รายงานวิจัยฉบับสมบูรณ์

โครงการ "การประเมินศักยภาพของพื้นที่ป่าไม้ในประเทศไทย ในการออกซิไดซ์ก๊าซมีเทน"

(Estimate of Methanotrophic Capacity of Tropical Forests in Thailand)

โดย

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สัญญาเลขที่ MRG4580019

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บัณฑิตวิทยาลัยร่วมด้านพลังงานและสิ่งแวดล้อม
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ก๊าซมีเทนเป็นการเรือนกระจกที่สำคัญและการออกซิไดซ์มีเทนโดยแบคทีเรียในดินถือเป็นแหล่งดูดกลับก๊าซมีเทนจากอากาศที่สำคัญแหล่งหนึ่ง ประเทศไทย ได้มีการประมาณการปลดปล่อยก๊าซมีเทนจากแหล่งต่างๆไว้ค่อนข้างมาก แต่ที่ผ่านมายังไม่มีการศึกษาเกี่ยวกับแหล่งดูดกลับและปริมาณการดูดกลับโดยแหล่งต่างๆเหล่านั้น การศึกษาครั้งนี้จึงได้วัดอัตราการออกซิไดซ์มีเทนโดยเปรียบเทียบในดินที่มีการใช้ประโยชน์แตกต่างกันในจังหวัดนครราชสีมา ได้แก่ ดินป่าธรรมชาติดิบแล้ง ดินป่าปลูกกระถินเทพา และดินที่ใช้ทำการเกษตร (ปลูกข้าวโพด) อัตราการออกซิไดซ์วัดโดยวิธี Close chamber method โดยทำการวัดทุกเดือนระหว่างมกราคม ถึง ธันวาคม 2546 นอกจากนี้ได้เก็บตัวอย่างดินเพื่อศึกษาลักษณะทางจุลศาสตร์ของการออกซิไดซ์ก๊าซมีเทนและลักษณะโครงสร้างประชากรของ methanotrophic bacteria โดยวิธี denaturing gradient gel electrophoresis (DGGE) จากชิ้นส่วนของยีนที่ควบคุมการทำงานของเอนไซม์มีเทนโมโนออกซิจิเนส ผลการศึกษาพบว่า ดินทั้งสามแห่งสามารถออกซิไดซ์ก๊าซมีเทนระดับความเข้มข้นตามธรรมชาติได้ แต่อัตราการออกซิไดซ์แตกต่างกันไป ขึ้นอยู่กับปัจจัยต่างๆ เช่น ฤดูกาล ลักษณะการใช้ที่ดิน และจุดที่ทำการเก็บตัวอย่าง ในฤดูแล้งอัตราการออกซิไดซ์ก๊าซมีเทนจะสูงกว่าในฤดูฝน นอกจากนี้ในฤดูฝนบางครั้งยังมีการปลดปล่อยก๊าซมีเทนออกมา แสดงว่าความชื้นของดินเป็นปัจจัยหลักอันหนึ่งในการควบคุมอัตราการออกซิไดซ์ก๊าซมีเทน จากการเปรียบเทียบ พบว่าการออกซิไดซ์ก๊าซมีเทนในป่าธรรมชาติและป่าปลูกในประเทศไทยอยู่ในระดับที่พบในดินแถบอบอุ่น จากค่าเฉลี่ยต่อปีพบว่า ดินป่าเป็นแหล่งดูดกลับก๊าซมีเทนสุทธิ (1.50 และ 1.17 มิลลิกรัมต่อตารางเมตรต่อวัน ในป่าธรรมชาติและป่าปลูก ตามลำดับ) สำหรับในดินที่ทำการเกษตร การปลดปล่อยก๊าซมีเทนโดยเฉพาะในช่วงฤดูฝนทำให้เฉลี่ยทั้งปีดินนี้เป็นแหล่งปลดปล่อยสุทธิ

ในดินป่าทั้งสองแห่ง พบว่าชั้นดินระหว่าง 15-40 ซม เป็นชั้นที่มีอัตราการออกซิไดซ์มีเทนสูงสุด ในขณะที่ในดินที่ทำการเกษตร ความแตกต่างในการออกซิไดซ์มีเทนตามความลึกไม่ชัดเจนนัก ความเข้มข้นที่สูงของไนเตรท ไนไตรท์และแอมโมเนียมในดินชั้นบนอาจมีผลยับยั้งการเจริญของแบคทีเรียที่ออกซิไดซ์มีเทน ทำให้ไม่พบการออกซิไดซ์ในดินชั้นบนนี้ จากการศึกษาทางจลศาสตร์ของการออกซิไดซ์มีเทน พบว่าดินป่าธรรมชาติมีความจำเพาะต่อมีเทนสูง (ค่า K_m = 52 ppmv) แต่ศักยภาพในการออกซิไดซ์ต่ำ (ค่า V_{max}= 0.82 นาโนโมลต่อกรัมดินต่อชั่วโมง) ในขณะที่ดินจากป่าปลูกมีความจำเพาะต่อมีเทนสูงรองลงมา (K_m= 723 ppmv) และดินจากพื้นที่เกษตรมีความจำเพาะต่อมีเทนต่ำสุด (K_m = 1454-2362 ppmv) ฉะนั้น ดินป่าสามารถออกซิไดซ์มีเทนที่ความเข้มข้นต่ำได้ดีกว่าดินที่ใช้ทำการเกษตร ลักษณะทางจลศาสตร์นี้สัมพันธ์กับลักษณะโครงสร้างประชากรของแบคทีเรียในดิน กล่าวคือผลของ DGGE ชี้ว่าโครงสร้างประชากรของ methanotroph ในดินป่าทั้งสองแห่งมีความคล้ายคลึงกัน และแตกต่างดินที่ทำการเกษตร ทั้ง *Methylobacter* spp. และ *Methylocystis* spp เป็นกลุ่มแบคทีเรียหลัก นอกจากนี้ ยังพบ methanotroph ที่จัดอยู่ใน cluster α (USC α) ซึ่งเชื่อว่าเป็นกลุ่มหลักที่สามารถออกซิไดซ์มีเทนที่ระดับความเข้มข้นตามธรรมชาติ ในดินป่าทั้งสองแห่ง และพบลำดับเบสของกลุ่มแบคทีเรียที่ยังไม่เคยพบมาก่อนที่อาจมีความสัมพันธ์กับแบคทีเรียในกลุ่ม Gammaproteobacteria หรือกลุ่มอื่นๆที่มียีนควบคุมการทำงานของเอ็นไซม์แอมโมเนียมโมโนออกซิจิเนส ของแบคที่เรียที่ออกซิไดซ์แอมโมเนีย จากผลที่กล่าวมาแสดงให้เห็นว่า นอกจากปัจจัยแวดล้อมเช่นความชื้นแล้ว ความแตกต่างด้านโครงสร้างประชากรของ methanotroph ที่พบในดินที่มีการใช้ประโยชน์ต่างกัน ก็เป็นเหตุผลที่สำคัญประการหนึ่ง อันนำไปสู่ความแตกต่างในศักยภาพและจลศาสตร์การออกซิไดซ์มีเทนดังที่กล่าวมาแล้ว

ผลการศึกษาครั้งนี้ แสดงให้เห็นว่าลักษณะการใช้ประโยชน์ที่ดินมีผลอย่างมากต่ออัตราการออกซิไดซ์มีเทน การเปลี่ยนจากป่าไปเป็นพื้นที่เกษตรส่งผลให้ดินสูญเสียคุณสมบัติการออกซิไดซ์ก๊าซมีเทน อย่างไรก็ตาม การปลูกป่าก็ช่วยทำให้ดินกลับมาออกซิไดซ์มีเทนได้อีกครั้ง การสูญเสียความสามารถในการออกซิไดซ์ก๊าซมีเทนนี้มีผลมาจากการเปลี่ยนแปลงโครงสร้างประชากรของ methanotroph และคุณสมบัติทางจลศาสตร์ของการออกซิไดซ์มีเทนของแบคทีเรียเหล่านั้น จากการประมาณการในเบื้องต้นพบว่า ดินป่าไม้ในประเทศไทยมีศักยภาพสูงในการออกซิไดซ์มีเทนที่ความเข้มข้นระดับธรรมชาติ การช่วยรักษาพื้นที่ป่าจึงมีความสำคัญในการรักษาลักษณะที่เป็นแหล่งดูดกลับมีเทนสุทธิของป่านเอาไว้

คำหลัก: มีเทนออกซิเดชัน ดินป่าไม้ในประเทศไทย การเปลี่ยนแปลงการใช้ประโยชน์ที่ดิน แบคทีเรียกลุ่ม methanotroph, DGGE

Abstract

Project Code: MRG4580019
Project Title: Estimate of Methanotrophic Capacity of Tropical Forests of Thailand
Investigator: Amnat Chidthaisong
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Project Period: July 2002-June 2004

Methane is an important greenhouse gas and oxidation in soils represents a significant sink. In Thailand, estimate of methane emission is well established but there has been no estimate available for methane sink. In this study, methane oxidation fluxes were measured with the closed chamber method in different land use types; natural forest (dry evergreenforest; SK site), re-grown forest (*Acacia mangium*, AC site) and corn cultivation (CF site). Monthly *in situ* fluxes were monitored during January – December 2003 in Nakornratchasrima Province, Northeast Thailand. Soil samples were taken for kinetic study in the laboratory and for analysis of methanotrophic bacteria (MB) community by denaturing gradient gel electrophoresis (DGGE) of *pmoA* gene fragments (encoding for a subunit of particulate methane monooxygenase) that were PCR-amplified from total soil DNA extracts. Results reveal that methane oxidation occurred in all land use types but oxidation rate varied according to season, land use types, and sampling spots. Both SK and AC forests showed the oxidation rates comparable to that found in temperate forests. High rate of methane oxidation was found during the summer months. In raining season, net methane emission was occasionally observed, indicating the importance of soil moisture as the controlling factor. On one-year average basis, soil at both SK and AC forests were the net methane sinks (1.50 and 1.17 mg CH_4 m^{-2} day^{-1}, respectively). On the other hand, high methane emission during raining season made soil at CF site became a net methane source on annual average basis.

In SK and AC soil a clear zonation for active oxidation layer was between 15-40 cm while in CF soil no clear active layer was observed. This was possibly due to high concentration of inorganic nitrogen compounds (NO_3^-, NO_2^- and NH_4^+) in the topsoil that inhibited the activity of methane oxidizing bacteria. Examining kinetic coefficients of these active layers revealed that soil at SK site had low methanotrophic capacity (V_{max} of 0.82 nmol·g $soil^{-1} \cdot h^{-1}$) but relatively high affinity for methane (K_m of 52 ppmv). Soil at AC and CF sites 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 while soil at SK site it was not. Difference in kinetic characteristics among these three sites is also reflected in structure of methanotrophic community. Cluster analysis based on the DGGE banding patterns indicated that the MB community of SK and AC sites were similar to each other but different from that of the CF site. Sequence analysis of excised DGGE bands indicated that *Methylobacter* spp. and *Methylocystis* spp represented genera of cultivated MB at the sampling sites. Sequences of upland soil cluster α (USC α), that has been suggested to represent organisms involved in atmospheric methane consumption in diverse soils, were detected in the native forest and reforested site. Such sequences formed a separate branch related to USC α. Other sequences that indicated the uncultivated groups of potential MB were related to methanotrophic Gammaproteobacteria or an unknown sequence cluster that may represent either *pmoA* or *amoA* (coding for a subunit of the ammonia monooxygenase of ammonia oxidizing bacteria). It is thus concluded that besides the environmental factors such as water content, different community composition of methanotrophic bacteria under different land use is one of the important factors leading to different in methanotrophic capacity and kinetic characteristics of methane oxidation.

In conclusion, the results obtained in this study indicate that land use change from forest to agriculture significantly reduces methanotrophic capacity of soils. However, conversion of agriculture back to forest and preserving the natural forest conditions can maintain high methane oxidation capacity. Loss of methanogenic capacity upon changing land use is associated with altering bacteria community and their characteristics of kinetic methane uptake. Preliminary estimate indicates that Thai forest soils have high capacity for atmospheric methane sink and thus maintaining forest area is important if their sink characteristics are to be preserved.

Keywords: Methane oxidation, Thai forest soil, Land use, Methanotroph, DGGE

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CHAPTER 1

INTRODUCTION

1.1 Background

Although greenhouse gases (CO_2, CH_4, N_2O, O_3 and CFCs) make up less than 1% of the atmosphere (99.9% of the atmosphere consists of N_2, O_2 and Ar), they play the important roles in global energy balance. The presence of these trace gases keeps the surface global temperature warm (through greenhouse effect phenomenon), thus allows lives to strive. Living organisms especially human being, in turn, can interact and exert a strong influence on trace gas concentrations and their spatial and temporal fluctuations. In fact, increasing concentration of trace gases in the atmosphere over the past centuries has been closely related with increasing human population, energy consumption and other human activities. This continuously increasing concentration causes an additional greenhouse effect and eventually the undesirable global warming.

The two most important greenhouse gases are CO_2 and CH_4. These two gases combined contribute approximately 70% of current global warming (Hütsch 2001). Annual increasing rate of CH_4 is about 1%, with current atmospheric concentration of 1.75 ppmv (Dlugokencky et al., 2003). The main sources of CH_4 include wetlands, rice paddies, livestock, landfills and biomass burning. All sources combined emit CH_4 about 515 Tg (10^{12} g) per year. On the other hand, the main CH_4 sinks are removal in the reaction initiated by hydroxyl radical in the troposphere (about 470 Tg per year), and removal by soils (30 Tg per year). Balancing the sources and sinks results in the atmospheric increase of about 32 Tg per year. Methane oxidation in aerobic soils is the only known net biological CH_4 sink, where CH_4 is oxidized to CO_2 and H_2O, or assimilated into microbial biomass. Thus, oxidation is environmentally beneficial, since CO_2 has much lower radiative absorbing potential when compared with CH_4 (32 times lower than CH_4). Although the soil sink strength for CH_4 is relatively small (30 Tg per year or about 6% of global methane sink), its absence would cause the atmospheric methane concentration to increase at approximately 1.5 times the current rate (Duxbury, 1994).

Thailand signed the United Nation Framework Convention on Climate Change (UNFCCC) at the United Nations Conference on the Environment and Development in Rio

de Janeiro on 12 June, 1992 and ratified the Convention in December 1994. The Convention became effective for Thailand in March 1995. As for the Non-Annex country, Thailand has committed to meet certain voluntary obligations. These include the issue such as; (1) submitting the national greenhouse gases inventory carried out with the support of UNFCCC to the Intergovernmental Panel on Climate Change (IPCC) and (2) cooperating with international community to reduce greenhouse gas emission based on its own capacity.

Current estimate of CH_4 emission from Thailand is 3.17 Tg (66.6 Tg CO_2-equilvalent or approximately 23.3% of total greenhouse gas emission in Thailand in 1994 (Ministry of Science, Technology and Environment, 2000). The main source (94%) is from agricultural sector such as rice paddies and livestock production. As mentioned above, methane is also taken by soil. Therefore, the net estimate of methane emission should take into account the sink strength. However, there is still no data on methane sink in Thailand. It is, therefore, important to study methane oxidation capacity in various potential ecosystems in Thailand. Knowing this will be possible when the data such as soil and land use classification are integrated with the results of methane oxidation measurements. Comparing the results from different ecosystems may be useful in term that we can know how and identify where to maintain and preserve methane oxidation capacity as the net CH_4 sink.

1.2. Literature Reviews

1.2.1 Basic processes involving biological CH_4 oxidation

In aerobic habitats, CH_4 is oxidized to CO_2 by the so-called methanotrophic bacteria (methanotrophs or CH_4 oxidizing bacteria, MOB, Conrad 1996; Hansan and Hanson, 1996). They are thus unique in their ability to utilize CH_4 as a sole carbon and energy source. These bacteria are ubiquitous in nature. The key enzyme involved in oxidizing CH_4; the methane monooxygenase, exhibits the remarkably low substrate specificity (especially in its soluble form, sMMO). Thus, MOB can use a wide range of substrates including those are xenophobic. The ability of MOB to oxidize ammonia is of ecologically significant because it serves as the linkage between carbon and nitrogen cycles in nature. The general pathways of methane and ammonia oxidation are given in Fig. 1. All methanotrophs are able to oxidize ammonia to nitrite, but the specific rate of

oxidation is much lower than that of ammonia oxidizers. Thus, it is not surprising that in the presence of ammonia, rate of methane oxidation by MOB is reduced.

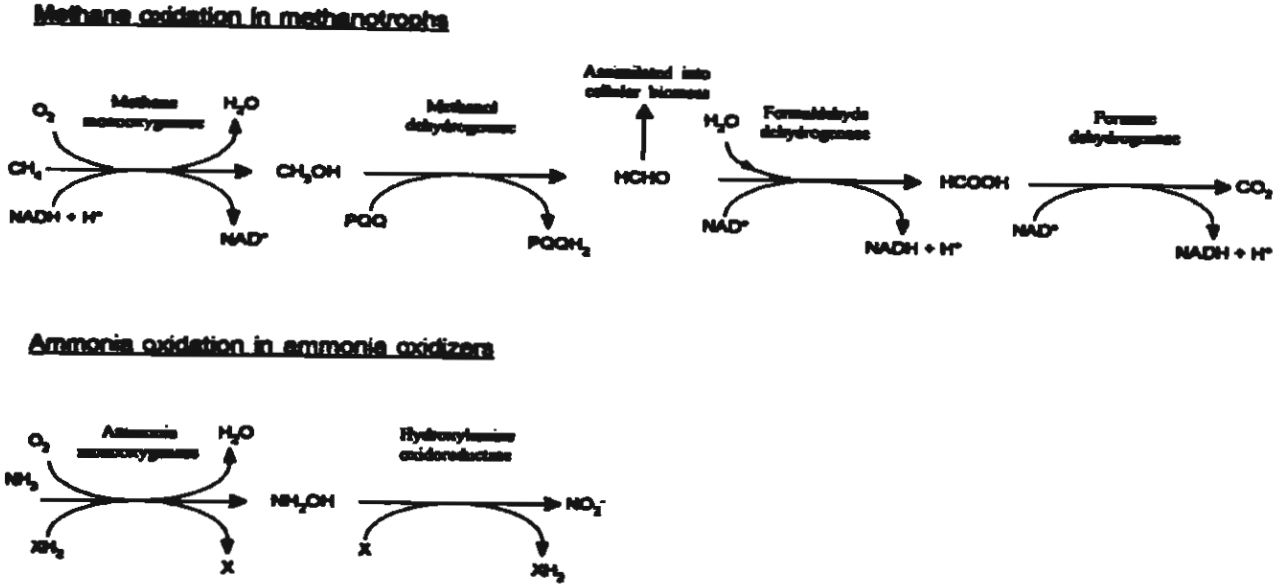


Fig. 1. Pathway of methane oxidation in methanotroph and ammonia oxidation in ammonia oxidizer, PQQ is pyrroquinoline quinone, X and XH_2 are the oxidized and reduced forms of an unknown electron donors (after Hütsch, 2001).

