 700-1000 above MSL at MDF site. From the records obtained from the adjacent Khun-Sathan Environmental Research Station, the minimum and maximum temperatures at study site was 17°C and 26°C, with annual rainfall of about 1300-1700 mm. For MEF site, the average elevation was between 20-40 m above MSL and the average temperature was 26.8°C. The relative humidity was 81.95%, with the average annual rainfall of about 3,000-4,000 mm. The maximum rainfall came in July, but the minimum rainfall was during April. Soil pH in the site was around 4.5-5.6. The soil was sandy clay loam at both HEF (clay = 27%) and MEF (clay = 27%), while at MDF site was clay.

For agricultural site, this was a cornfield (AG) situated adjacent to ARF (2 km away). It was deforested more than 40 years ago and maize had been continuously cultivated at this site during the last 16 years. The soil texture was sandy clay loam (clay content = 21%). Soil preparation for corn plantation at AG site was usually started in the mid of June, and seeds of corn were sown on July. Chemical fertilizer (16-20-0, N-P₂O₅-K₂O) was applied at the rate of 125 kg ha⁻¹.

2.2 Flux measurements

The net methane flux across the soil surface was determined using a closed-static chamber method as described by Knief et al. [11]. Chambers were consisted of a top made of transparent acrylic glass (30 cm width × 30 cm length × 15 cm height) that could be attached to a fixed stainless steel collar. The collar was inserted 10-15 cm deep into the soil and remained there during the whole study period. Each chamber was 10-15 m away from each other. During measurement, the chamber top was inserted into a 1-cm-deep water-filled channel of the fixed collar. Using 30-ml syringes inserted through silicon stoppers in the chamber lids, 6-10 gas samples of 25-30 ml were taken from each chamber during 1 h after capping. Methane mixing ratios of the gas samples were determined on a Shimadzu gas chromatograph (Model 14B), equipped with a flame ionization detector (FID), and an Unibead C packed column (injector temperature, 120°C; oven temperature 100°C; detector temperature 300°C). The first-order uptake rate constant was estimated from the exponential decrease of methane over time. The methane oxidation rate was calculated from the first-order uptake rate constant by multiplication with the initial methane mixing ratio in the chamber headspace.

2.3 Kinetic study

The air-dried and 2-mm sieved soil was used. Firstly, oxidation activity at different soil depth was determined. Soil layers were divided according to soil depth of 5 cm intervals. Soil samples (30 g) were weighed into the vials, moisture adjusted to 25% and then they were incubated at 25°C (room temperature). Concentration of methane in the vial's headspace was subsequently determined. Decrease in headspace methane concentration was then plotted against incubation time and rate constant of methane consumption was determined from the slope of log-transformation plot. Oxidation rate was calculated by multiplying the rate constant with the initial methane concentration.

The most active oxidation layer was then selected for further determination of kinetic coefficients. The 2-mm sieved soil (30 g) were weighed and put into the 100-mL vials. Soil moisture was adjusted to 25% by weight. The vials were then closed with a rubber stopper and the headspace methane concentrations were adjusted to the range from ambient to 1800 ppmv. The vials were incubated at room temperature (25-26°C) and headspace methane concentration was determined at appropriate time intervals to follow a decrease in methane concentration with times. Incubation of duplicate samples for each soil layer was made. Kinetic coefficients (V_{max} , K_m value) associated with CH₄ uptakes were estimated from the plot between the initial CH₄ concentrations versus oxidation rates. Such plot was fitted to a Michaelis-Menten hyperbolic model of pseudo-first-order enzyme kinetics using the least-square iterative fitting procedures of Origin 5.2 (Microcal Software, Inc., Northampton, Maine).