Methanotrophs are generally classified into two types according to their carbon assimilation pathways. Type I methanotrophs assimilate cell carbon entirely from CH_4 through the RuMP pathway while type II methanotrophs use the serine pathway to assimilate cell carbon. For type II methanotrophs, 50-70% of the cell carbon is derived from CH_4, and the rest (30%) is from CO_2 (Hanson and Hanson 1996). The presence of methane monoxygenase (MMO) renders the molecular detection of methanotrophic community in natural samples possible. This can be achieved by targeting the gene encoding the MMO (*pmoA* gene, Henckel *et al.*, 2000). Analysis of *pmoA* gene libraries from forest soils has demonstrated the existence of a new group of methanotrophs (Henckel *et al.*, 2000, Homes *et al.*, 1995). Knowing the diversity of methanotrophs in various ecosystems is necessary if we want to understand the process of methanotrophy.

1.2.2 Factors affecting methane oxidation

Temperatures Most of culturable methanotrophs are mesophiles. However, oxidation of methane have been reported in wide range of temperature; ranging from about 0 °C in Arctic tundra (Whalen and Reeburgh, 1990; Reeburgh *et al.*, 1997, Dunfield *et al.*, 1993) to 35°C (King, 1992). In laboratory, some strains of methanotrophs are capable of growth up to 50°C. These data indicate that populations of methanotrophs can adapt to and maintain activity in the wide range of temperatures. The optimum temperature of methane oxidation in general is 25 °C.

Soil pH Oxidation of methane has been detected in a board range of pH, from acidic to neutral pH in most case. Born *et al.* (1990) reported high oxidation rate at pH values of 3.5 and 7.7 in sandy forest soils. Bender and Conrad (1995) observed CH_4 oxidation activity at pH as low as 2.3 in forest soil. In reviewing methane fluxes in central Europe (50 sites), Dörr *et al.* (1993) found no significant correlation between CH_4 uptake and pH. However, Brumme and Borken (1999) investigated seven forest ecosystems and concluded that there was a strong positive correlation between annual CH_4 oxidation and soil pH ($r^2 = 0.77$).

Nitrogen fertilization Ammonium fertilization inhibits methane oxidation both in field and laboratory study of pure culture of methanotrophs (Hanson and Hanson 1996). Thus, chemical fertilization is one of the key factors limiting CH_4 oxidation by soils. Mosier *et al.* (1991) were the first who reported the inhibition of CH_4 uptake by N fertilization. An annual fertilization of 22 kg N ha^{-1} as NH_4NO_3 reduced CH_4 oxidation by 41% when compared to the unfertilized control. Hütsch (2001) pointed out that N fertilization has both long term and immediate effects on CH_4 uptake. Immediate effects involve the interference of NH_4^+ with the methanotrophic enzyme system (MMO). Due to a low substrate specificity of MMO, methanotrophs metabolize NH_3 as soon as it is applied to the soil. Thus, NH_4^+ acts as a competitive inhibitor of the methanotrophic bacteria and the effect was not fully reversible. However, it is noted that inhibition of CH_4 oxidation by NH_4^+ is quite complicated. For example, in rice fields, landfills and perhaps in some soils, NH_4^+ becomes limited for growth of MOB. In this case, CH_4 oxidation is stimulated by small amounts of NH_4^+. CH_4 oxidation is not inhibited before a certain NH_4^+ concentration

is reached; namely when NH_4^+/CH_4 ratio gets higher than a particular value (Conrad, personal communication).

The long-term effects result from repeated N applications in the long run cause changes in microbial ecology and probably a shift in microbial population (Adamsen and King, 1993). Hütsch *et al.* (1996) investigated the long-term effects of N fertilization in the "Broadbalk Wheat Experiment" at Rothamsted, UK. They found that the highest CH_4 oxidation rate was observed in the no nitrogen treatment, and the rates declined progressively for increases in mineral N application up to 144 kg N ha^{-1} per year. They concluded that CH_4 oxidation was greatly decreased in soils with a history of inorganic N application. On the other hand, Hütsch *et al.* (1994) and Willison *et al.* (1996) demonstrated that inhibition of methane oxidation by N fertilization was in fact due to the presence of NH_4^+, but not NO_3^-. Fig. 2 shows an example of one of their results in the experiment receiving the same about of N and similar pH. The CH_4 oxidation rates were 0.008 and 1.46 mg CH_4 m^{-2} per day in the presence of NH_4^+ and NO_3^-, respectively. They explained how the complete loss of CH_4 oxidizing ability after long-term NH_4^+ application that this could be due to a change in the kinetics of methanotrophic bacteria in relation to the threshold value. The threshold value is the CH_4 concentration below which no oxidation occurs and is a critical determinant for the total CH_4 uptake. Application of NH_4^+ for decades could have shifted the threshold to the much higher values.

Application of organic manure Hütsch *et al.* (1994; 1996) demonstrated that long term application of farmyard manure resulted in inhibition of CH_4 oxidation, although the effect was less strong than application of mineral N application. This can be explained by the fact that much of N especially in form of NH_4^+ can be absorbed in organic complexes, therefore, makes it only partly accessible to methanotrophs and other microorganisms.

Land use Changes in land use, especially cultivation of the formerly undisturbed soils, strongly reduced the soil's sink strength for atmospheric CH_4 (Keller *et al.* 1990, Ojima *et al.* 1993, Reay *et al.* 2001). Measurements of CH_4 uptakes usually reveal several times higher CH_4 uptake rates in the undisturbed forests than in regularly cultivated arable lands. Dobbie *et al.* (1996) found that mean reduction resulting from conversion of land to agriculture in Europe was 60%. This probably results from the disturbance of the original soil structure by cultivation (Conrad 1996, Bender and Conrad 1994). According to Conrad (1996), trace gas metabolism occurs in certain unites in aerobic soils, either

individual soil curmbs, non-aggregated soils, or sand grains covered by microbial films. Agricultural practices such as plowing of soils may easily destroy some of these "activity centers".

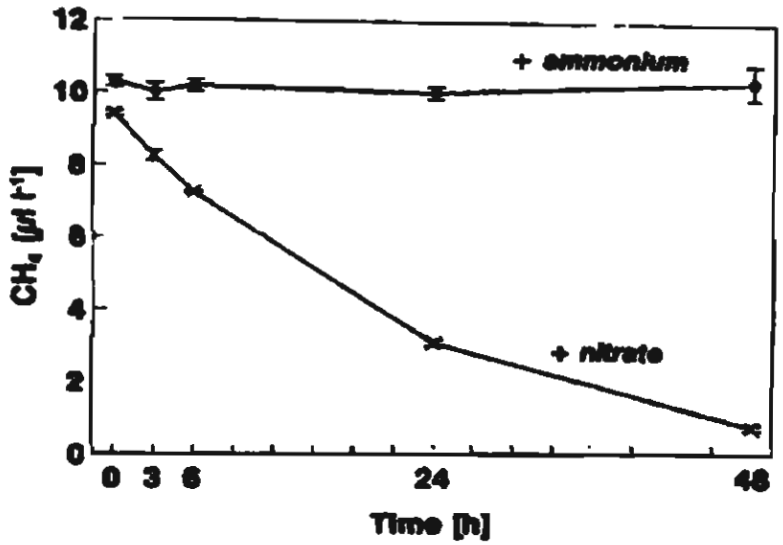


Fig. 2. Effects of ammonium and nitrate-based fertilization on oxidation of CH_4 by soils (after Hütsch, 2001).

1.3 Objectives

The objectives of current study are;

1. To quantitatively estimate methane oxidation capacity of forest and some selected arable lands in Thailand, thereby providing the preliminary information for adjusting emission inventory of the country.
2. To study the effect of agricultural practice that affects methane oxidation capacity.
3. To study the kinetics of methane oxidation under tropical Thailand ecosystem.
4. To investigate community structure and diversity of methanotrophic bacteria in Thai forest soil.
5. To know and compare geographical distribution of methane oxidation capacity and methanotrophic community structure.

CHAPTER 2
MATERIALS AND METHODS

2.1 Study Sites

The survey of study sites was carried out on November 17, 2002. Besides checking the site of evergreen forest at Sakaerat Experimental Station, some areas nearby were also surveyed to use as reference site (agricultural area, Fig. 3). Accordingly, three sites with different land-use types were selected. Details for each site are described below.

Sakaerat Experimental Research Station (SK site) This was the primary sampling site in this project (Fig. 3). It is located in Amphur Wang Nham Keaw, Nakorn Ratchasrima province and belonged to Ministry of Science and Technology. Majority of the area is made up of gentle slope mountains of between 10 and 30%. The average elevation is between 730 m and 760 m above the mean sea level. This experiment station was established in 1967, covering the area of 50 km^2. The sampling plots were selected in area of dry evergreen forest because it covers the majority (60%) of the forest area in this site. Under the monsoon climate, the annual temperature at the site is 26 °C with average annual rainfall of 1240 mm. Dry season in this area covers the period from December to February during which monthly rainfall is usually less than 50 mm (Sakurai *et al.*, 1998; Wacharakitti *et al.*, 1980) and wet season from mid of May until the end of September. Average relative humidity is around 75%. Soil at this site is classified as Phra Wihan formation of the Korat group. The soil is low in fertility by nature. Some physical and chemical characteristics of soils were also investigated in during the course of this project study.

The natural evergreen forest at SK site comprises of various native species (Sakurai *et al.*, 1995). Some of the dominant species include *Hopea ferrea, Pterocarpus marcrocarpus Kurz, Xylia xylocarpar, Dalbergia cochinchinensis, Lagerstroemia duppereana, Shorea henryana.*

Re-afforestation Research and Training Station (AC site) This site is maintained and managed by the Royal Forest Department, Ministry of Natural Resources and Environment. It is the forest restoration area after deforestation about 20 years ago, planted with the fast growing, and nitrogen fixing tree species such as: *Acacia mangium*,

Pterocarpus marcrocarpus, *Xylia xylocarpa*, *A. auriculaeformis*. In this study, the area planted to *A. mangium*, a nitrogen fixing and fast growing tree was selected. This *A. mangium* (age 16 years old) plantation is the main type of reforestation at the AC site.

Cornfield (CF site) The site is situated next to Re-afforestation Research and Training Station. This area was selected due to its proximity to other sites but with different land use type. Thus, all sites are located within the same climates and some other variables can be removed. Corn cultivation has been continuously practiced for more than 16 years after deforestation. In 2003 when this project study was initiated, soil preparation for corn plantation started in the mid of June and seeds of corn were sown on July 10, 2003. On this date, fertilizer (16-20-0) was also applied at the rate of 20 kg per Rai (1 Rai = 0.2 ha).

2.2 Construction of chambers and chamber base

Close-static chamber method was applied to quantify the rate of methane oxidation in this study. Chamber was made of a transparent-acrylic glass with the dimension of 30 cm x 30 cm x 15 cm (width x length x height). On top side of each chamber, two small holes were made; one for inserting thermometer in order to monitor the temperature during gas sampling and another for facilitating gas sampling by using a syringe and needle inserting through the silicon stopper (Fig. 4). Small capillary tube was inserted during air sampling to allow pressure equilibration. The chamber base was made of stainless steel and custom constructed with an upper trough that exactly fits the size of the chamber (Fig. 5). The bases were inserted into the sampling location at 10-15-cm deep into the soil and were remained in the same proximal and distal locations during the entire study period. Before using, all possible leakages were checked to ensure the complete air-seal during gas sampling. When gas samples were taken, the chamber was placed on the water-filled channel of this chamber base to prevent gas exchange between inside and outside of enclosure.

2.3 Gas sampling procedures

For the gas sampling in January and February 2003, ten gas samples at 0, 5, 10, 15, 20, 25, 30, 35, 45 and 60 min after the enclosure were taken. However, for the remaining

months, six gas samples at 0, 10, 20, 30, 45 and 60 min were collected, since oxidation was not rapid and six sampling points are considered enough to follow the decreasing concentration in the chamber headspace. For each sampling time, 25-30 mL of gas was taken from the headspace by using a 30-mL gas syringe and then transferred immediately to vials. The vials were prepared in the laboratory prior to the field trip. They were filled with the autoclaved-saturated NaCl solution. The gas sample taken from the chamber's headspace was injected into the vials to replace the NaCl solution. Together with gas collection, other environment parameters such as soil and air temperatures, soil moisture along soil depth to 1 m were also recorded. After being taken, the gas samples were kept in a cool box to maintain the stable temperature. Upon returning to the lab, the concentration of CH_4 was determined by GC as soon as possible following the procedures described below.

2.4 Calculation of methane flux

In situ biological methane oxidation rate was estimated from the first order rate constant multiplied by initial concentration of methane, according the equation 1 below;

$$F = k_1[CH_4] \quad (1)$$

where

F is methane oxidation rate (ppm CH_4 min^{-1})

k_1 is the methane oxidation rate constant (min^{-1})

$[CH_4]$ is the initial methane concentration in the chamber headspace (ppm)

The methane oxidation rate constant (k_1) was calculated from the plot between methane concentration and sampling time as shown equation 2.

$$ln[CH_4]_t = - k_1 t + \ln[CH_4]_0 \quad (2)$$

where

$[CH_4]_t$ is the headspace methane concentration at time t (ppm)

$[CH_4]_0$ is the initial methane concentration in headspace (ppm)

t is sampling time (min)

k_1 is oxidation rate constant (min^{-1}).

When there is a significant correlation between increasing methane concentration and sampling time in the chamber headspace, methane emission rate was also determined. The flux (F) is calculated from the change of the methane concentration inside the chamber over sampling time as:

$$F = \rho(V/A)dC/dt \quad (3)$$

where

ρ is the density of methane at pressure and temperature inside the chamber (mg m^{-3})

V is the headspace volume of the chamber

A is the area of surface that cover by the chamber

dC/dt is the increase of methane concentration inside the chamber as a function of time determined from linear regression (ppm min^{-1}).

2.5 Chemical analysis

Gas Chromatograph (GC) setup A Shimadzu Gas Chromatograph (Model 14B) equipped with FID detector and Methanizer was used to measure CH_4 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. After being separated by column, CO and CO_2 in the samples were converted to CH_4 by the Methanizer and thus enabling them detectable by FID. With the Methanizer and Unibead C column, this GC setup can detect three main gas components in the samples: CO, CH_4 and CO_2 (Fig. 6) with retention time at 3.6 min, 7.0 min and 13.9 min, respectively. When determining CH_4 concentration for each set of measurement, new calibration curve was made. The example of typical calibration curve is given in Fig. 7.

Soil texture Soil texture was determined by using hydrometer method (Bouyoucous Particle Size Analysis) on air-dried soils that were passed through a 2-mm sieve to remove small rock, roots, pebbles, and debris followed by wet sieving to separate sand fraction. Sand, silt, and clay were expressed as a percentage of oven dry weight. This method determines the physical proportions of three sizes of primary soil particles as determined by their settling rates in an aqueous solution using a hydrometer. The hydrometer method

of estimating particle size analysis (sand, silt and clay content) is based on the dispersion of soil aggregates using a sodium hexametaphosphate (calgon: $(NaPO_3)_6$) solution and subsequent measurement based on changes in suspension density (Gee et al., 1982).

Soil bulk density Determinations of bulk density were done by measurement of soil volume and weight. Soil bulk density is the ratio of the mass of dry solids to the bulk volume of the soil (Blake and Hartge, 1986). The bulk volume includes the volume of the solids and of the pore space. The termination of bulk density consists of drying (105°C) at less than 48 hours and weighing a soil sample, the volume of which is known (core method) or must be determined.

Soil moisture Soil moisture content was measured by using the automatic soil moisture sensor; Theta Probe type ML2x (Eijkelkamp, The Netherlands) for measuring soil moisture in the soil surface (at 6 cm from soil surface) and Profile Probe type PR1 for vertical soil moisture profile. A single probe can measure soil moisture at 6 different depths down to 1 meter. Moisture meter displays soil moisture in % water by volume, included is internal calibration for mineral and organic soils.

Soil pH Soil pH was determined in the laboratory with a glass-electrode and pH meter (HACH sensIon378, USA) using 1:1 ration of air-dried and 2-mm sieved soil : deionized water.

Determination of nitrate, nitrite and ammonium content Dissolved inorganic nitrogen compounds (NO_3^-, NO_2^- and NH_4^+) were determined by Ion Chromatograhp (Dionex DX600, USA) equipped with an electrochemical detector. Ten grams of 2-mm sieved soil from each layer were weighed and placed into an Erlemeyer flask and 70 mL of deionized water was added. The flask was shaken on a shaker for 45 min and the supernatant was filtered through a 0.45 μm cellulose acetate filter membrane. Concentrations of inorganic nitrogen in filtered liquid were then determined and expressed as mg of an inorganic nitrogen compound per kg of dry soil.

2.6 Kinetics of CH_4 oxidation

Soil samples were collected on July 27, 2003 in the proximity of the gas sampling sites for kinetic study. Firstly, oxidation activity at different soil depth was determined. The most active oxidation layer was then selected for further determination of kinetic coefficients. 30 g of 2-mm sieved soil were weighed and put into the 100-mL vials. Soil moisture was adjusted to 15-30% by volume. The vials were then closed with a rubber stopper and the headspace methane concentrations were adjusted to the range from ambient to about 1800 ppmv. The vials were incubated at room temperature (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 CH_4 uptake were estimated from the plot between the initial CH_4 concentrations versus oxidation rates (see Section 2.4). 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 Solfware, Inc., Northampton, Maine).

2.7 Analysis of methanotrophic structure

2.7.1 DNA extraction

DNA was extracted from 0.5 g of soil using the BIO 101 "FastDNA SPIN Kit for Soil" (Carlsbad, CA, USA) with modifications. Cell lysis was performed in a cell disruptor (model FP 120 FastPrep, Savant Instruments Inc., Farmingdale, NY, USA) for 2 × 30 s at 6.5 m s^{-1}. After centrifugation (10 min, 20,000 × *g*) the supernatant was collected and the soil bead mixture was re-suspended in kit-supplied buffers for a second extraction. Proteins and debris were precipitated as described by the manufacturer instructions and 1 ml of Binding Matrix was added. For purification, the matrix-bound DNA was washed 3 times with 1 ml of 5.5 M guanidine-thiocyanate solution. After the last centrifugation step (1 min, 14,000 × *g*) the supernatant was removed and the Binding Matrix was resuspended in 600µl guanidine- thiocyanate solution. The Binding Matrix was loaded onto the kit-supplied SPIN™ Filters and further steps were carried out as described by the manufacturer instructions. The final elution of the DNA was performed twice with 100 µl

of DNAse free water. DNA was further purified and concentrated with PVPP and the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany (Knief *et al.* 2003).