2.4 Chemical analysis

A Shimadzu Gas Chromatograph (Model 14B) equipped with FID detector was used to measure CH₄ in the present study. The GC operating conditions were: FID temperature: 300°C, Injection temperature; 120°C, Column temperature; 100°C, Carrier gas; Helium (99.99% purity), Carrier gas flow rate; 65 mL min⁻¹, Column; Unibead C packed column. Inorganic nitrogen contents (NH₄⁺, NO₂⁻, NO₃⁻) were determined by Ion Chromatograph (Dionex DX600, USA) equipped with an electrochemical detector. Ten grams of 2-mm sieved soil from each layer were weighed and placed into an Erlenmeyer flask and 70 mL of deionized water was added. The flasks were shaken on a shaker for 45 min and the supernatant was filtered through a 0.45 μm cellulose acetate filter membrane. Deionized water was used as a blank control. Extraction was carried out in triplicates for each soil layer. Concentrations of inorganic nitrogen in filtered liquid were then determined and expressed as mg of an inorganic nitrogen compound per kg of dry soil.

3. Results

3.1 Inorganic nitrogen content

Inorganic N content (NO₂⁻, NO₃⁻ and NH₄⁺) of all soils was measured along the soil depth. Results are given in Fig. 1. In forest soils, NO₃⁻ content was higher in topsoil than subsoil. Relatively high NO₃⁻ contents were found in topsoil of HEF and DEF, while it was almost the same amount for all forest soils below 30 cm (Fig. 1a). Content of NO₃⁻ throughout soil profile at MEF and MDF sites was fairly constant around 1-10 mgNO₃⁻ kg soil⁻¹. NO₃⁻ content throughout soil profile at both ARF and AG sites varied between 30 and 75 mg kg soil⁻¹. However, very low concentration of nitrate was found below 30 cm in ARF soil. No clear trend along the profile was observed for both ARF and AG sites.

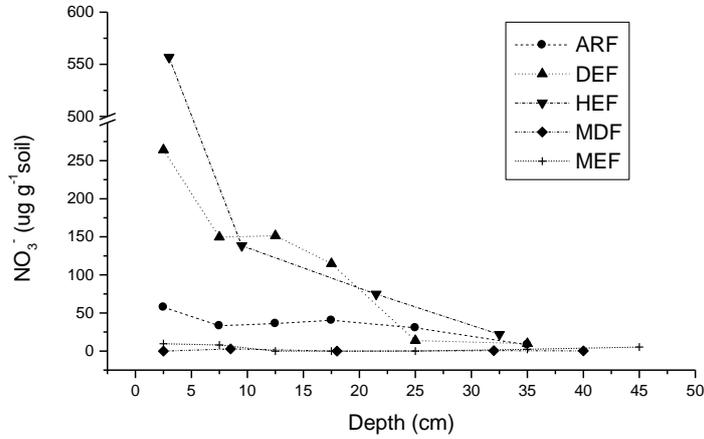
For forested and reforested sites, NO₂⁻ was found only in DEF and ARF soils, but it was found only in small amount in range of 0.29-16.26 and 0.40-14.63 mg NO₂⁻ kg soil⁻¹ for DEF and ARF sites, respectively (Fig. 1b). Compared to NO₃⁻, NO₂⁻ content was approximately more than 10 times lower for both DEF and ARF soils. It was lower than 3 mg kgsoil⁻¹ below 10 cm in DEF site as well as below 15 cm for ARF site. In contrast, NO₂⁻ content was not detected across the soil profile for other forest soils. For cultivation site (AG), the NO₂⁻ was detected across the soil profile at AG site, although its concentration was generally lower than 10 mg NO₂⁻ kg soil⁻¹, which range was 1-6 mg NO₂⁻ kg soil⁻¹ (data not shown).

The NH₄⁺ profile for forest soils was similar to the trends found in NO₃⁻ content, which tended to decrease as soil depth increased. However at ARF site, NH₄⁺ concentration was quite fluctuated in the top 20 cm surface layer (Fig. 2C). The

concentration of NH_4^+ in DEF and HEF were sharply decreased during the top 15 cm but below this layer the concentration was not much different, which was below $50 \text{ mg kg soil}^{-1}$. In contrast, a fairly constant amount of NH_4^+ was observed throughout the profile at both MDF and MEF soils, which was approximately

less than $40 \text{ mg kg soil}^{-1}$. The highest concentration was found in DEF soil but was not significantly different from other forest soil types. The NH_4^+ concentration at AG site at all depths was below $40 \text{ mg kg soil}^{-1}$ and it trended to increase in the deeper layers (data not shown).

(a) Nitrate



(b) Ammonium

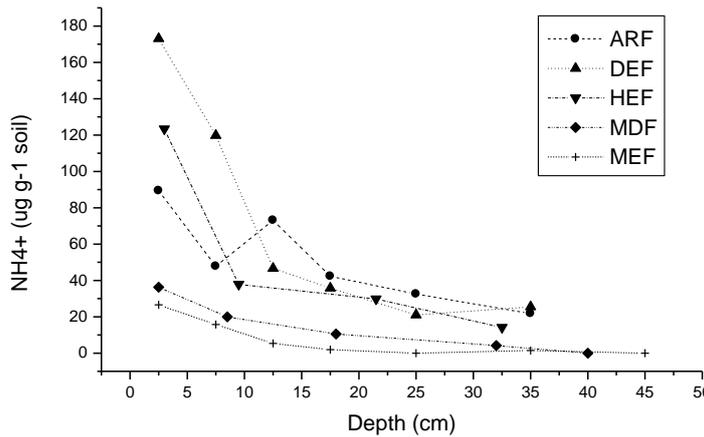


Figure 1. Nitrate (a) and ammonium (b) contents along the soil profile in different land use.

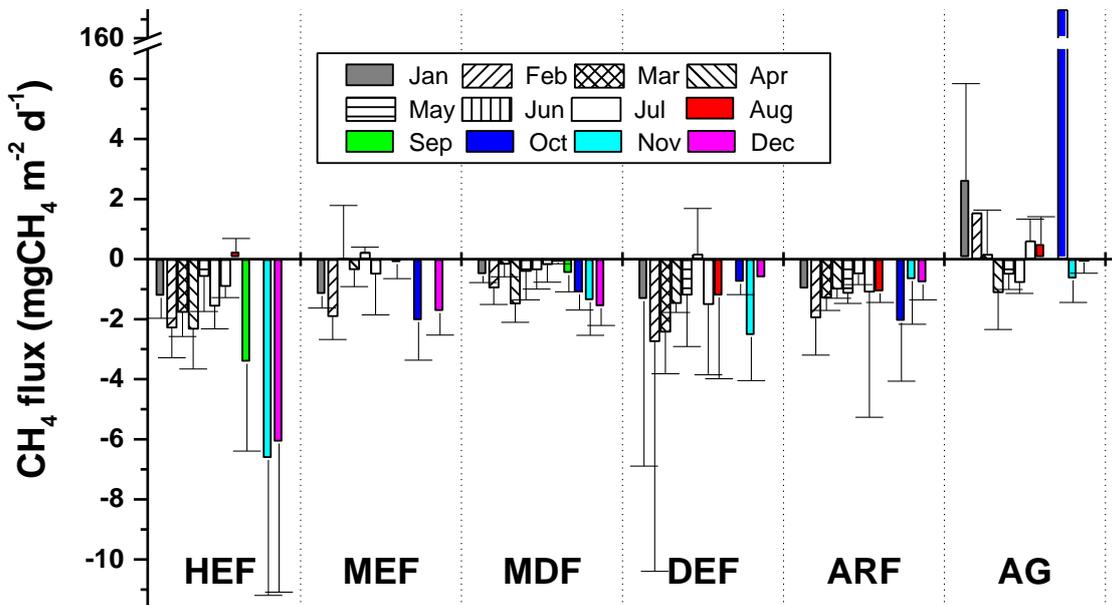


Figure 2. Monthly flux of methane under different land use type. The negative and positive flux values denote the net methane uptake and emission, respectively. Error bars represent standard deviation of three replications.

3.2 Methane fluxes

The methane uptake rates obtained from different chambers within the same sampling site showed large variation. The variations of net fluxes among chambers measured within the same day were usually >50% of the mean. Not all chambers at a site showed a net consumption of methane. Likewise, in some chambers neither net emission nor consumption was observed. However, in general net atmospheric methane consumption was observed in all months at forest sites, except in some months of evergreen forest which were observed in August, May June at HEF, MEF and DEF respectively. The high oxidation rate was found at HEF site, although these were not significantly different among all forest sites. The monthly average were in order of HEF > DEF > MEF \approx MDF, which were -2.44 ± 2.20 , -1.45 ± 0.88 , -0.88 ± 0.90 and -0.76 ± 0.56 mg CH₄ m⁻² d⁻¹, respectively.