2.7.2 Amplification of functional marker genes

A partial fragment of *pmoA* was amplified with the primer sets A189f / A682b (these primer sets amplify both *pmoA* and *amoA* gene, McDonald and Murrell, 1997; Knief *et al.*, 2003) and A189f / mb661 (these amplify only *pmoA* gens, Costello and Lidstrom, 1999). The *pmoA* encodes part of the α subunit of pMMO which is present in all methanotrophs (except *Methylocella palustris* and *Methylocella silvestris*). Targeting *pmmoA* of pMMO rather than the gene encoding sMMO is advantageous, since only a few sequences are available and sMMO is presents only in some methanotrophs. For denaturing gradient gel electrophoresis (DGGE) a GC-clamp was attached to the 5' end of the A189f primer (Henckel *et al.*, 1999). A touchdown PCR program (Henckel *et al.*, 1999) with decreasing annealing temperatures from 64°C to 57°C was applied with 35 cycles. A fragment of the *mmoX* gene, encoding the active-site subunit of soluble methane monooxygenase (sMMO), was amplified using a newly designed forward primer (mmoX f945: 5'-TGGGGYGNAATCTGGAT-'3) with a GC-clamp published by Iwamoto *et al.* (2000) and the reverse primer of Auman *et al.* (2000). A touchdown PCR program with decreasing annealing temperatures from 63°C – 56°C within the first 15 cycles and 20 cycles with an annealing temperature of 56°C was applied. It consisted of an initial denaturation step of 5 min at 94°C, followed by 35 cycles of 94°C for 1 min, 63°C – 56°C for 1 min, and 72°C for 1 min, with a final extension step of 72°C for 7 min. The PCR reactions had a total volume of 50 µl and contained 5 µl of Accutaq-LA 10× buffer (Sigma-Aldrich, Steinheim, Germany), 0.5 µg μl^{-1} BSA (Roche Diagnostics, Mannheim, Germany), 3.5 mM $MgCl_2$, 200 µM of each dNTP (Promega, Mannheim, Germany), 1 µM of each primer, 0.05 U μl^{-1} REDAccuTaq LA DNA Polymerase (Sigma), and 1 µl of template DNA. PCR reactions were performed on a GeneAmp PCR System 9700 (Perkin Elmer Applied Biosystems, Weiterstadt, Germany) or a Primus 96 Cycler (MWG Biotech, Ebersberg, Germany).

2.7.3 DGGE and Sequencing of excised DGGE-bands

Separation of mixed PCR products was done by DGGE as described previously (Henckel *et al.*, 1999; Knief *et al.*, 2003). 40 µl of PCR products were loaded onto the gel. Visible bands were excised, DNA was reamplified by PCR, and PCR products were purified for sequencing (Knief *et al.*, 2003). Reamplification of some bands lead to non-specific PCR products, which appeared in the agarose gel as a second band or were visible after sequencing. The former problem was solved by cutting the *pmoA* PCR product out of the agarose gel (Knief *et al.* 2003). Otherwise, PCR products of were separated by cloning using the TOPO TA cloning kit (Invitrogen, Karlsruhe, Germany). The *pmoA* PCR product of positive clones was sequenced. For some bands, primers mb661 and A650 (Bourne *et al.* 2001) were used for reamplification. These primers are more specific for *pmoA* and thus prevent amplification of non-*pmoA* targets.

2.7.4 Phylogenetic analysis

Phylogenetic tree reconstructions, based on deduced amino acid sequences of partial *pmoA* and *amoA* sequences, were performed using the ARB software package (Strunk *et al.*, 1998). Original tree construction included sequences of all DGGE bands, all available *pmoA* sequences in the GenBank database (www.ncbi.nlm.nih.gov/GenBank, 15 December 2003), and selected public-domain *amoA* sequences. A selection was then made of 24 representative sequences from this study plus 42 public-domain sequences. The presented tree was calculated based on 130 deduced amino acid positions using a neighbor-joining algorithm with a Kimura correction. Bootstap values were generated by 1000 data reassemblings. The general tree topology was confirmed by a maximum-likelihood tree.

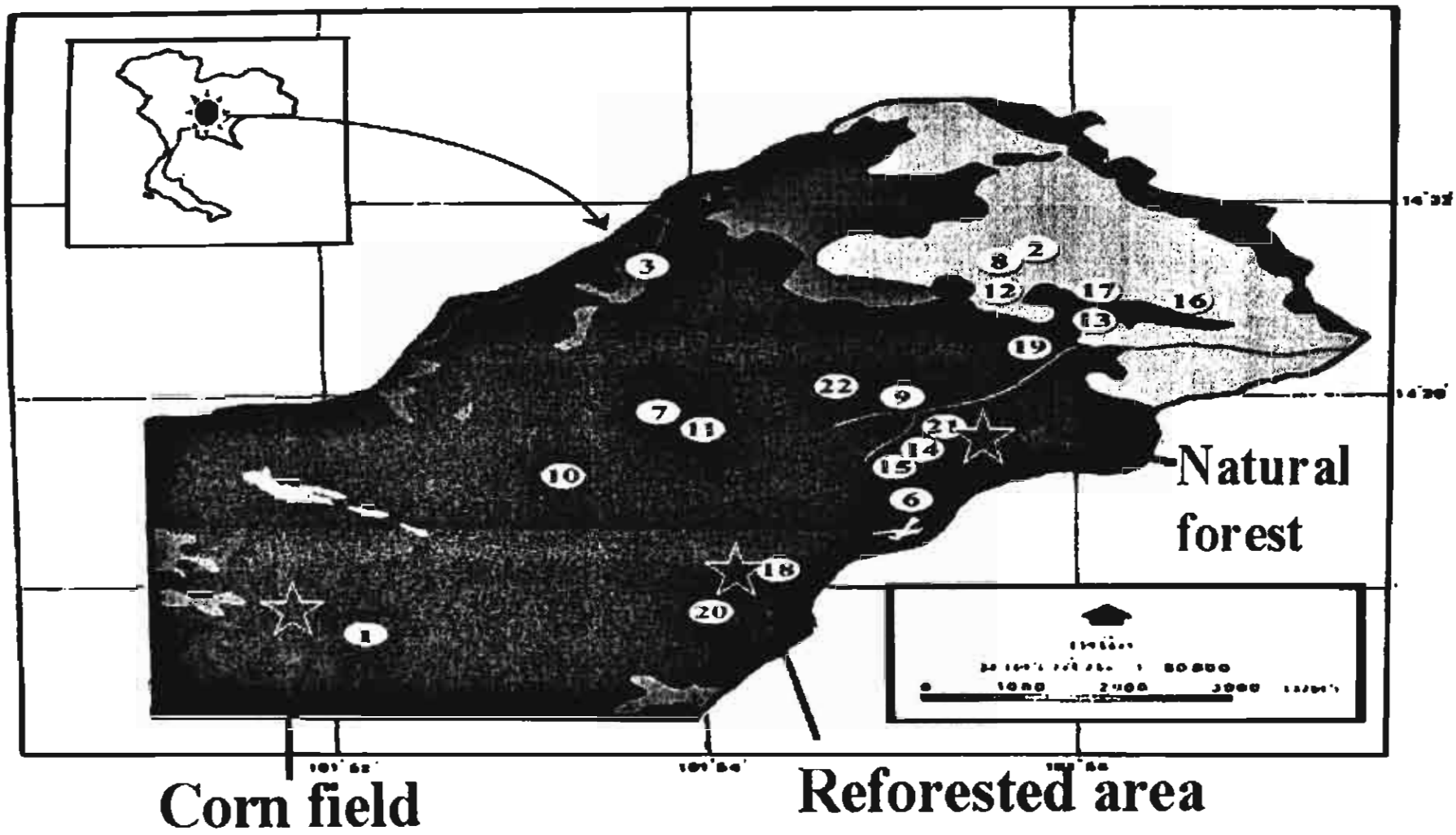


Fig. 3 Map showing the study areas; 1) Sakaerat Forest Research Station, 2) Reforested areas under the management by Royal Forest Department, Ministry of Natural Resources and Environment, and 3) agricultural area in which corn plantation has been continuous for more than 16 years.

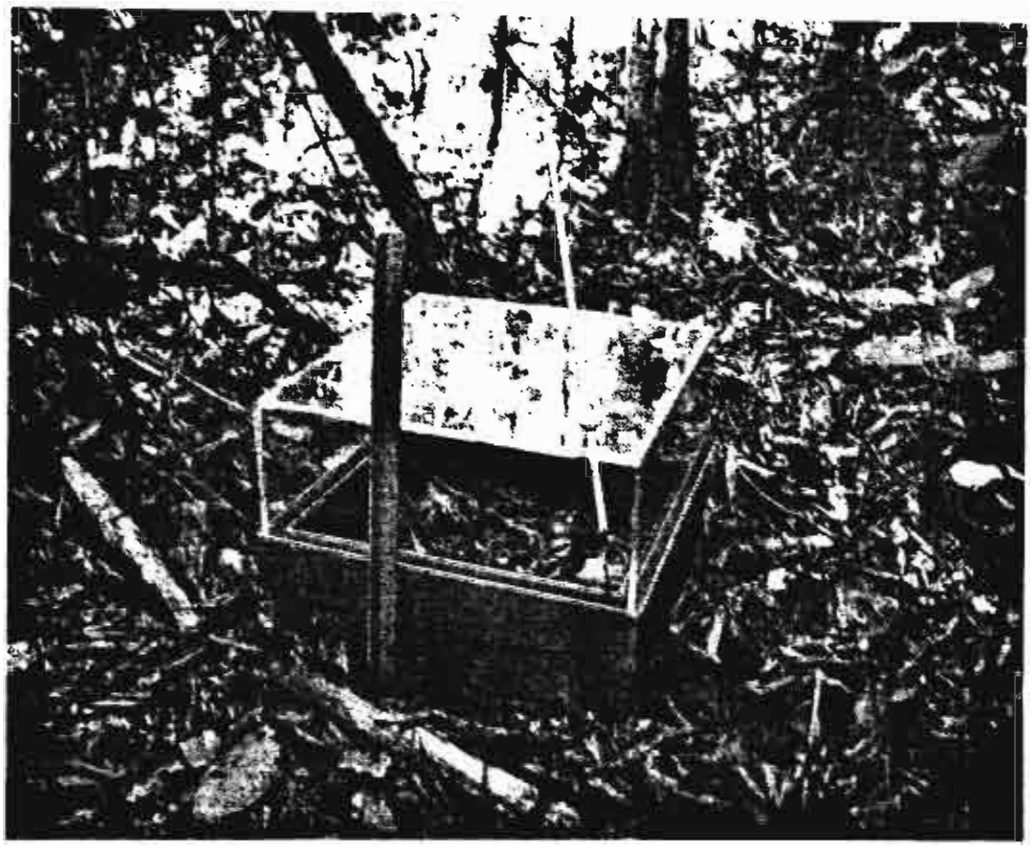


Fig. 4 Setups for sampling the chamber headspace gas.

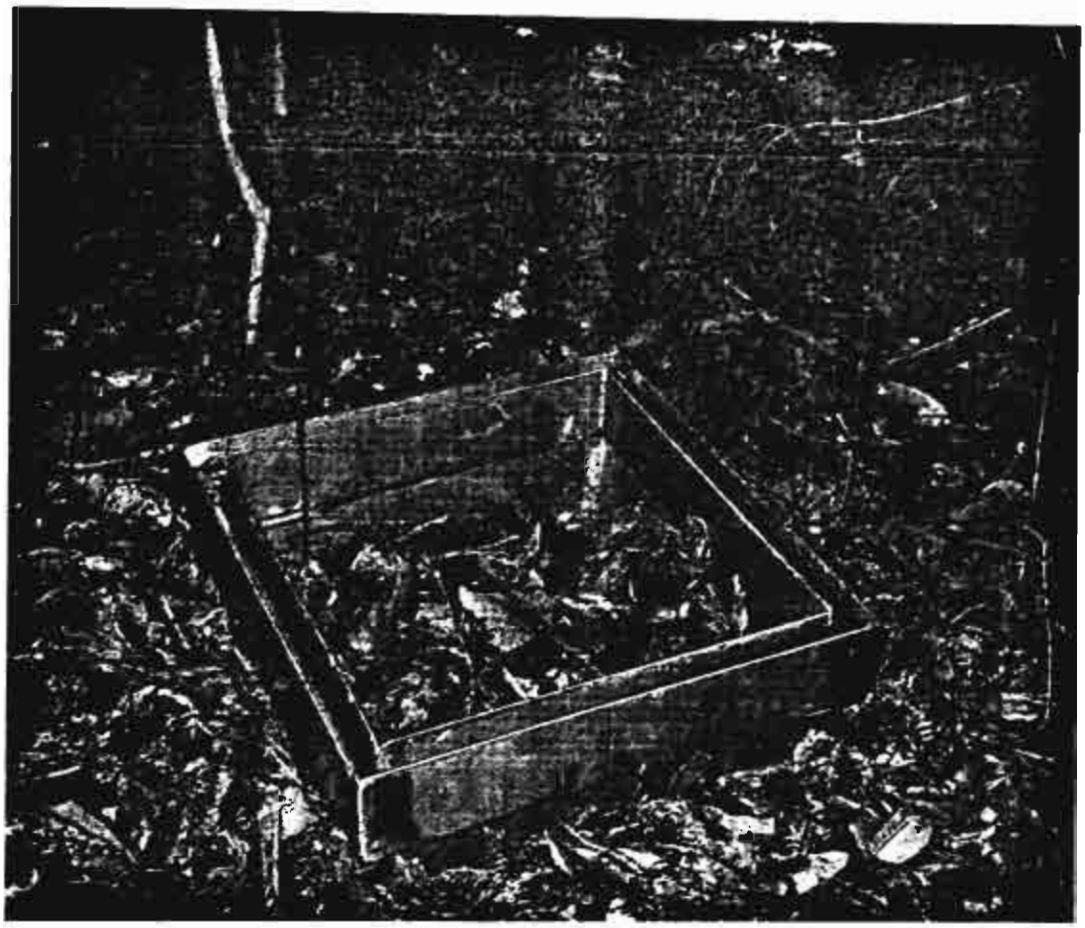


Fig. 5 Stainless steel chamber base used as the basement for chamber placement.

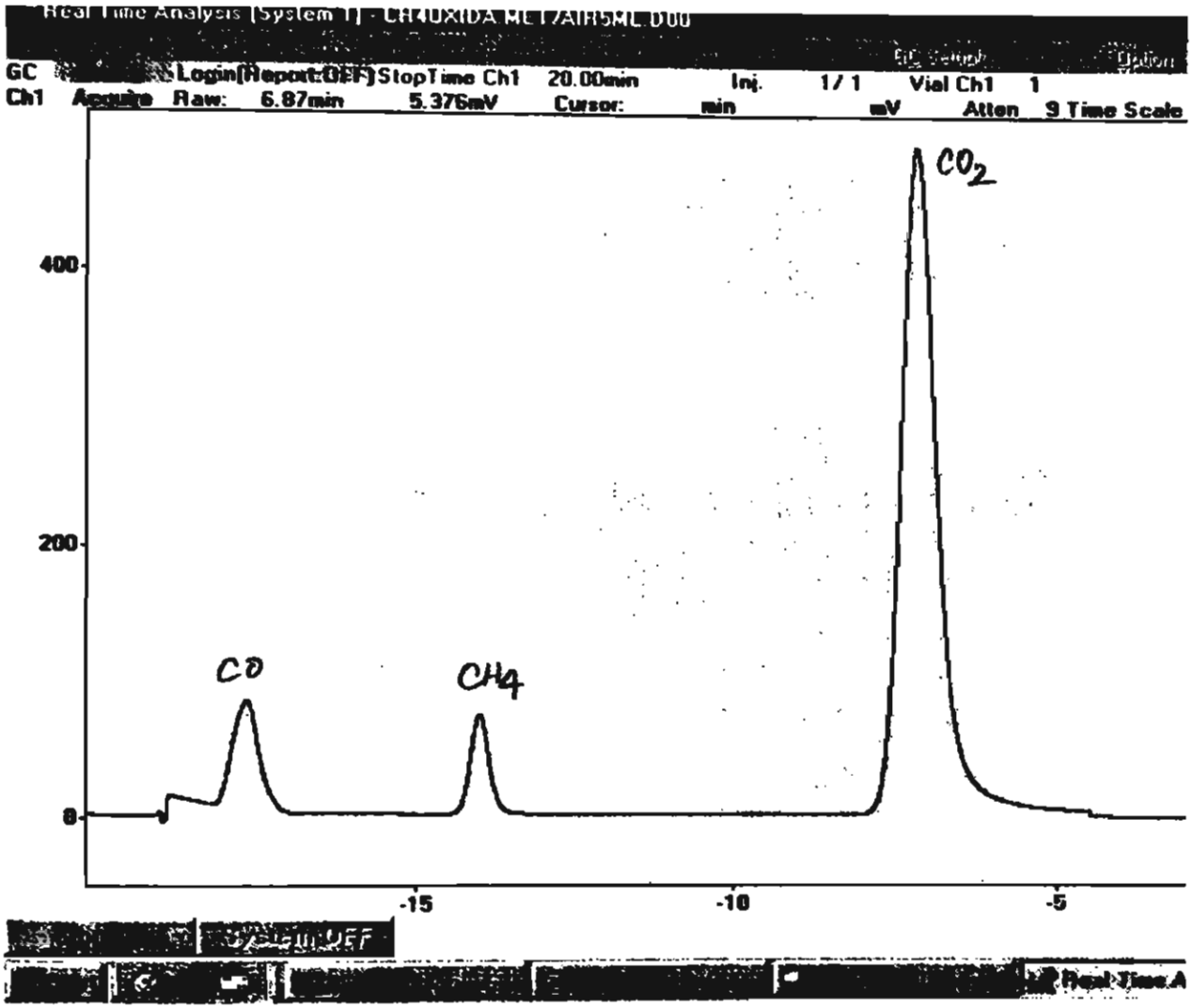


Fig. 6 Chromatogram and peak identification of gas sample components. The GC operating conditions are as indicated in the text. The sample type shown here is the ambient air (1.7 ppmv CH_4 concentration) with injection volume of 5 mL.

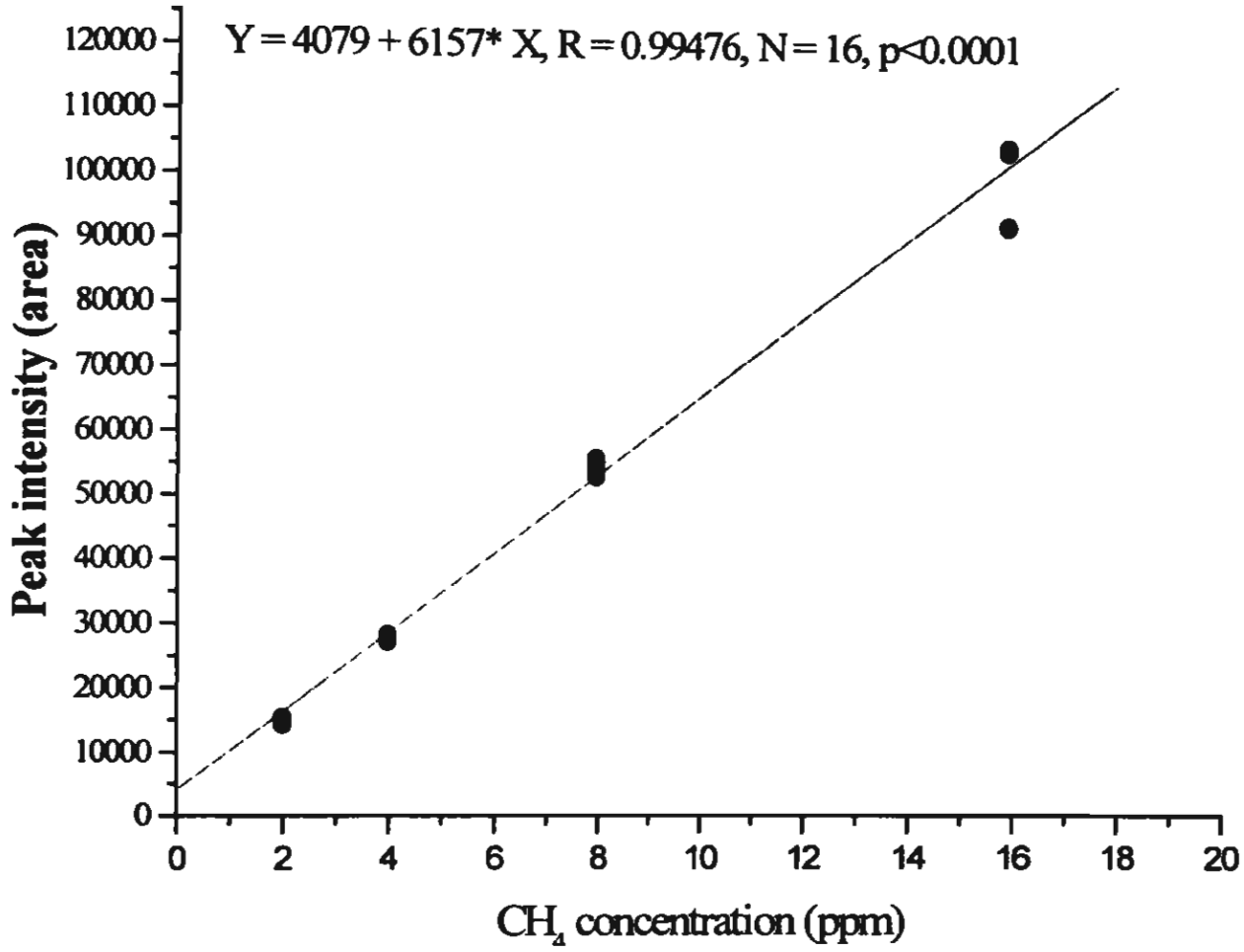


Fig. 7 Example of calibration curve for calculating CH_4 concentration in the samples.