Net oxidation of methane was only observed during April-June and August-November at AG site. Except in June, in all cases net methane uptake was observed at DEF, with oxidation rate ranging between -0.7 and -3 mg CH₄ m⁻² day⁻¹. At ARF, there was no net emission observed but oxidation occurred in all cases with the value ranged between -0.5 and -2 mg CH₄ m⁻² day⁻¹. At AG site, although occasionally net oxidation of atmospheric methane was observed, in October the relatively high flux ($+161$ mg CH₄ m⁻² day⁻¹) made it became the net methane emitter. The average net methane fluxes over the measurement period at each site were -1.53 ± 0.88 , -0.57 ± 1.53 and $+13.58 \pm 46.60$ mg CH₄ m⁻² day⁻¹ at DEF, ARF and AG sites, respectively. To test for significant difference in the net methane flux among the sampling sites, the non-parametric Mann-Whitney test was applied. For the comparison, the flux data from both forested and reforested sites of both months were combined and compared to the data from the farmland site. This analysis results indicates that the

net methane flux in the two forested sites was significantly different (more negative) than the flux in the AG site ($p < 0.01$). However, there was no significant difference among uptake rates among forested sites.

3.3 Methane oxidation rate as a function of soil depth

Soils from forested and reforested sites were incubated under ambient methane concentration and then consumption was followed over times. Since soil from AG site showed low oxidation activity, incubation was carried out under 5 ppmv methane. Each soil depth in all sites were measured in two replicates. Most of soil layers exhibited the methane oxidation activity, except at below 30 cm depth of MEF site. However, the most active methane oxidation layers for forested soils were 15-30, 24-40, 13-30 and 15-20 cm at DEF, MDF, HEF and MEF respectively. Although *in situ* methane flux measurements indicate that both of oxidation and emission, methane oxidation was occurred in all depth profile at HEF and DEF. Almost no oxidation activity was observed in the top 5-cm and below 40-cm layers at DEF site. The oxidation layer at MEF was found above 30 cm but production was observed at below 30 cm. At forested active layers, the oxidation was found approximately 13, 14, 30 and 47 pmol CH₄·g dry soil⁻¹·hr⁻¹ in MEF, DEF, AHE and MDF respectively (Fig. 3). For ARF site, oxidation activity was found in all layers and the highest rate was found in the 5-10 cm layer (9 pmol CH₄·g dry soil⁻¹·hr⁻¹, Fig. 3C). Similarly, most layers of soil from AG site show oxidation activity (Fig. 3D). Oxidation rate was not significantly different among different soil layers, ranging from about 5 to 7.5 pmol CH₄·g dry soil⁻¹·hr⁻¹. However, the most active layer was at 0-5 cm and 30-40 cm (7.5 pmol CH₄·g dry soil⁻¹·hr⁻¹ for both layers).

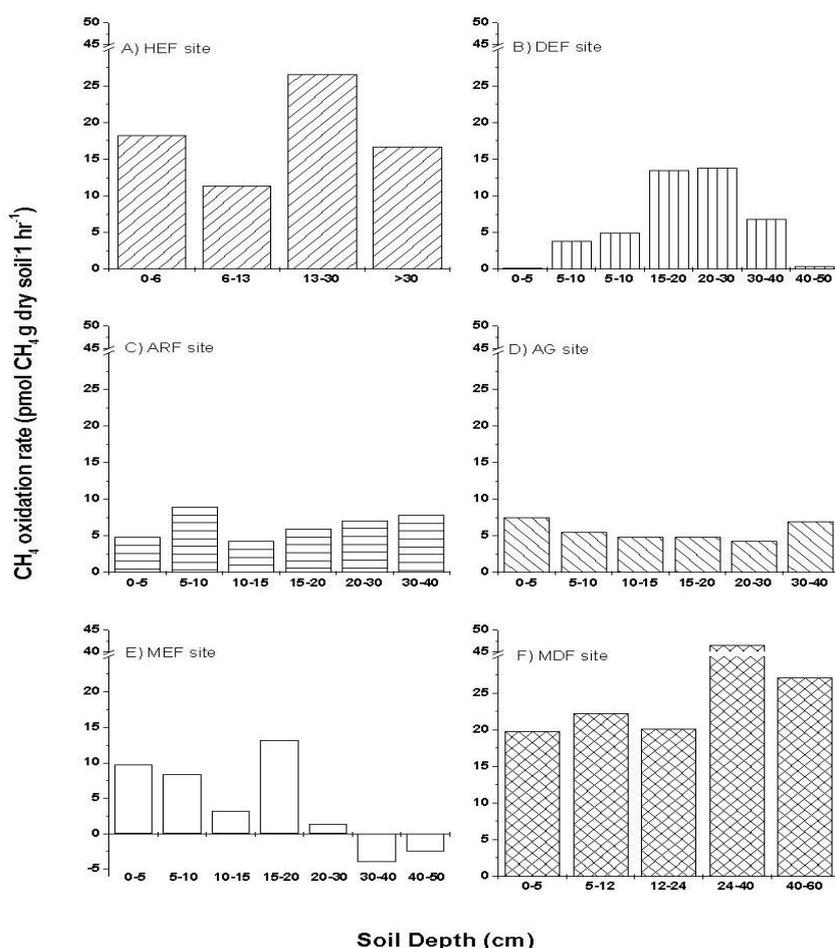


Figure 3. Oxidation rate of methane in incubated soils taken from different soil depths.

3.4 Kinetics of methane oxidation

In order to calculate the kinetic coefficient for methane oxidation, soil from the most active layer was incubated under various methane concentrations. Methane oxidation rate at each concentration was then estimated by linear regression between CH_4 mixing ratios versus time. A plot of initial methane concentration and methane oxidation rate is given in Fig. 5. The initial methane concentrations used in the experiment were 5, 10, 20, 50, 100, 200, 300, 700, 800, 1000 and 1800 ppmv. The plot between consumption rate and substrate concentration followed an expected shape of enzymatic kinetics with initially increase oxidation rate paralleling increase concentration. In tropical Thai forested soils, V_{\max} were observed at 0.82 to 1.76 $\text{nmol CH}_4 \text{ g soil}^{-1} \text{ hr}^{-1}$. The K_m of CH_4 oxidation was 52.02, 126.50, 99.43 and 188.39 ppmv in DEF, MDF, MEF and HEF soils, respectively. These mean that higher affinity was found at DEF than MEF, MDF and HEF respectively. At the initial CH_4 concentration below 50 ppmv, the oxidation rate was not different (Fig. 4). However, small difference among forest soils was observed. At CH_4 concentration higher than 100 ppmv, oxidation rate differed significantly, being highest in HEF, followed by MDF, MEF, and DEF soils, respectively. Therefore the capacity of methane oxidation as a function of methane concentrations in DEF was lower than MEF, MDF and HEF soils, based on the V_{\max} values.

Throughout different land uses, high affinity was found at forested (DEF), reforested (ARF) and agricultural (AG) soils respectively. For DEF site, the oxidation rate was relatively high comparing to other soils under methane concentration below 50 ppmv. However for ARF and AG soils, oxidation rate increased significantly at concentration above 50 ppmv. The oxidation rate was no longer showed concentration dependence at above 400 ppmv CH_4 concentration. However, in ARF and AG soils it seemed that at concentration below 50 ppmv, there was no clear increase in oxidation rate when concentration of methane in the headspace increased. Significant increase of oxidation rate in these two sites was observed at concentration above 50 ppmv (Fig. 4). However, above 500-600 ppmv and 1000 ppmv, no clear increase in oxidation was observed in ARF and AG soils, respectively. For a better visualization, an expanded-scale was plotted between oxidation rate and methane concentration below 300 ppm as given in Fig. 5. It is clear that at methane concentrations was below 250 ppmv, rate of methane oxidation in DEF soil was higher than at both ARF and AG site. Thus, soil at DEF could oxidize methane at low concentration better than soils at ARF and AG sites. However, above about 250 ppmv, the oxidation rate became concentration independence at DEF site. Thus, methane oxidation rate in DEF soil would not increase significantly if its methane concentration is higher. In ARF and AG sites oxidation rate still increased and then stabilized around 800 and 1000 ppmv, respectively. At ambient and 50 ppmv concentration, however, soil at ARF site showed higher oxidation rate than at AG site, though the difference was not clear.