Table 1 Geographical variations of CH_4 oxidation rates under different field condition (modified from Hütsch, 2001 and Ishizuka et al., 2000).

Location	Methane oxidation rate (mg CH_4 m^{-2} per day)			References
	Arable	Grassland	Forest	
North America				
USA	0.17	0.35		Mosier et al., 1991
	0.03-0.20	0.35-0.84		Mosier and Shimel, 1991
			3.20-4.16	Steudler et al., 1989
			3.84-5.44	Castro et at., 1994
	0.91-1.03	1.16		Kassavalou et al., 1998
			0.8-6.4	Castro et al., 1995
			2.1-6.9	Goldmann et al., 1995
Canada	0-0.13		0.04-1.10	Lessard et al., 1994
			2.7	Adamsen and King, 1993
Central America				
Costa Rica			0.3-2.3	Keller and Reiners, 1994
Europe				
Denmark	0.04-0.08	0.03-0.25	0.14-0.33	Ambus and Christensen, 1995
	0.2		0.7	Dobbie et al., 1995
Germany			0.12-0.96	Butterbach-Bahl et al., 1998
	0.04-0.05			Ruser et al., 1998
	0.11			Flessa et al., 1996
			0.03-0.68	Brumme and Borken, 1999
	0.01-0.55		0.25-3.66	Born et al., 1990
	0-0.62		0-1.79	Koschorreck and Conrad, 1993
			2.19-2.97	Dobbie and Smith, 1996
UK			0.80-1.4	Sitaula et al., 1995
Norway			0.86-1.06	Macdonald et al., 1996
Scotland	0.02-0.12	0.85	1.4	Dobbie et al., 1996
	0.7		0.19-0.36	Macdonald et al., 1997
				Dobbie at al., 1996
Poland	0.2			
Asia-Pacific				
New Zealand			0.04-0.05	Van der Weerden et al., 1999
India			6.12-17.0	Singh et al., 1997
Japan			0.69-10.7	Ishizuka et al., 2000

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Results of Soil Analysis

3.1.1 Soil bulk density

Bulk density of a natural forest, reforestation and corn cultivation soils is shown in Fig. 8. In the natural forest, reforestation and corn cultivation, the values ranged from 1.46 to 2.01, 1.56 to 2.04 and 1.62 to 1.94 g cm^{-3}, respectively. The bulk density tended to increase when the soil depth increased. Except at depth 15-20 cm, bulk density in corn cultivation soil was higher than both natural and re-grown forests. Relatively higher bulk density in cornfield soil is possibly due to field preparation and other cultivation practices. This may affect methane oxidation through limitation of atmospheric methane and oxygen diffusion into the oxidation layers as described below (Section 3.2 and 3.3).

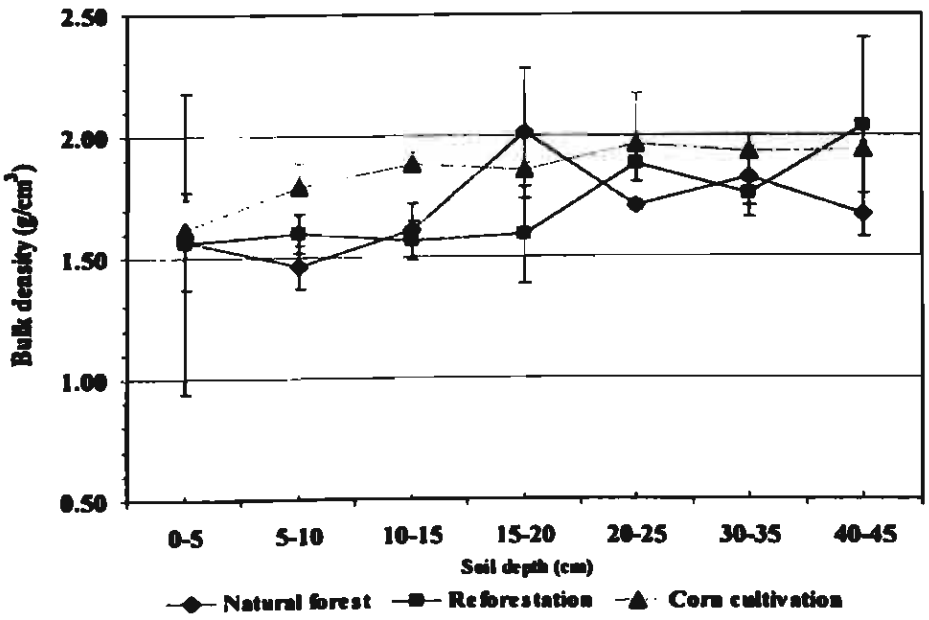


Fig. 8 Changes in bulk density along the soil depths and comparison among different land use. Error bars indicate S.D. of three soil core measurements.

3.1.2 Soil texture

Soil texture was determined based on the composition proportion of sand, silt and clay particles (Table 2). Throughout the 50-cm upper layer, soil at SK site contains more clay particle and soil at CF contains more sand particle than other sites. Thus, soil at SK is a clayey soil while at CF is a sandy soil. Soil texture at AC site comes in between SK and CF sites. Among them, the soils at 30-40 cm and 40-50 cm in natural forest show the highest clay content throughout the profile (60.08 %). Changes in soil particle composition and thus its texture after a long period conversion of natural forest soil to cultivated soil are possible under the intensive agriculture and high amount of rainfall (Phopinit and Limtrakul, 1999). This is due to the mixing of surface and subsurface soil during field preparations such as plowing. Such practices usually expose soil to leaching by rainfall. Clay particle is thus transported from upper to the deeper parts, resulting in an accumulation of clay particle as observed in this study (Table 2). On the other hand, the presence of forest and its dense network of roots and the litter layer in the forest floor prevent water transport of soil particle along the soil profile. Thus relatively large proportion of clay particle can be found near the soil surface when compared to the cultivated soils.

3.1.3 Soil pH

Soil pH was measured in deionized water (soil : water = 1:1), after drying the soil samples at room temperature. Forest soils (both at SK and AC sites) were acidic, especially in surface layers (Fig. 9). The SK soil at surface was highly acidic with the pH of about 2.0 while at AC site the pH at surface is 3.5. However, pH values at both forest sites increased to about 4 at 10 cm and then stayed fairly constant at this value throughout a 50-cm profile. Contrarily, land with corn plantation showed a different pH profile compared with forest soils. In cornfield soil, the highest pH was found at surface (pH 6.2) and pH decreased to and stayed constant around 5.2 below 5 cm.

Table 2 Soil texture along the soil depth at three different land use types.

Site	Soil depth (cm)	Particle Size (%)			Texture
		Sand	Clay	Silt	
CF	0-5	72.56	19.44	8.00	Sandy loam
	5-10	70.56	19.44	10.00	Sandy loam
	10-15	69.20	21.44	9.36	Sandy clay loam
	15-20	68.98	21.44	9.58	Sandy clay loam
	20-30	70.20	21.44	8.36	Sandy clay loam
	30-40	68.84	21.44	9.72	Sandy clay loam
	40-50	68.20	21.44	10.36	Sandy clay loam
SK	0-5	49.56	35.44	15.00	Sandy clay
	5-10	42.56	42.44	15.00	Clay
	10-15	37.98	47.44	14.58	Clay
	15-20	32.98	53.08	13.94	Clay
	20-30	32.56	57.08	10.36	Clay
	30-40	28.56	60.08	11.36	Clay
	40-50	30.56	60.08	9.36	Clay
AC	0-5	54.56	23.08	22.36	Sandy clay loam
	5-10	50.56	26.44	23.00	Sandy clay loam
	10-15	50.92	28.44	20.64	Sandy clay loam
	15-20	48.56	31.44	20.00	Sandy clay loam
	20-30	40.56	40.44	19.00	Clay
	30-40	39.56	44.44	16.00	Clay
	40-50	46.92	39.44	13.64	Clay

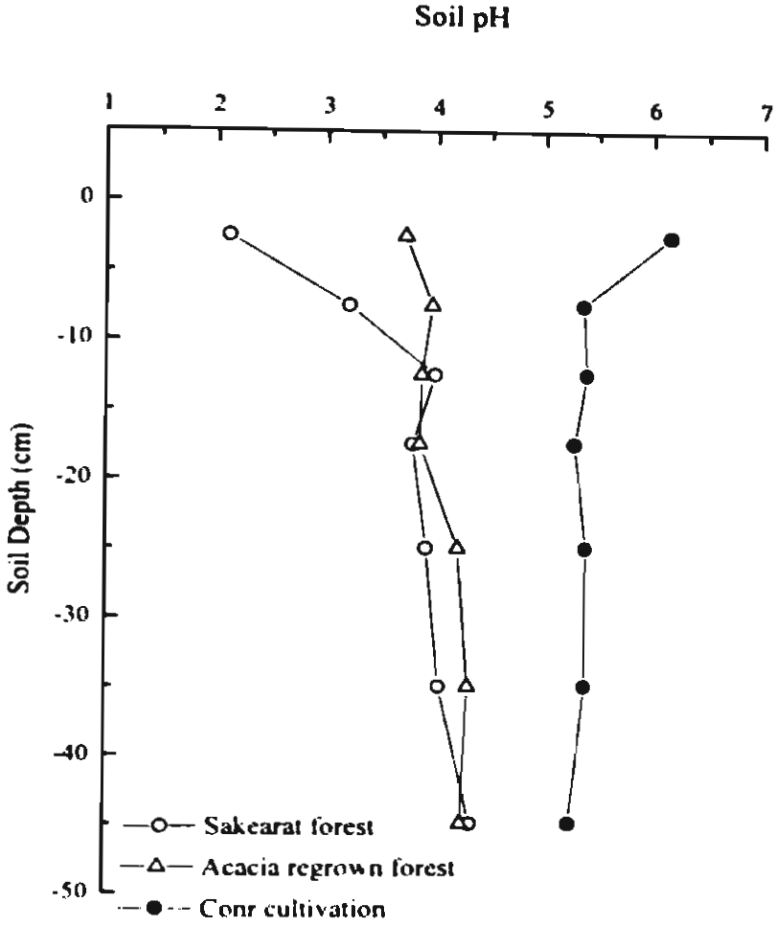


Fig. 9 Soil pH along soil depth at SK, AC and CF sites.

3.1.5 Inorganic N content

Inorganic N content (NO_2^-, NO_3^- and NH_4^+) of all soil was measured along the soil depth. Results are given in Fig. 10A-C. The top layer of natural forest soil showed the highest nitrate content (about 260 mg NO_3^- kg $soil^{-1}$, Fig. 10A). High level of nitrate was detected down the profile to 10-15 cm and below this layer nitrate content was below 25 mg kg $soil^{-1}$. On the other hand, nitrate content throughout soil profile at both AC and CF sites was fairly constant around 50-75 mg kg $soil^{-1}$. However, very low concentration of nitrate was found below 30 cm in AC soil. No clear trend along the profile was observed for both AC and CF sites.

In contrast to nitrate, nitrite content along the soil profile and across the sampling sites was not different. Compared to nitrate, nitrite content was approximately more than 10 times lower (Fig. 10B). It was lower than 3 mg kg $soil^{-1}$ below 10 cm in SK site and below 15 cm in AC site. In contrast, NO_2^- was detected across the soil profile at CF site, although it concentration was generally lower than 10 mg kg $soil^{-1}$.

Ammonium content in all sites is given in Fig. 10C. Similar to the trends found in nitrate content, natural forest soil at SK had the highest ammonium content. The highest concentration at SK was found in the top 10 cm surface layer (173 mg kg soil^{-1} at 0-5 cm depth). In both forest sites, ammonium concentration trended to decrease as soil depth increased and below 10 cm the typical concentration was below 50 mg kg soil^{-1}. In contrast to the forest site, a fairly constant amount of ammonium was observed throughout the soil profile at CF site. However, the concentration at CF site at all depths was below 50 mg kg soil^{-1} and it trended to increase in the deeper layers.

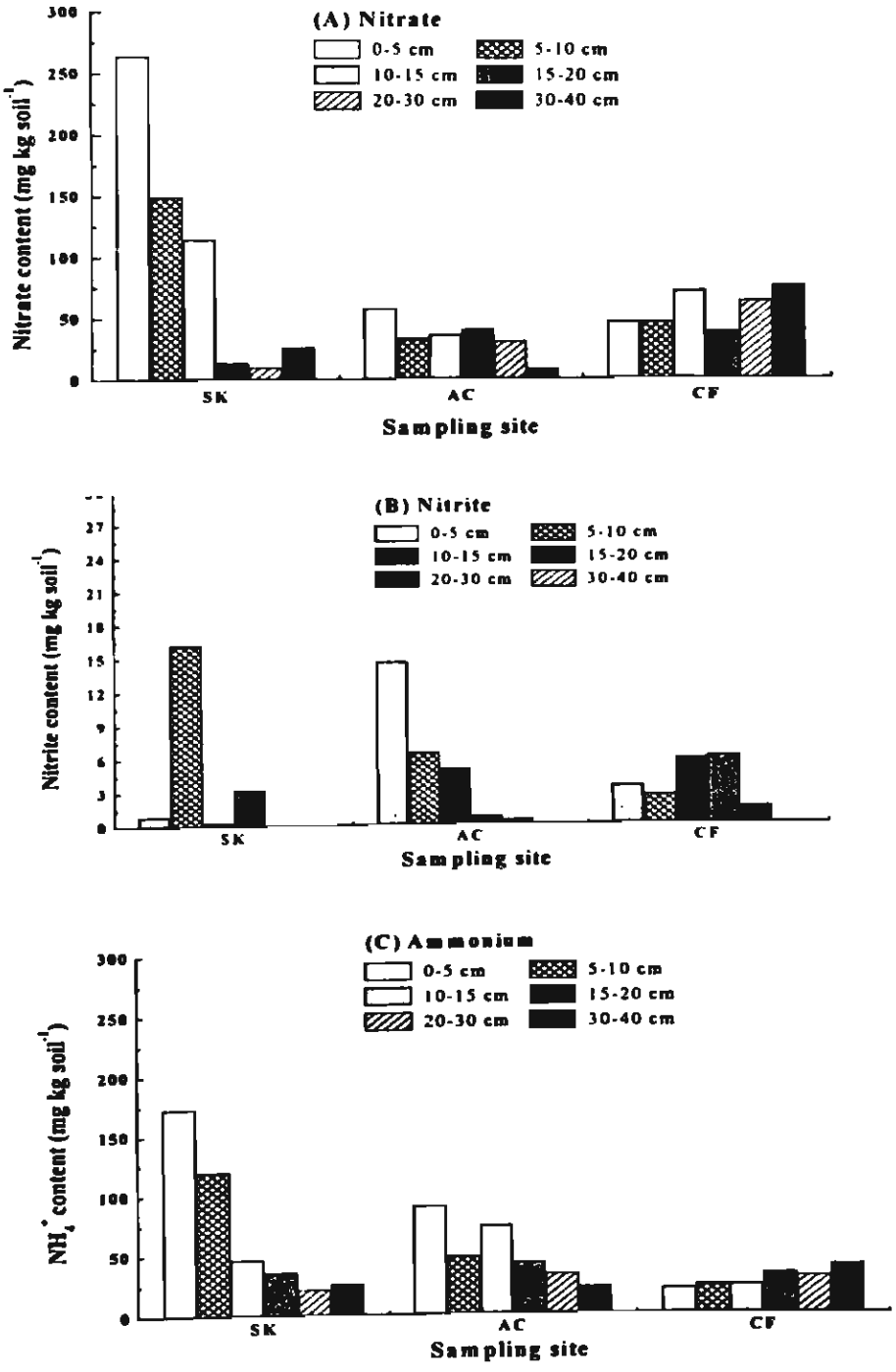


Fig. 10 Nitrate (A), nitrite (B) and ammonium (C) contents along the soil profile in different land use.

3.2 *In situ* CH_4 oxidation

3.2.1 Seasonal variations of methane oxidation among different land use type

Monthly methane flux was measured during January –December 2003. However there was no measurement made in September 2003. The results show that atmospheric methane oxidation varied both temporally and spatially. Type of land use strongly affects methane oxidation. On average over one year, the highest oxidation rate was observed in forest sites but rates were usually associated with large variations among chambers. Although the oxidation varies seasonally, at AC site net methane oxidation was observed consistently in almost all chambers measured. This is different from SK site where sometimes the positive methane flux (net methane emission) was observed. On the other hand, land use as corn plantation results in a drastically decrease in methane oxidation rate. Occasionally, net methane emission appeared, particularly when soil moisture content was high (see results below). Thus, soil at CF site soil could become either source or sink of methane depending largely on environmental conditions and cultivation practice such fertilization. Details of measurement results are described below.

a). Sakaerat forest site. At this site, ten chambers were used to monthly monitor the methane oxidation. However, not all chambers showed methanotrophic no methanogenic activity. Only those chambers showing a significant increase or decrease in headspace concentration over time were include in flux estimation. During January to December 2003 (no measurement was carried out in September), the highest average oxidation rate was found in February and November as 2.8 and 2.6 mg CH_4 m^{-2} day^{-1} respectively, Fig. 11). Except in June and July when net emission of methane was observed, forest soil acted as the net methane sink throughout the years. The relatively high oxidation rates appeared during the dry months and low rates were found during the raining season. The average value during one year measurements was 1.50 mg CH_4 m^{-2} day^{-1}, indicating the fact that forest soil act as the net atmospheric methane sink although in some period of the year it may emit certain amount of methane.