Table 1. Kinetic coefficients measured for methane oxidation by soil samples taken from different land use types.

| Site | V_{\max} ($\text{nmol g}^{-1} \text{ hr}^{-1}$) | K_m (ppmv) |
|----------|---|--------------|
| DEF | 0.82 | 52.02 |
| MDF | 1.72 | 126.50 |
| HEF | 1.76 | 188.39 |
| MEF | 1.25 | 99.44 |
| ARF | 5.72 | 723.96 |
| AG | | |
| 0-5 cm | 10.66 | 1454.88 |
| 30-40 cm | 9.97 | 2361.76 |

The estimated kinetic coefficients associated with methane oxidation as affected by different land use are given in Table 1.

The highest affinity for methane was found in DEF soil ($K_m = 52 \text{ ppmv}$). Soil from ARF and AG showed lower methane affinity compared with DEF site (K_m values of 724 ppmv and 155-2362 ppmv, respectively). The lowest V_{\max} was found in DEF soil. However, there was no significant difference in V_{\max} between ARF and AG soils.

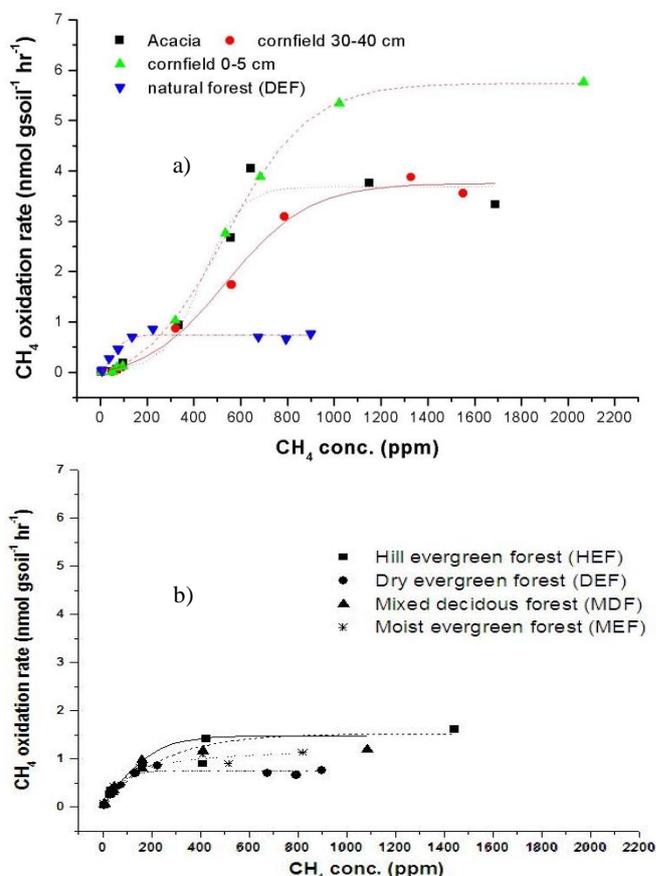


Figure 4. Relationship between initial methane concentration vs. methane oxidation rate, a) in ARF (reforested to acacia), AG (cornfield) and natural forest (DEF) and b) in other natural forests.