It is noted that the rates of methane oxidation found in Thai forest soil are comparable to those found in other forests (Table 1). For example, methane oxidation rate of 0.3-2.3 mg CH_4 m^{-2} day^{-1} in Costa Rica (Keller and Reiners, 1994), 0.8-6.4 mg CH_4 m^{-2} day^{-1} in USA (Castro et al., 1995), 0.67-10.7 mg CH_4 m^{-2} day^{-1} in Japan (Ishizuka et al.,

2000) were reported. However, the rates obtained in the present study are usually higher than those measured in most European temperate forests. This indicates that soil under dry evergreen forest in Thailand possess a relatively high potential for oxidizing the atmospheric methane.

b). Reforested site (Acacia plant, AC site). Similar to the SK site, net methane consumption by AC forest was observed (Fig. 11). The rates of methane oxidation are similar to those observed for SK site, ranging from 0.5 to 2.1 mg CH_4 m^{-2} day^{-1}. High oxidation rate was observed in dry season, the same trend observed in SK site. The highest methane oxidation rate was found in October (2.1 mg CH_4 m^{-2} day^{-1}) followed by February (2.0 mg CH_4 m^{-2} day^{-1}). Soil at AC site always showed the net methane sink throughout the year. The average oxidation rate of all months was 1.17 mg CH_4 m^{-2} day^{-1}.

c). Agricultural site (Cornfield site, CF). CF soil acted as both methane sink and source throughout the year (Fig. 11). Even in dry months such as in January and February, net methane emission was observed. There was neither net methane emission nor consumption in March. However, CF soil turned to be the net methane sink during March to June. When cultivation of corn began in July, it became net methane source again and high amount of methane emission was observed in October, concomitant with heavy rainfall and sharply increased in soil water content (see below). Because of this emission peak, on the average basis throughout the measurement period the CF soil became a net methane source (+12.9 mg CH_4 m^{-2} day^{-1}). Difference in emission or oxidation pattern in this site compared to forest soil site indicates that different factors may control methane production and oxidation. Even during the dry season, it seemed that some part of soil profile at CF site remained anoxic, allowing methanogenesis and emission of methane to occur as observed in the present study.

When the results of averaged oxidation rates from all of these three land-use types are compared, it was found that the oxidation capacity is in the order of Sakaerat forest (-1.50 mg CH_4 m^{-2} day^{-1}) > Acacia reforest (-1.17 mg CH_4 m^{-2} day^{-1}) > agricultural area (cornfield, +12.9 mg CH_4 m^{-2} day^{-1}). Thus highly acidic pH in SK and AC soils (Fig. 9) is not the inhibitory to methane oxidation. On the other hand, soil texture (Table 2) which is one of the important parameters limiting gas diffusing seems not to relate much to the different oxidation rate among these three sites. The soils at SK and AC sites contain more

clay particle than at CF site but bulk density at SK and AC site is generally lower than at CF site, indicating that gas diffusion (O_2 and CH_4 from the atmosphere into soil layers) at SK and AC sites may be faster than at CF site. High bulk density at CF site may be resulted from soil cultivation practices.

Another factor that affects methane oxidation activity of soils is the presence of inorganic nitrogen species. Of these, ammonium and nitrite have been known for their strong effects on methane oxidation rates (Dunfield and Knowles, 1995). Ammonium acts as a competitive substrate for MMOs while nitrite is toxic to methanotrophic bacteria. Gulledge and Schimel (1998) showed that ammonium concentration of 94 mg kg soil^{-1} (as Ammonium sulfate) inhibited methane oxidation by 67% in temperate hardwood forest. Nitrate becomes inhibitory to methane oxidation only at high concentration (usually > 10 mM dissolved nitrate, Adamsen and King, 1993). However, concentrations of all nitrogen species are higher in SK and AC soils than in CF soil and such levels of ammonium might be sufficiently high to inhibit methane oxidation. Nevertheless, relatively high oxidation throughout the year was observed at both SK and AC sites. Thus, nitrogen content alone in surface soil cannot explain the oxidation rate differences between forests (SK and AC) and agriculture soil (CF) in this case.

It was observed that the distribution pattern of nitrogen content along the soil profile differed among three sites. In SK and AC 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 SK and AC site. On the other hand, soil at CF 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 CF 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 CF site. Such distribution of N and at level of concentration found may lead to a relatively uniform inhibition of methane oxidation throughout the soil profile, while such inhibitory effects of N is not prevail in SK and AC soils due to their relatively low concentration in subsoil. As a result, net methane oxidation was observed in SK and AC site throughout almost most of the time. In addition, forms of ammonium present in the soil also have significant effects on methane oxidation. Gulledge and Schimel (1998) found in temperate hardwood forest soil that NH_4Cl inhibited methane oxidation by 88% while $(NH_4)_2SO_4$ only inhibited methane

oxidation by 67, compared to the control. Inputs of nitrogen through fertilization at CF site, thus, may lead to difference in nitrogen forms present in the CF soil from those found in SK and AC site. This may lead to inhibitory effects on methane oxidation although its concentration is not as high as that found in SK and AC soils.

Seasonal variations in oxidation found in all sites during the study period may be attributed mainly to changes in soil moisture. Soils with heavy texture such as SK and AC soils usually contain more water per soil weight than soil with light texture such as CF soil in the present study. However, it is likely that when moisture increases to certain level, soil texture plays only a minor role in regulating methane oxidation. Relatively low activity of methane oxidation observed in all sites during the raining months might have been caused by high soil moisture content (see Section 3.3). Difference in draining capacity of soil between at SK and CF might result in different in methane oxidation, especially in June. This is supported by the distinct soil texture between these two sites, especially in the top 20 cm.

From these results, some distinct characteristics of methane oxidation associated with different land use types can be concluded as followed;

1. methane oxidation capacity of soils is strongly affected by land use types and season-associated factors such as soil moisture and cultivation practices,
2. undisturbed forest has the highest atmospheric methane oxidation capacity,
3. methane oxidation capacity can be recovered by reforestation, but not yet fully the same level as in undisturbed forest,
4. land use change from forest to agriculture (corn plantation in this case) reduces methane oxidation activity significantly.

3.2.2 Spatial variations of methane oxidation

a). SK site. In this site although on average net methane consumption was observed but large spatial variations among chambers are noted (Fig. 12). For example, within each sampling time oxidation rate among all chambers could be as high as 86% above the average values (in February). However, sometimes such as in April the spatial variations of methane oxidation among chambers was as low as 13%. Similarly, if we follow the same chamber through out the study period, oxidation also varied seasonally. These results indicate that there are the true large variations in oxidation rates of methane in different locations of forest floor and this may be due to the heterogeneous nature of soils.

b). AC site. Methane oxidation rates among all five chambers at AC site are shown in Fig. 13. In contrast to SK site, except in July all chambers showed net methane consumption. However, variations in methane consumption rates among chamber are remarkable. For example, in January range of oxidation rate among five chambers is from 1 to about 2.7 mg CH_4 m^{-2} day^{-1} (Fig. 13) and in February is from about 2.7 to 3.7 mg CH_4 m^{-2} day^{-1}. Methane oxidation different among chambers was relatively small during March to June, and in August and November, respectively.

c). CF site. As expected, large variations among chambers was found at CF site. Almost in all cases during raining season net emission was observed. Net emission became obvious in July and this lasted until October. Net methane consumption in all chambers was found in only May, June and November. In all other occasions, at least one chamber showed the net methane emission (Fig. 14). It is noted that in October, three out of five chambers emitted more than 100 mg CH_4 m^{-2} day^{-1}. This was probably due high soil water content, especially below 40 cm where water content was always above 40% (Fig. 18).

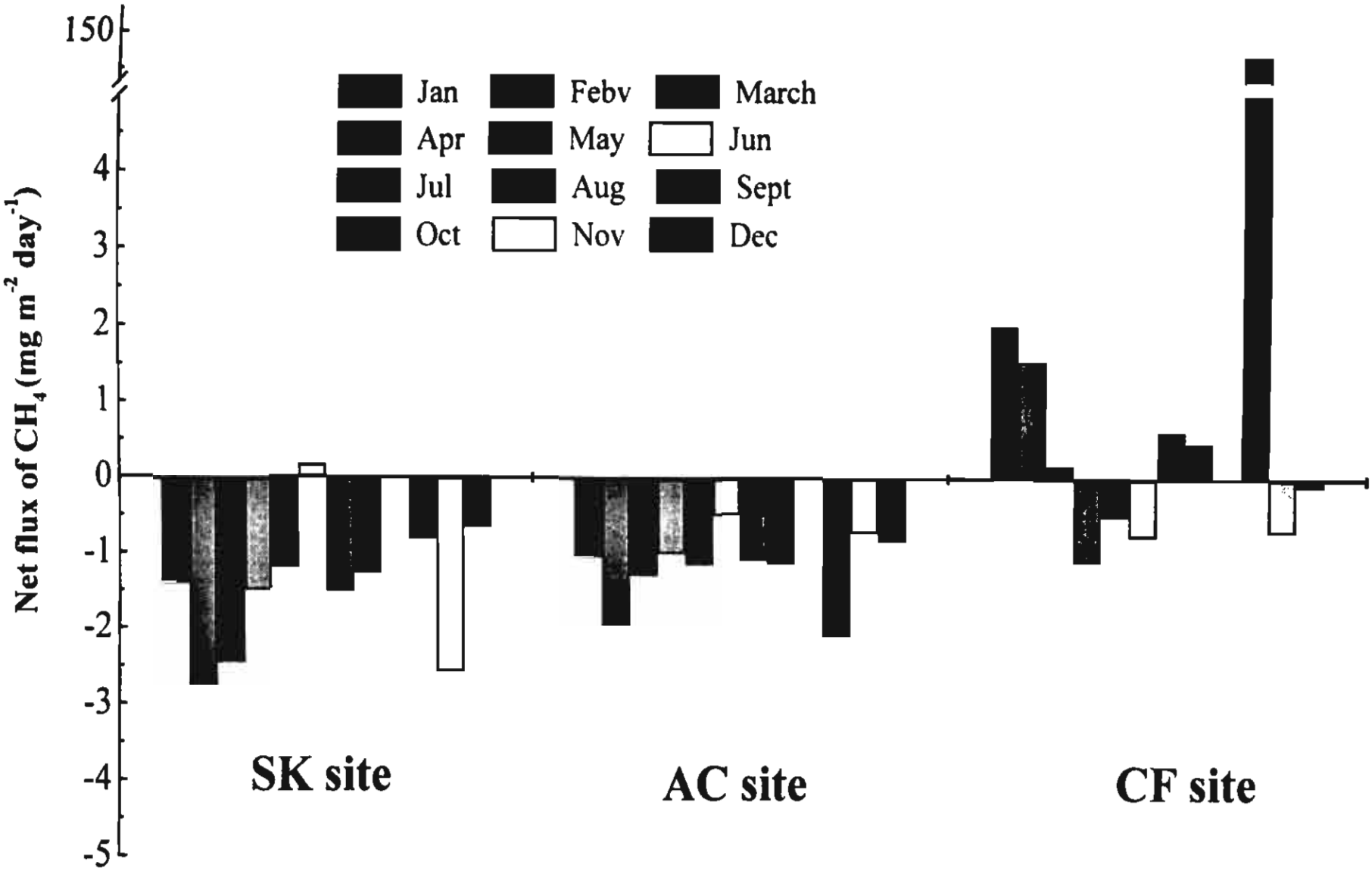


Fig. 11 The monthly average of methane oxidation rates in different land use types.

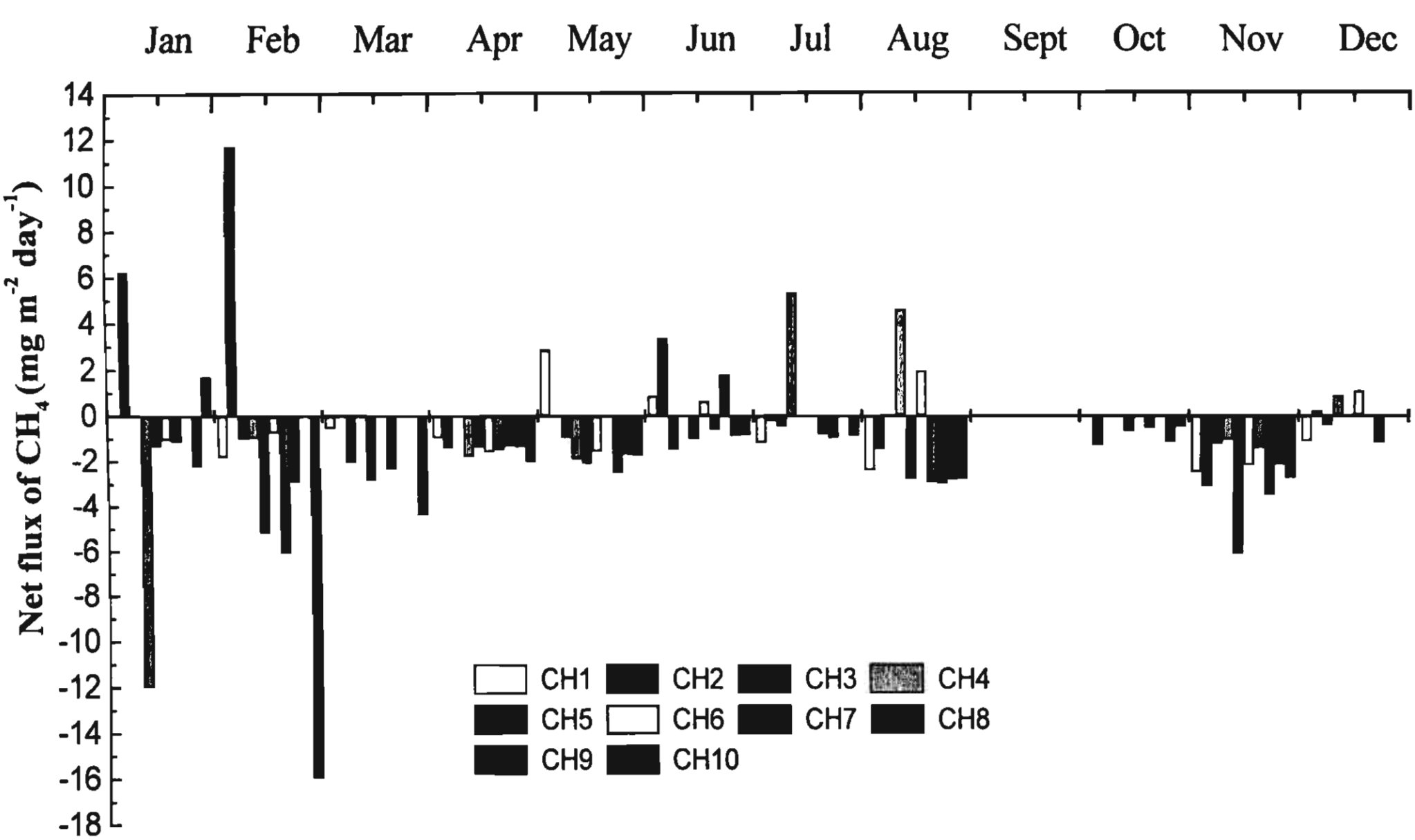


Fig. 12 Variations in methane oxidation rates among ten chambers at SK site. (CH1 to CH10 designate Chamber No. 1 to Chamber No. 5). In some months, results from certain chambers are omitted due to lack of significant changes in headspace concentration of methane.

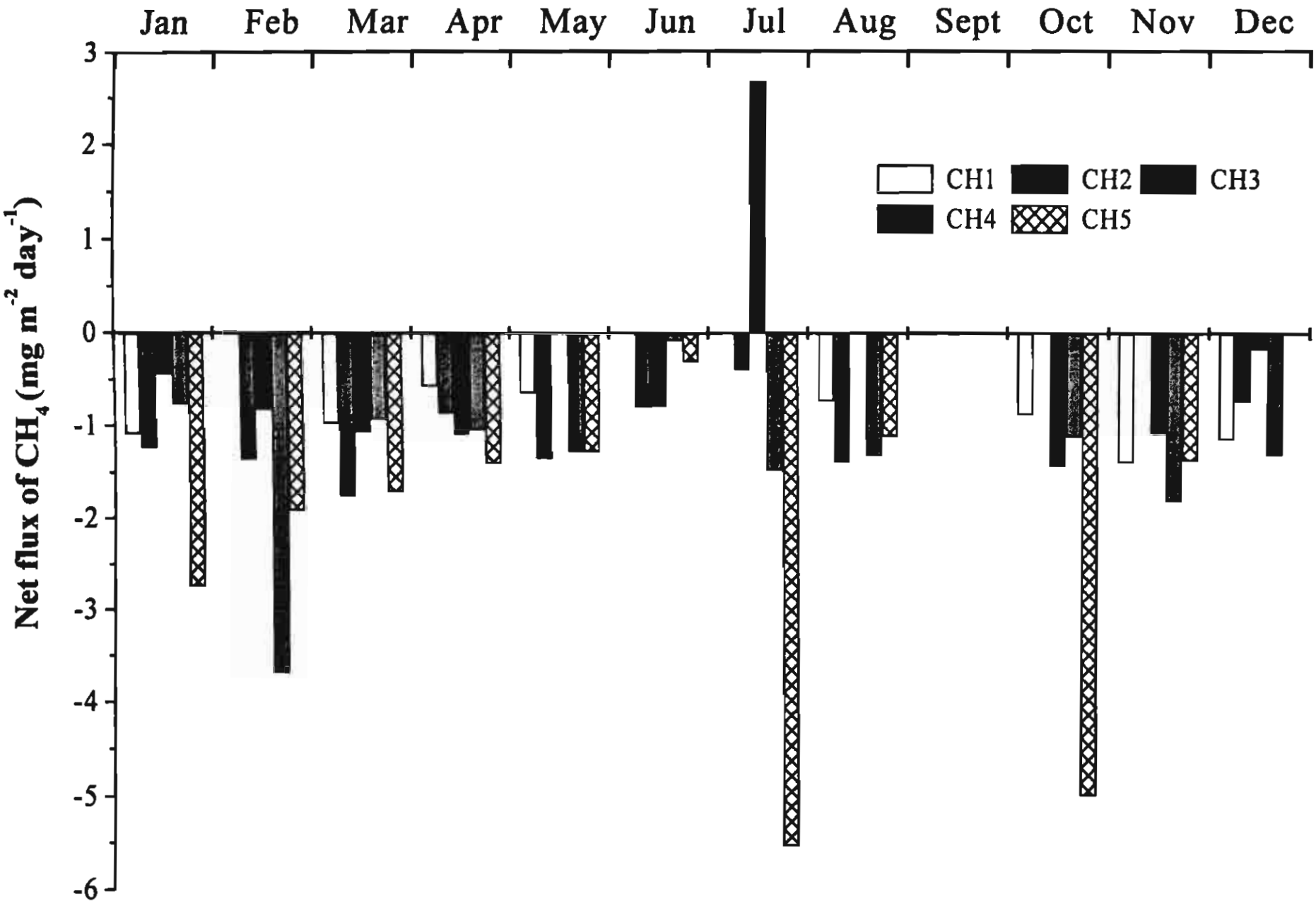


Fig. 13 Variations in methane oxidation rate among chambers at AC site. (CH1 to CH5 designate Chamber No. 1 to Chamber No. 5). In some months, results from certain chambers are omitted due to lack of significant changes in headspace concentration of methane.

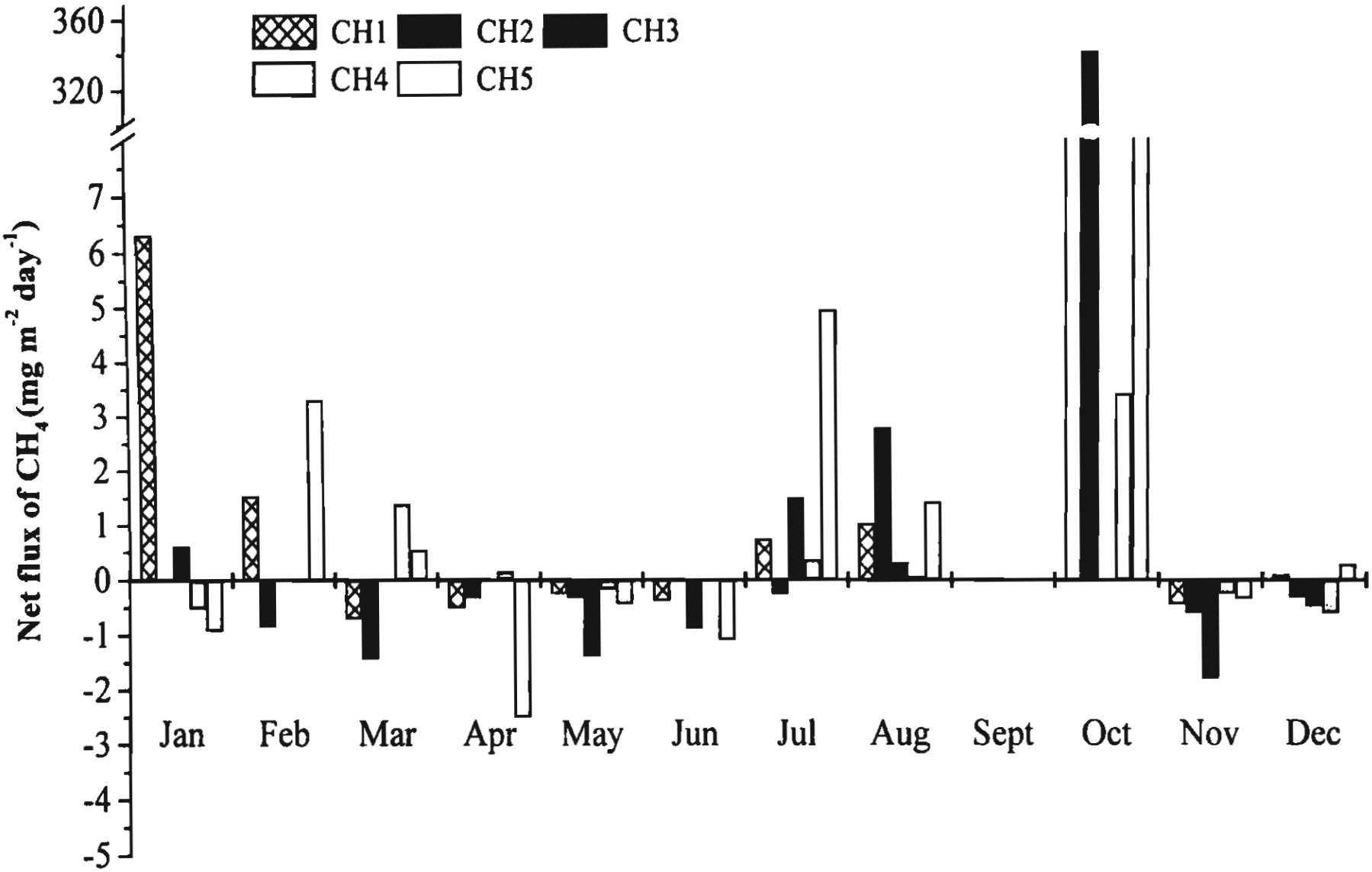


Fig. 14 Variations in methane oxidation rate among chambers at CF site. (CH1 to CH5 designate Chamber No. 1 to Chamber No. 5). In some months, results from certain chambers are omitted due to lack of significant changes in headspace concentration of methane.

3.3 *In situ* soil moisture

Soil moisture was measured during April 2003-Feb 2004. Earlier measurement was not carried out due to unavailability of instruments. Moisture was measure at 6 cm below soil surface and at every 10 cm after that down to 100 cm below the soil surface.

3.3.1 Soil moisture at 6 cm

At 6 cm depth, soil moisture in all sites ranged from about 4 to 30% by volume (of soil pore). Relatively higher seasonal variations were observed at CF site than in other sites. Almost in most cases, high soil moisture developed during July to October after the beginning of the raining season. Many occasions that soil moisture in CF site was below 10% or more than 20% while such were not usually observed in both AC and SK site.

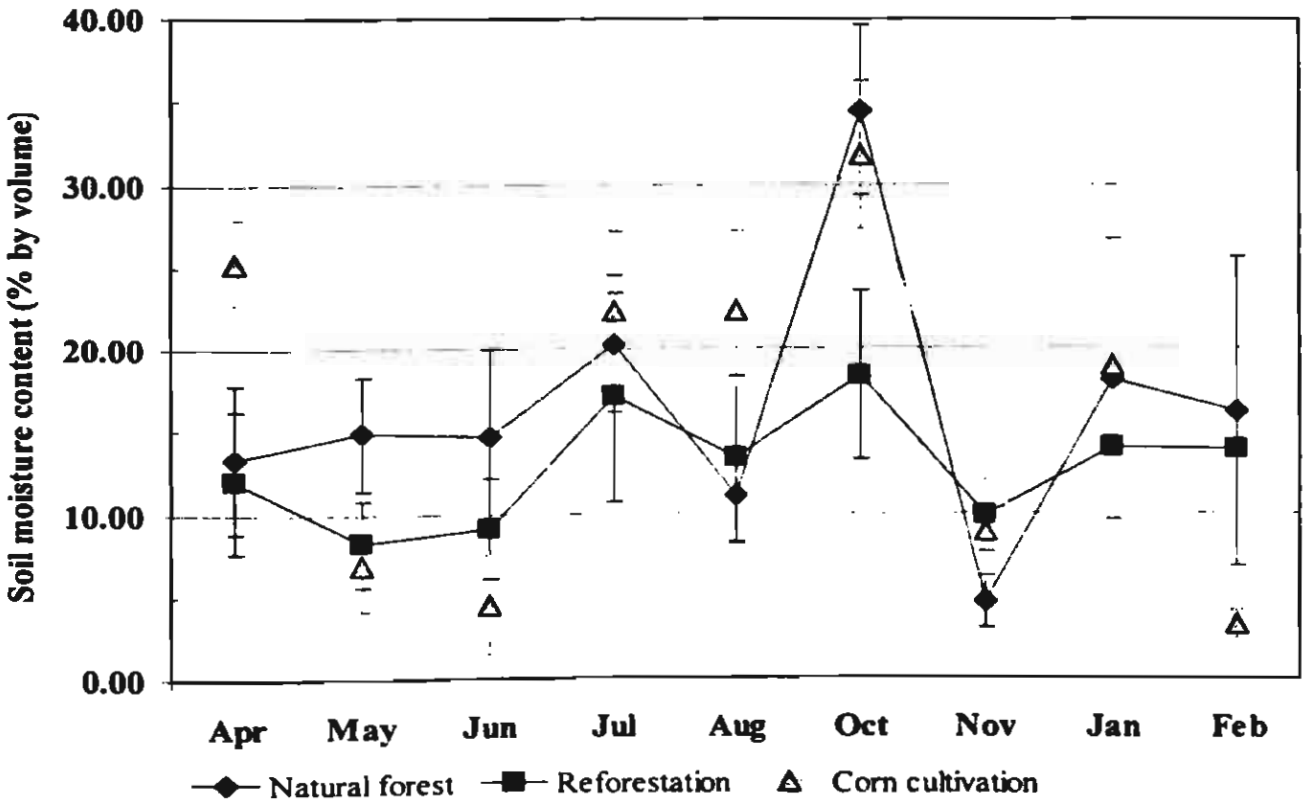


Fig. 15 Soil moisture at 6 cm soil depth in different land use type. Each point is the average from 10 measurements and errors bars indicate S.D.

3.3.2 Soil moisture along the soil profile

a) Sakaerat Forest (SK site). Except in October, soil moisture at this site stayed below 30% throughout the soil profile (Fig. 16). However, moisture tended to increased from January towards July and decreased again in August. It then increased and peaked in October when through the profile the moisture content was more than 80%.

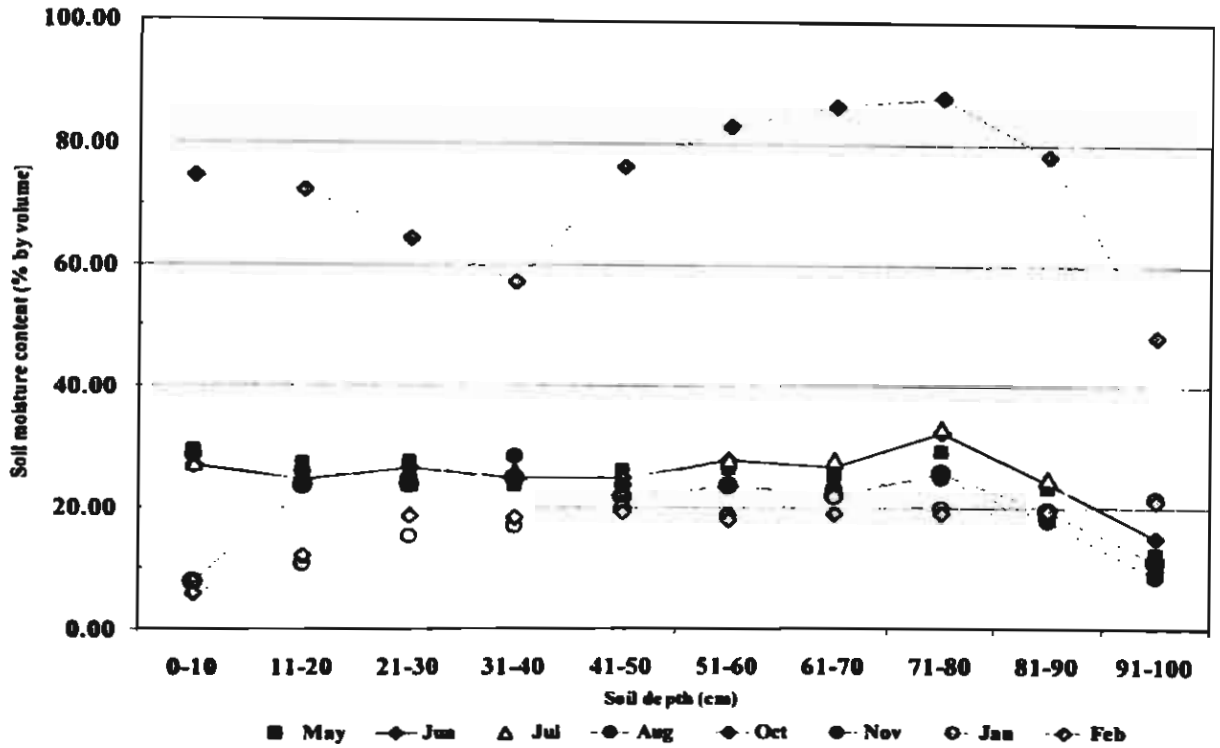


Fig. 16 Temporal and spatial variations in soil moisture along soil profile at SK site.

b) Acacia plantation (AC site). At AC site, the soil moisture profile was quite different from at SK site (Fig. 17). Soil moisture increases along with soil depth. In a given month, moisture at surface was always lowest throughout the soil profile. From all data available, moisture content in the soil profile at this site never exceeded 45%. This is in contrast with SK site where in October the soil was nearly saturated with water. Thus, the soil at AC site is well drained compared with other two sites. This may be the reason why this site acts as the net methane sink most of the time.

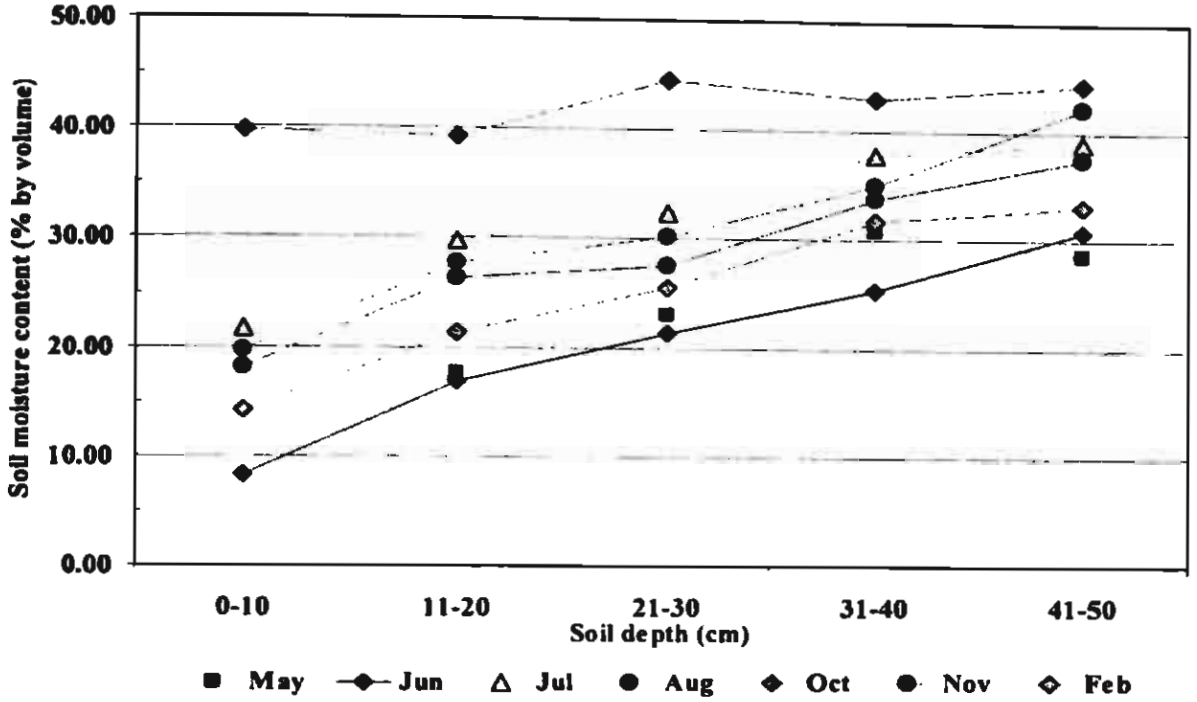


Fig. 17 Temporal and spatial variations in soil moisture along soil profile at AC site.

c) Corn cultivation (CF site). Similar to AC site, moisture at CF site increase along with soil depth. However, in the deeper layer (below 80 cm), soil moisture in every month was more than 40%. In October and November below 50 cm the moisture content was relatively high (above 60%). This high moisture content explains the net methane emission characteristic as mentioned above, possibly due through activation of methanogenesis.

3.3.3 Relationship between soil moisture and methane oxidation rate

Relationship between soil moisture at different depths and rate of methane oxidation measured on the soil surface was analyzed. At SK site, it seemed that oxidation rate decreased as water content at 0-10 cm and 20-30 cm increased from 5% to about 20%, and from 25-35%, respectively (Fig. 19A). There was no clear correlation at 10-20 and 30-40 cm soil depth in this site. On the other hand, at AC site in all soil depths, methane oxidation rate increased as water content increased from about 7% to 45% (Fig. 19B). No correlation between soil moisture and methane oxidation rate in all soil depth at CF soil

was observed. Thus, it seems that interplay between site characteristics and moisture content influences methane oxidation differently.

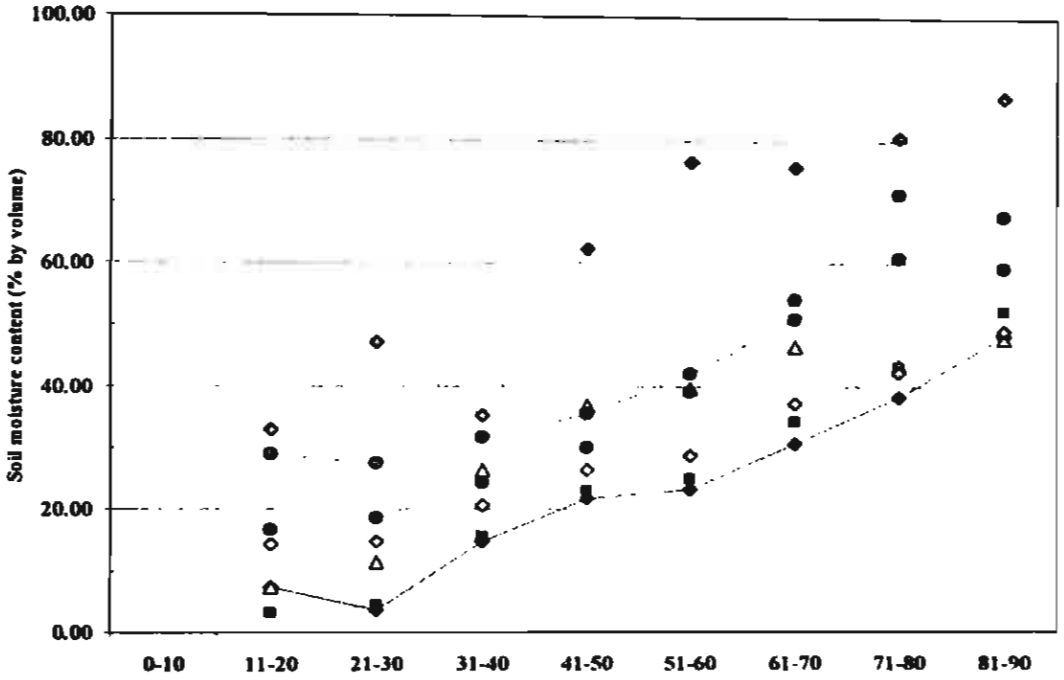


Fig. 18 Temporal and spatial variations in soil moisture along soil profile at CF site.

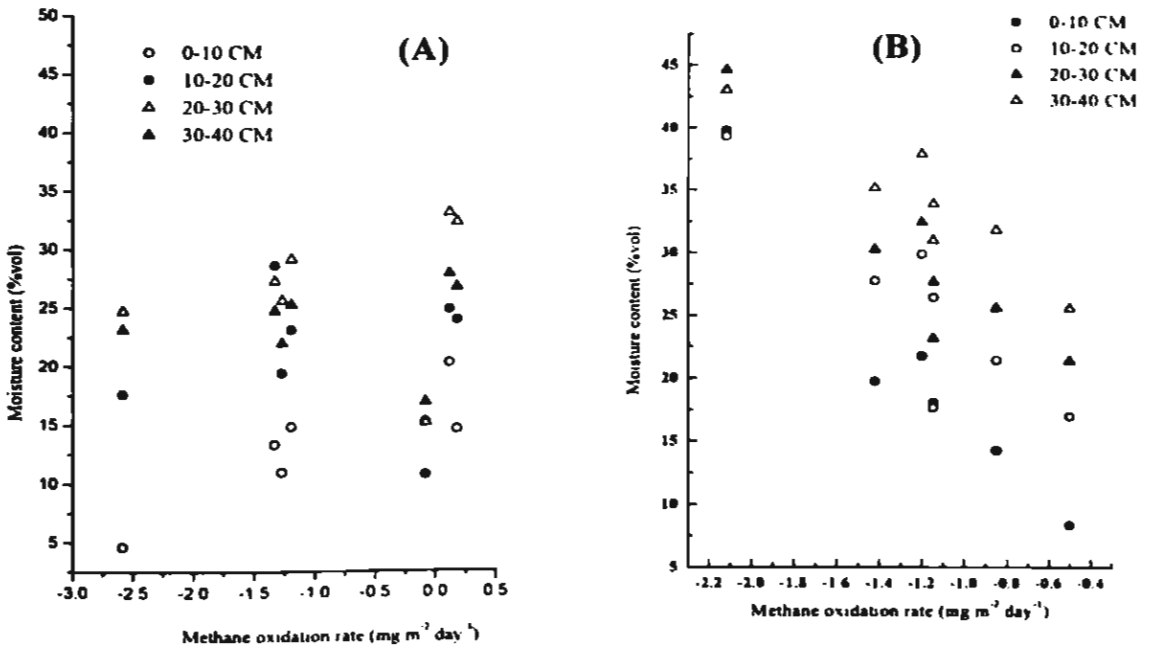


Fig. 19 Relationship between soil moisture and methane oxidation rate at SK site (A) and AC site (B).