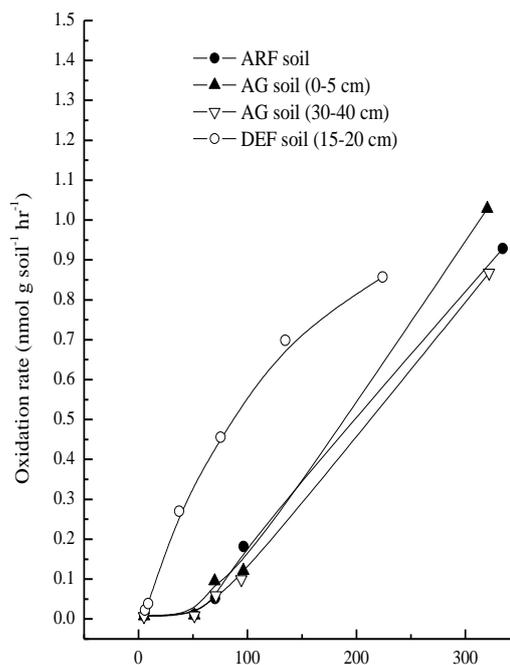


Figure 5. An expanded plot of relationship between oxidation rate and methane concentration below approximately 350 ppmv.

4. Discussions

Results from previous studies indicate that methane oxidation in soils varies according to several factors. Among these, land use type and mode of agricultural practices are among the most influencing ones [3, 14-15]. Conversion of natural forest to cultivated lands usually results in partial loss of methanotrophic activity. Powlson et al. [16] reported that continuous cultivation of 150 years for arable crops led to a reduction of methane consumption rate by 85%, compared to the soil under woodland. Several explanations have been suggested for the loss of methane oxidation associated with land use change.

Repeated application of nitrogen fertilizers have been shown to inhibit methane oxidation [16-18]. Among types of nitrogen compounds, ammonium seems to have the strongest effects. Although nitrite is directly toxic to methanotrophs, its low concentration usually encountered in soils and being highly unstable in nature makes it less important than ammonium regarding its role in inhibiting methane oxidation. For ammonium, inhibitory mechanisms on methane oxidation is suggested to be through the competition between ammonium ions and methane for the binding site of methane monooxygenase which is the key enzyme catalyzing the first step of methane oxidation in methanotrophs [12]. Besides the competitive mechanism, salting effects on methane oxidation accompanied with fertilization is also important [19]. Gullledge and Shimel [19] (1998) showed that application of nitrogen fertilizer in form of ammonium chloride had stronger effects on methane oxidation than ammonium sulfate. They also showed that ammonium concentration of 94 mg kg soil⁻¹ (as Ammonium sulfate) inhibited methane oxidation by 67% in temperate hardwood forest. On the other hand, nitrate becomes inhibitory to methane oxidation only at high concentration (usually > 10 mM dissolved nitrate)

Results in the present study agree well with the findings in the previous studies that when high concentration of inorganic nitrogen presents (Fig. 1), low methane oxidation was observed. However, concentrations of all nitrogen species were higher in DEF and ARF soils than in AG soil and such levels of ammonium might be sufficiently high to inhibit methane oxidation. Nevertheless, relatively high oxidation rate was observed at both DEF and ARF sites. Thus, nitrogen content alone in surface soil cannot explain the oxidation rate differences between forests (DEF and ARF) and agriculture soil (AG) in this case. As shown by Gullledge and Shimel [19], forms of ammonium strongly affect methane oxidation. Chemical fertilization in cornfield thus might have added ammonium in the form that strongly affects methane oxidation.

It was observed that the distribution pattern of nitrogen content along the soil profile differed among three sites. In DEF and ARF soils, high content of inorganic N were observed only in the top 10-15 cm and significantly lower concentrations was found below this depth. If the inhibition of such nitrogen compound is considered, oxidation of methane may occur at depth below 15 cm in DEF and ARF site. On the other hand, soil at AG site showed a fairly uniform distribution of inorganic N along soil depth and a slight increasing trend along the soil profile was observed. Such distribution pattern in AG soil may be resulted from mixing between top and subsoil by cultivation practices during corn plantation. Leaching of nitrogen from the topsoil to subsoil may explain the increasing trends along the soil profile found at AG site. Such distribution of N and at level of concentrations found may lead to a relatively uniform inhibition of methane oxidation throughout the AG soil profile (Fig. 2). Such inhibitory effects of N are not prevail in DEF and ARF soils due to their relatively low concentration in subsoil. As a result, net methane oxidation was observed in DEF and ARF site throughout almost most of the time.