3.4 Soil Temperature

Air and soil temperatures (at 2 cm and 5 cm) were measured during project period. The results are given in Fig. 20 below. It is clear that different land covers and land use type strongly influence temperature characteristics of soils. Highest soil and air temperatures were found at CF site. Larger seasonal variations were also observed at CF than at SK and AC sites. The average air temperature and soil temperatures at 2 and 5 cm depth during February to December are 25.4, 24.0 and 23.5 °C at SK site, 26.9, 25.6 and 25.3 °C at AC site, and 33.3, 30.7 and 28.7°C, respectively. Temperature difference between air and soil is higher at CF site than at both forest sites.

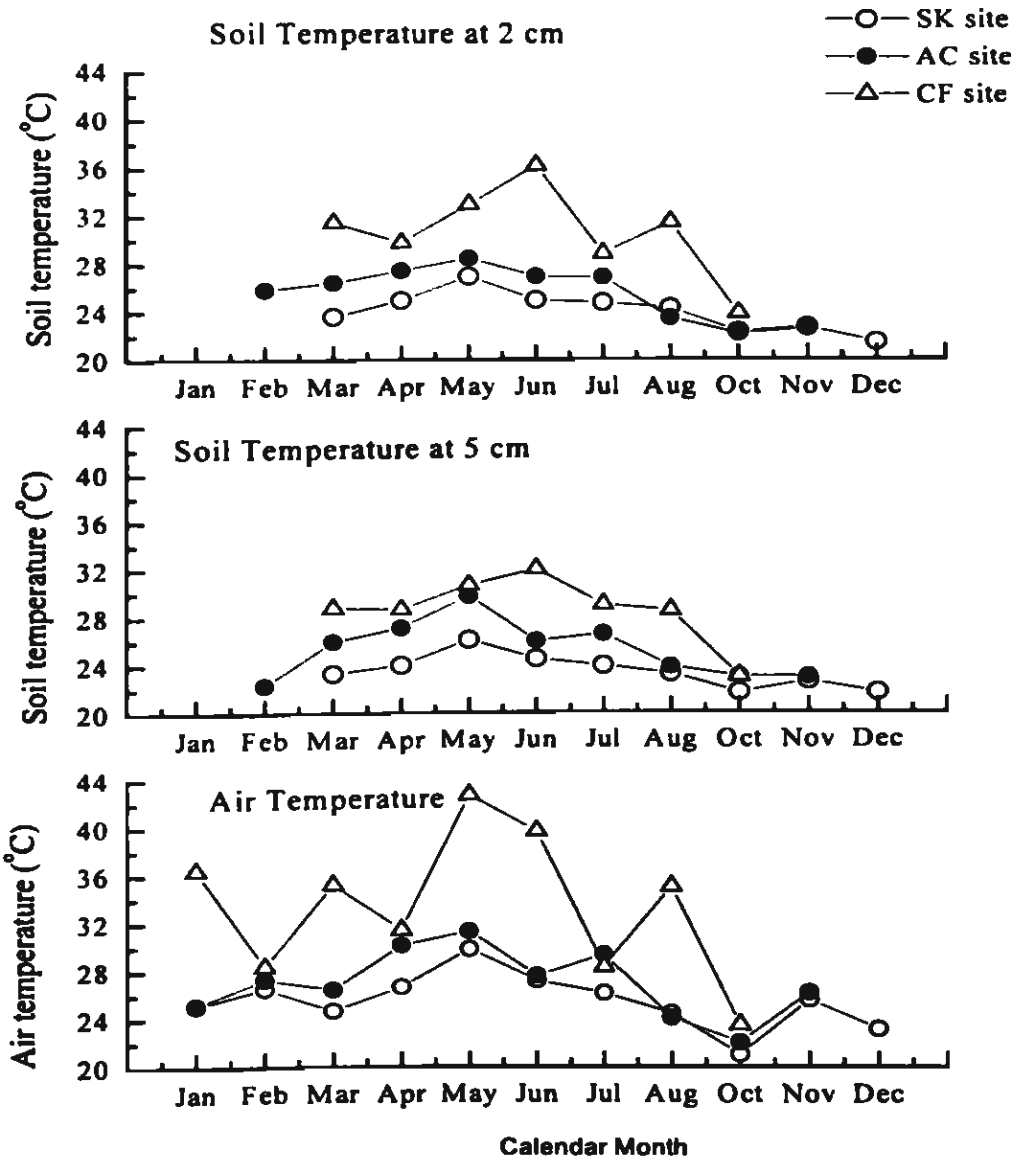


Fig. 20 Seasonal variations in soil temperature at 2 and 5 cm and air temperature in 2003

3.5 Results from Laboratory Study

3.5.1 Oxidation profile

Soils at different depths taken from all three sampling sites were studied in the laboratory. Soils from SK and AC sites were incubated under ambient methane concentration and then consumption was followed over times. Since soil from CF site shows low oxidation activity, incubation was carried out under 5 ppmv methane. Results of these incubation experiments are given in Fig. 21-24. Most of soil layers exhibit the methane oxidation activity. However, the most active methane oxidation layers for SK site is between 15 and 30 cm (Fig. 21 & 24). For AC site clear oxidation activity was found only at 10-15 and 20-30 cm (Fig. 22 & 24) and changes in concentration of methane in the incubation vials were erratic. Repeated experiments gave the similar results. Similarly, all layers of soil from CF site show oxidation activity (Fig. 23 & 24). There was no distinct active layer for in this site. However, the most active layer was still in the subsurface at 15-20 cm. From these results, it is concluded that the active layers for methane oxidation lie below the soil surface in forest soils. The reason for this is not know. However, it is typical that oxidation in the organic layers of forest soils is usually lower than the mineral soil layers (Roslev et al., 1997; Bradford et al., 2001; Reay et al., 2001). This is due to the fact that mineralization in organic layers release inorganic nitrogen species that 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 is indeed due to high ammonium and nitrate contents (Fig. 10). In addition, the pH at the surface layers is very low in the present study (Fig. 9). Thus, The soil at the surface layers may be too acidic for methane oxidation.

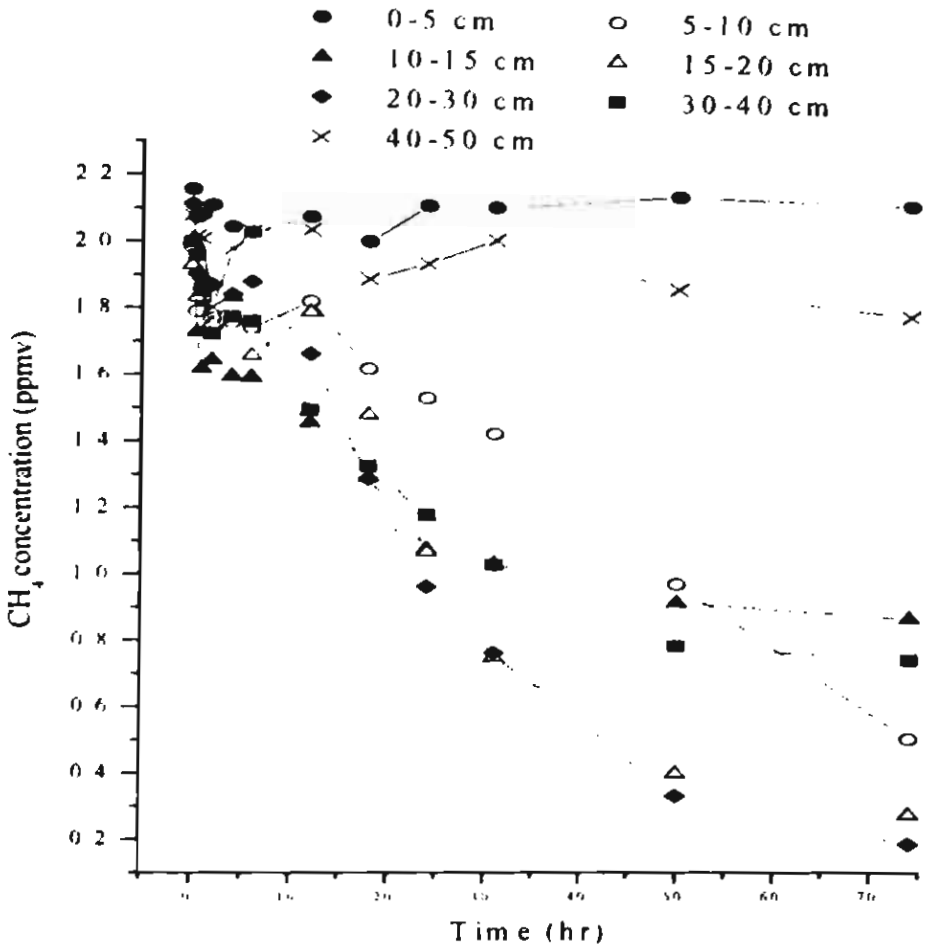


Fig. 21 Oxidation of methane by different soil layers taken from SK site and incubated under ambient methane concentration.

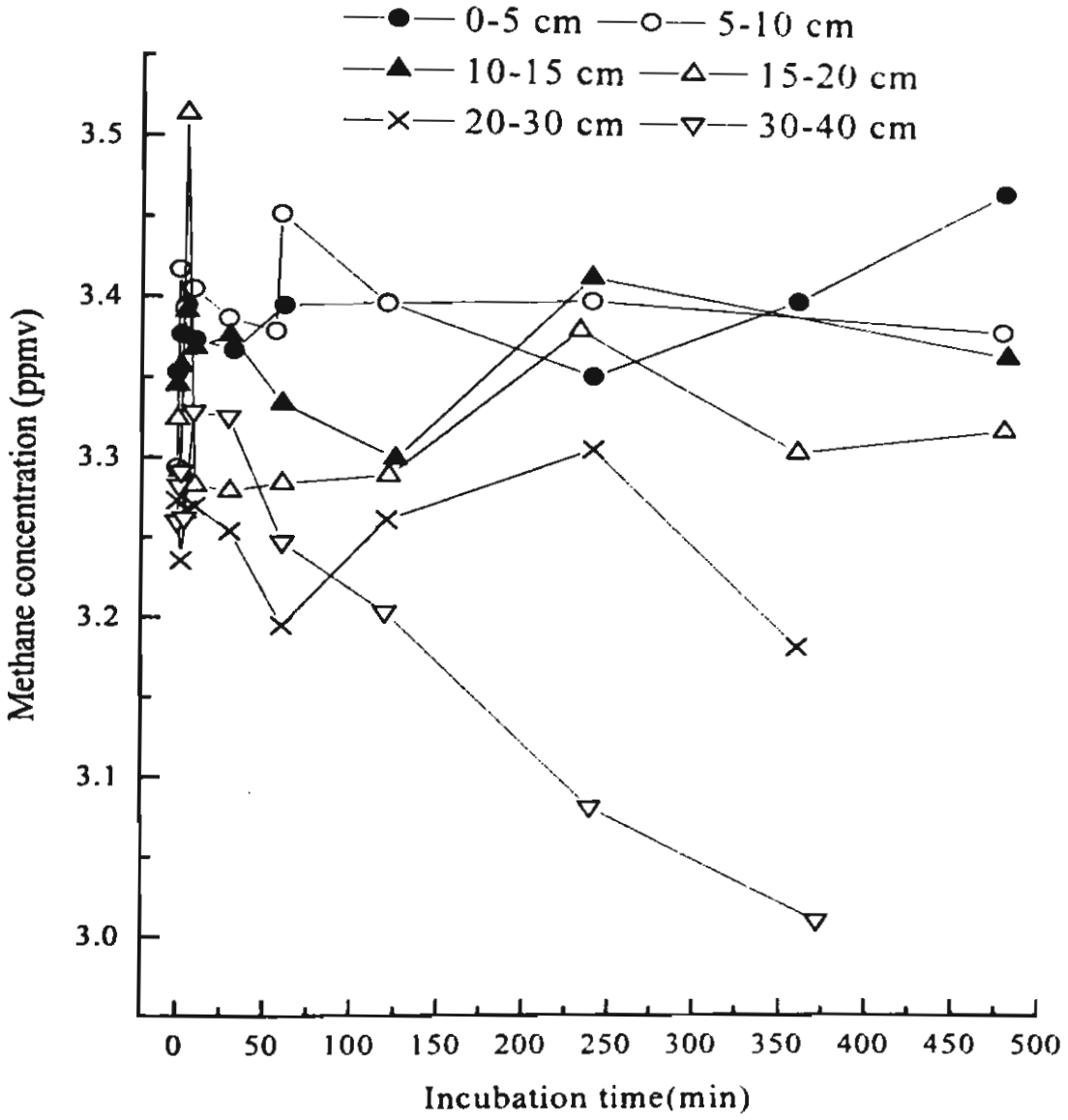


Fig. 22 Oxidation of methane by different soil layers taken from AC site and incubated under ambient methane concentration.

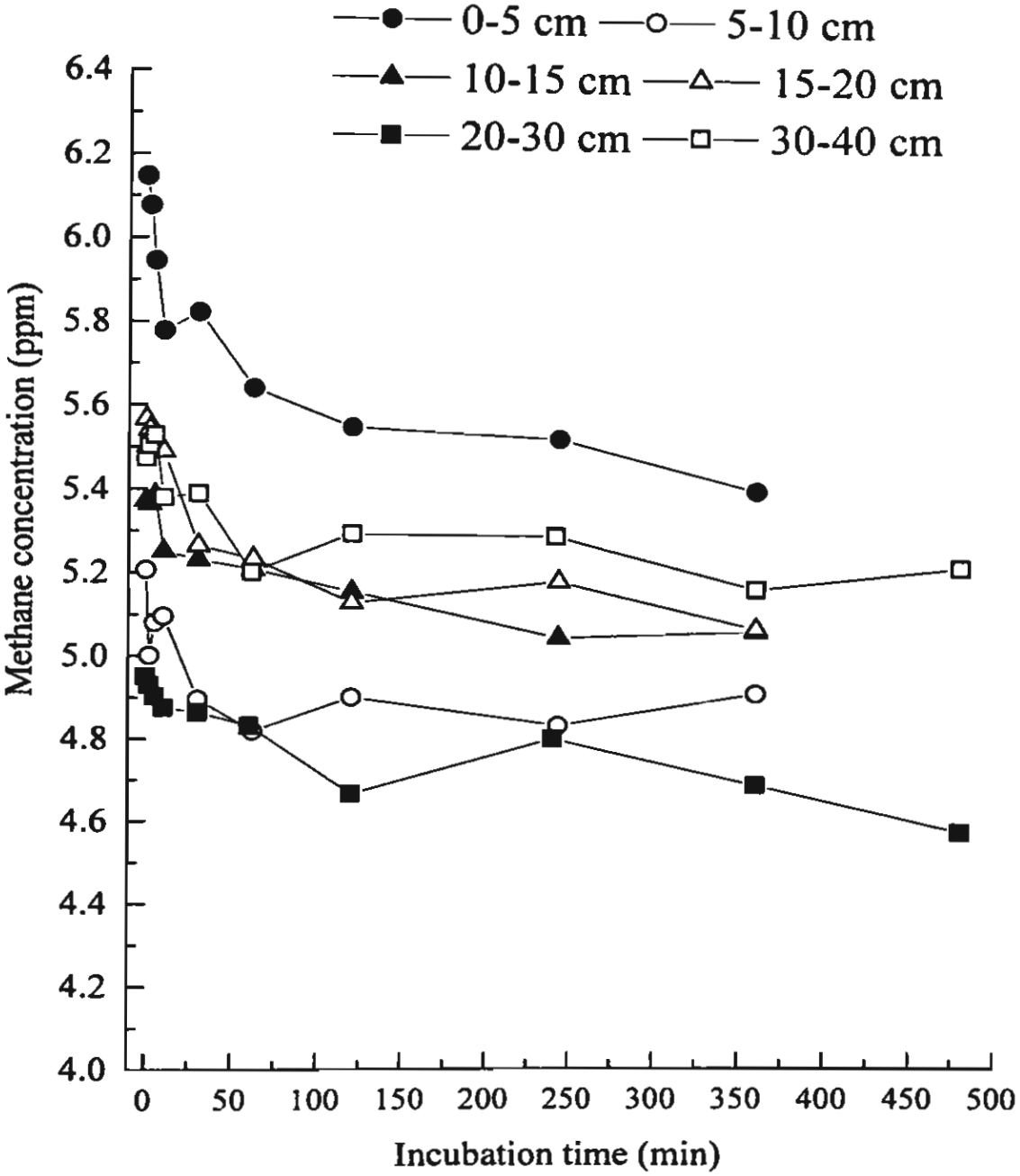


Fig. 23 Oxidation of methane by different soil layers taken from CF site, incubated under 5-6 ppmv of methane.