From these results, it is thus concluded that the active layers for methane oxidation lie below the soil surface in forest soils. It is typical that oxidation in the organic layers of forest soils is usually lower than the mineral soil layers [9, 20-21]. This is due to the fact that mineralization in organic layers releases inorganic nitrogen species that may exert the inhibitory effects on methane oxidation. The depth profile of nitrogen content especially ammonium and nitrate confirms that relatively low methane oxidation in surface layers of forest soil may be indeed due to high ammonium and nitrate contents (Fig. 1). In addition, the pH at the surface layers is very low in the present study. Thus, the soil at the surface layers may be too acidic for methane oxidation.

Hütsch [14] (2001) suggested that repeated application of ammonium could result in change in the kinetics of methanotrophic bacteria with consequences for the threshold value (concentration below which no oxidation takes place). Although incubation was not long enough to allow the estimate of threshold value, kinetic coefficients clearly indicated that methane oxidation rate was not increased at concentration above 200 ppmv in DEF soil, and seemed that no oxidation occurred below 50 ppmv in ARF and AG soils. These results agree well with Nesbit and Breitenbeck [22] who found that cultivated soil consumed methane only when it was exposed to relatively high concentration of methane (>1000 ppmv) and no oxidation was observed at ambient concentration. In Thai forest soils, the V_{max} and the K_m are within the range found in temperate forests. For example, the K_m for forest soils reported by Bender and Conrad [23] was 22 ppmv and the V_{max} associated with this methane oxidation was 3.6 nmol g⁻¹ hr⁻¹. Environmental factors and land use, thus, lead to change in kinetics of methane oxidation. Such change in kinetics may be associated with changes in microbial community. However, it is noted that only apparent kinetic coefficients are measured in a study with a mix microbial community such as in the current soil incubation study. Thus, it cannot be assumed that these represent true enzyme properties.

Due to the porous nature of soil, methane oxidation in subsurface layer is limited by gas diffusion (oxygen and methane) from the atmosphere [15]. Thus, methane oxidation of such soil is controlled mainly by substrate (oxygen) availability. Methanotrophic community in DEF subsoil may have experienced and well adapted to such limitation. Therefore, DEF soil develops high affinity to methane but oxidation rate could not increase beyond certain levels. On the other hand, land use usually leads to changes in various chemical, physical and biological properties of soil, such changes may in turn affect methane oxidation activity. Change in gas diffusibility and water regime may be also altered by land conversion from forest to agriculture [15, 24]. On the other hand, low affinity for methane in AG soil may be due to the existence of different methanotrophic community which is classified as the first group as mentioned above and as reported in previous study [8]. Methanotrophic community in AG soil may have experienced high concentration of methane during certain period of crop cultivations. Since corn plantation has been continuous for over 16 years, such cultivation practices may help establish the different methanotrophic community that well adapt to high concentration of methane. It is interesting to note that kinetic coefficients for ARF soil come in the middle between DEF and AG soil, as for the history of land use. This may indicate that soil at ARF site is in the transient period from previously disrupted by human activity (agriculture) towards the natural conditions. Methanotrophic community in ARF soil, thus, may be the mixture of those found in DEF and ARF sites, possibly means it could oxidize both methane at low (as observed in DEF site) and high methane concentration (as observed in AG site).

In conclusion, kinetics of methane oxidation strongly depends on land use type. In tropical upland soil, methanotrophic community with high affinity for methane establishes in undisturbed-natural forest soils. Conversion of such forest soils into agriculture leads to the loss of soil capacity to oxidize atmospheric levels of methane. However, it seems that when agricultural land is converted back to forest, methane oxidation capacity and potential of soil can be somehow recovered. Present study shows that after planting for 16 years to *A. mangium*, oxidation rate and kinetics of soils are still not yet fully returned to the previous levels in natural forest. However, such forest plantation improves methane oxidation capacity substantially compared with agricultural lands.

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