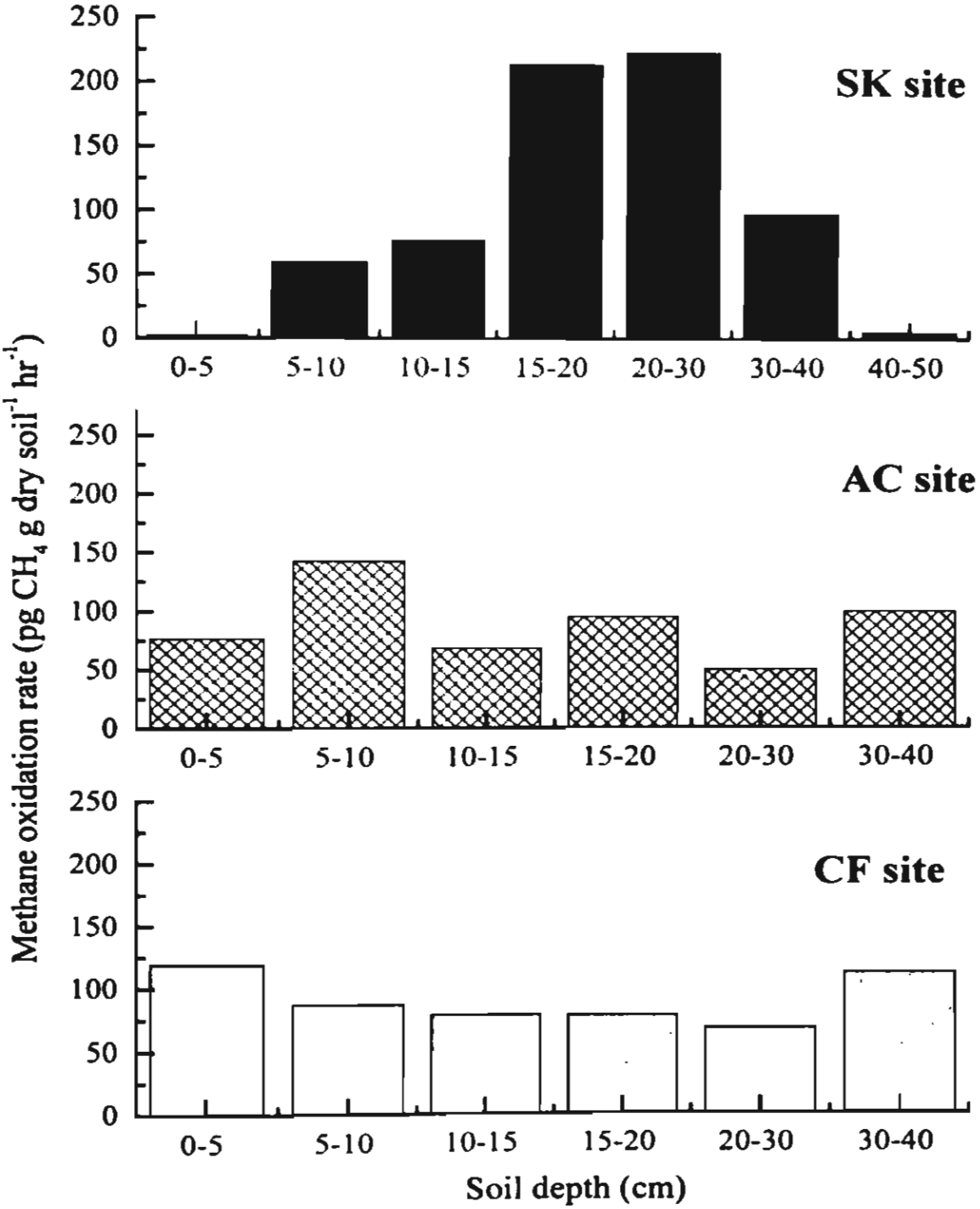


Fig. 24 Oxidation of methane along soil profile and in different land use type.

3.5.2 Kinetic study

From the results of section 3.5.1, soil from the active layer was used for kineticstudy of methane oxidation. The results in are described below.

Methane oxidation rate at each concentration was 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. 24. The initial methane concentrations used in the experiment were 5, 10, 20, 50, 100, 200, 300, 700, 800,1000 and 1800 ppmv. For SK site, the plot between consumption rate and substrate concentration followed an expected shape of enzymatic kinetics with initially increase oxidation rated paralleling increase concentration, followed by a rate that no longer shows concentration dependence. This is true even at low methane concentration. However, in AC and CF soil it seemed that at concentration below 50 ppmv, there was no clear correlation between oxidation rate and methane concentration as suggested by pseudo-first order kinetic model of Michaelis-Menten. Increase of oxidation rate in these two sites was observed at concentration above 50 ppmv (Fig. 26).

From Fig. 26, it is clear that at methane concentration below 250 ppmv, rate of methane oxidation in SK soil was higher than at both AC and CF site. Thus, soil at SK can oxidize methane at atmospheric concentration better than soils at AC and CF site. However, above about 200 ppmv, the oxidation rate becomes concentration independence at SK site. Thus, SK soil will not oxidize methane if its concentration is too high. In AC and CF sites oxidation rate still increased and then stabilized around 800 and 1000 ppmv, respectively. Comparing between AC and CF soils, at ambient and 50 ppmv concentration, however, soil at AC site shows higher oxidation rate than at CF site, though the difference was not clear.

The estimated kinetic coefficients associated with methane oxidation as affected by different land use are given in Table 3. The highest affinity for methane was found in SK soil (K_m = 52 ppmv). Soil from AC and CF show lower methane affinity compared with SK site. It is interesting that kinetic coefficients for AC soil come in the middle between SK and CF soil, as for the history of land use. This may indicate that soil at AC site is in the transient period from previously disrupted by human activity (agriculture) towards the natural conditions. Methanotrophic community in AC soil, thus, may be the mixture of those found in SK and AC sites, possibly means it could oxidize both methane at low (as observed in SK site) and high methane concentration (as observed in CF site).

From these results, it is apparent that land use type affects oxidation kinetics of methane oxidizing bacteria. 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. Difference in kinetic parameters among land use types could be due to various factors associated with land use such as nitrogen fertilization (Dunfield and Knowes, 1995). In additions, availability of substrate which is limited by methane and oxygen diffusion into the oxidation site, different in methanotrophic community as shown in the Section 3.6 below, all could account for such variations. In Thai forest soil, 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 (1994) was 22 ppmv and the V_{max} associated with this methane oxidation was 3.6 nmol g^{-1} hr^{-1}.

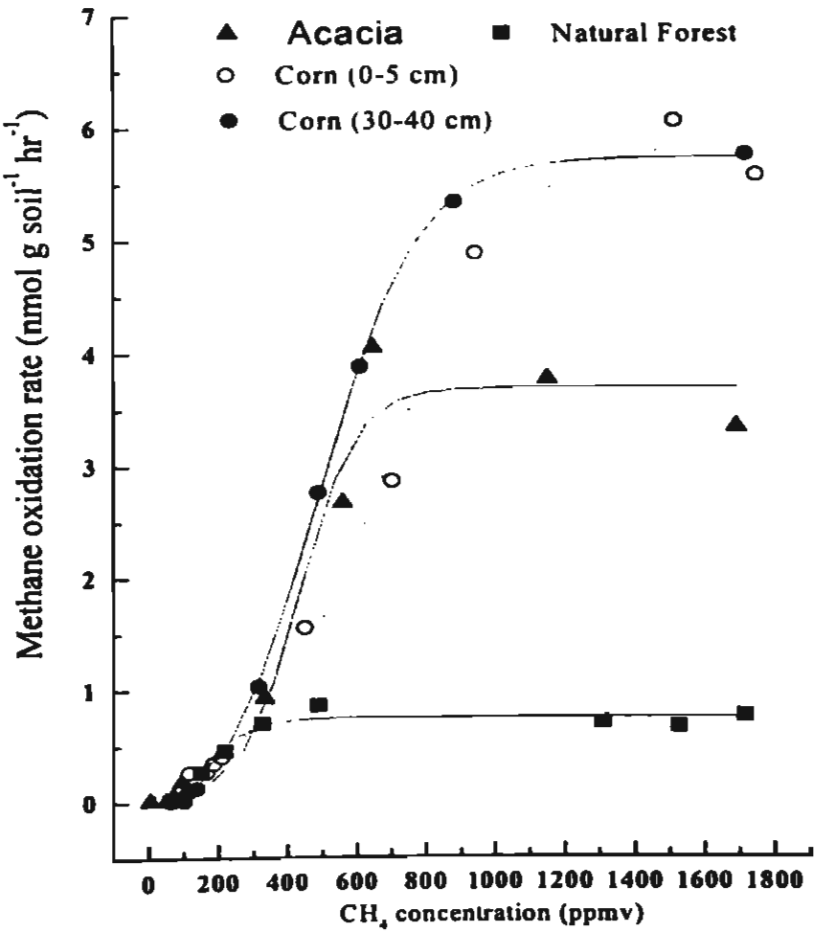


Fig. 25 Kinetic plots for initial methane concentration vs methane oxidation rate. Soil samples used in experiments at SK and AC sites were taken from 10-20, 5-10 cm depth, respectively, while at CF site two depths (0-5 and 30-40 cm) were examined.

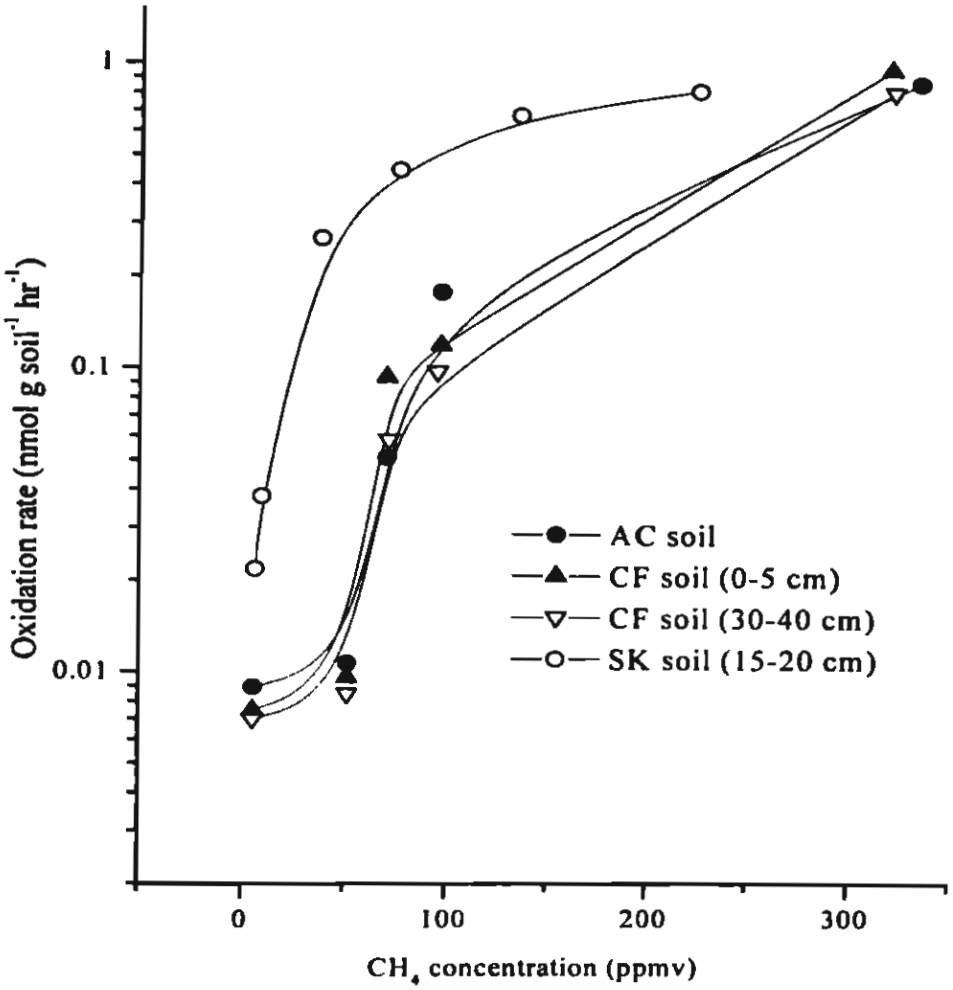


Fig. 26 Exponential plot of relationship between oxidation rate and methane concentration below approximately 350 ppmv.

Table 3 Kinetic coefficients measured for methane oxidation by soil samples taken from different land use types.

Site	V_{max} (nmol g^{-1} hr^{-1})	K_m (ppmv)
SK (10-20 cm)	0.82	52.02
AC (10-20 cm)	5.72	723.96
CF		
0-5 cm	10.66	1454.88
30-40 cm	9.97	2361.76

3.6 Molecular biology aspects of methanotrophs

3.6.1 DGGE

DGGE photographs of the A682b DGGE and the mb661 DGGE are shown in Fig. 27. Black bars indicate the excised bands. More bands were obtained with primer A682b than the primer mb661. Some of these bands (bands 1 and 4) contained neither *pmoA* (encoding a subunit of pMMO) nor *amoA* (encoding a subunit of ammonia monooxygenase). This is due to the fact that primer A682 is not as specific as mb661. For some bands (A682b DGGE) more than one PCR product was identified. This is due to the different procedures applied to identify the bands (cloning, application of different backward primers). These procedures, especially the application of different backward primers, may lead to the detection of "background smear". Therefore, most bands were additionally cloned to identify the PCR product(s). The reverse primer mb661 is more specific for methanotrophs. Each visible band in the DGGE gel represented only one methanotrophic taxon. *Methylocystis* and *Methylobacter* sequences were the only taxa detected with both primer systems, but only in some of the soils (AC and CF, respectively, Table 2). Application of the reverse primer A682b lead to a preferential amplification of *amoA* genes, of genes with sequences which cluster in between those of *pmoA* and *amoA* (= PmoA/AmoA), and of USC α sequences.

3.6.2 Amplification of the pmoA gene (encoding the α subunit of pMMO)

Using cultivation-independent methods, the communities of MB were characterized in soil samples taken from 10 – 20 cm soil depth during the second half of the wet season. Duplicate soil samples were taken from each sampling site. Two different reverse primers were used for the amplification of the *pmoA* gene fragment. Primer A682 is the less specific of the two, since it was designed to amplify both *pmoA* and *amoA* gene fragments (Holmes et al., 1995). Primer mb661 was designed to amplify *pmoA* gene fragments specifically, excluding amplification of *amoA* gene fragment (Costello and Lidstrom, 1999). The *pmoA* gene fragment, used as a functional marker for the characterization of the methanotrophic community, was successfully PCR-amplified from all soil DNA extracts (Fig. 27). A gene fragment of the *mmoX* gene could not be amplified. This is in accordance with results from upland soils from Central Europe (Knief et al., 2003) and

suggests two different explanations. Either the primer systems used are not very sensitive for the detection of *mmoX* genes, or the dominant MB in upland soils do not possess *mmoX* genes. The latter is reasonable considering that MB in upland soils have to live on very low substrate concentrations. Under these oligotrophic conditions it should be more preferable to use pMMO over sMMO, since it has a lower energy demand for the oxidation of methane to methanol. MB expressing pMMO have higher growth yields and show a higher affinity for methane (Hanson and Hanson, 1996).

3.6.3 Detected taxa in soils from different land use

The PmoA sequences related to the nitrifier *Nitrosospira muliformis* was only detected in CF soil (Fig. 28, Table 4). Those closely related to the genera *Methylocyctis*, *Methylobacter*, *Methylococcus*, and distantly related to *Methylocapsa*, were detected. *Methylocystis* sp. (Type II) was not detectable at site SK (Fig. 28, Table 4). However, at site AC it was detected with both primer systems. USC α was represented by band 3 in the A682b DGGE. The USC α is a novel sequence cluster usually called the "fiorest sequence cluseter" and as indicated in this study and previous reported (Bourne *et al.*, 1997, Holmes *et al.*, 1999), it is present only in SK and AC soil samples (For sample AC a "clean" PCR product could not be obtained, therefore this sequence is missing in the phylogenetic tree). The most closely reated pmoA sequence from a pure culture is that of *Methylocapsa acidiphila*, a type II methanotrop isoloated from acidic peat. Interestingly, the soils under forest (both SK and AC) are also highly acidic (pH below 4 between 10 and 20 cm-depth, Fig. 9). This may indicate that this methanotroph prefers the acidic environment. The disappearance of USC α at site CF (a cornfield) confirms the result of Knief *et al.* (2003) and, who did not detect sequences of this cluster in farmlands (correlated with low methane oxidation activity). This also supports the hypothesis that this bacterium prefers an acidic habitat. Primer mb661 does not amplify the *pmoA* gene of the USC α (Bourne *et al.* 2001, this study). But PCR products were obtained with this primer that formed a cluster related to the sequences of USC α . The highest amino acid identity of "Thailand AC3141, band A" and "Thailand SK3141, band B" was 83% to "uncultured bacterium, DGGE band EL4-4 (Fig. 26). The corresponding DGGE band (band 2) was very intensive in soils SK and AC but not detectable at site CF. This correlates with the results for the USC α sequences, obtained by primer A682b. *Methylobacter* sp. sequences were

represented by a visible band in the mb661 DGGE in one of the replicates at all different sites (band1). Band 3-5 of the mb661 DGGE represented a *pmoA* sequence, which indicates for as-yet uncultivated methanotrophs (related to methanotrophic Gammaproteobacteria). The amino acid identity was below 92% to uncultivated bacteria represented by "clone Zhenjiang 5" and "clone Beijyuan 2" (Hoffmann *et al.* 2002) and to the cultivated methanotrophs of the genera *Methylococcus* and *Methylocaldum*. This *pmoA* sequence was present in all samples that were analyzed with mb661. With primer A682b, it was detected at site CF 3141 only (band 6).

3.6.4 Community composition as revealed by DGGE analysis

The DGGE banding patterns from the three different sampling sites indicated for a very similar community structure within one sampling site. But there were clear differences in the community composition of the different sampling sites. Based on the DGGE banding pattern of the two gels, a cluster analysis was performed with SYSTAT version 9 (SPSS Inc., Richmond, Calif.). The presence or absence of a band in each sample was used to calculate Jaccard dichotomy coefficients. These coefficients were used to calculate a hierarchial tree based on average linking and Euclidean distances.
The resulting tree confirmed the similarity of samples from one sampling site and differences between the different sampling sites (Fig. 29). All trees indicate for higher similarity between SK and AC than between SK and AC to CF. The tree includes all different detected sequences from each sampling site (with one exception, see above). Band names shown in capitals indicate for *pmoA* sequences obtained from the mb661 DGGE, while the others represent the bands of the A682b DGGE. There are three sequences added (methanotrophic isolates arc1, arc2, and sakb1), which belong to methanotrophic isolates, obtained from the soil samples. *3.6.1 DGGE.*

The detection of the third cluster representing uncultivated bacteria, Cluster 2, has as yet been restricted to upland soils. Since the sequences of this branch are only distantly related to PmoA sequences of recognized genera of MB, it cannot be excluded that these sequences code for the AmoA of uncultivated ammonia-oxidizing bacteria. However, the growth of the organisms harboring these sequences was possibly stimulated in sieved soil samples after incubation under elevated methane mixing ratios (Knief, unpublished data). In this study, only *Methylocystis* spp. were isolated. These isolates probably did not represent the dominant *Methylocystis* spp. in the soil samples, since PmoA sequences

identical to those of the isolates were not detected by cultivation-independent methods in the soil samples.

The different land-use of the sampling sites was reflected by different methane uptake rates and different soil pHs, especially for the cornfield soil. The characterization of the methanotrophic community indicated that the community composition reflected the land use change as well. The *pmoA* sequences of USC α and Cluster 5 were detected in samples from sites SK and AC, but not in the soil samples from the cornfield. The absence of USC α at site CF is in agreement with the results of other studies, in which the methanotrophic community of some farmland soils was analyzed and in which USC α sequences were not detected, although they were present in adjacent grassland and forest soils (Knief et al., 2003). The pH shift in the farmland soil is probably not the reason for the disappearance of USC α sequences at this sampling site. The organisms harbouring USC α sequences, although they appear to occur more frequently in acidic than in pH-neutral soils, do occur in soils of a wide pH range (4.0 – 8.0). Further sequences that led to the different community compositions of the different sampling sites were those of *Methylocystis* spp., Cluster 2 and of ammonia-oxidizing bacteria (Table 4). Sequences of *Methylobacter* and Cluster 3 were detected at all sampling sites. A cluster analysis was used to compare the degree of similarity across sites among the communities of MB plus ammonia-oxidizing bacteria. The resulting tree confirmed the high similarity of replicate samples from one sampling site and differences between the different sampling sites (Fig. 29). The differences within the replicate samples from one site were due to faint DGGE bands visible in one sample but not in the other ones. Thus, *pmoA* sequences with abundance near the detection limit of the methods were mainly responsible for the dissimilarity of duplicate samples. The main purpose of this cluster analysis was the comparison of the different sampling sites. The community composition of SK and AC sites were more similar to each other than either was to CF site. This finding is in accordance with the methane uptake rates and pHs of the sampling sites, which also indicated a high degree of dissimilarity of site CF compared to sites SK and AC.

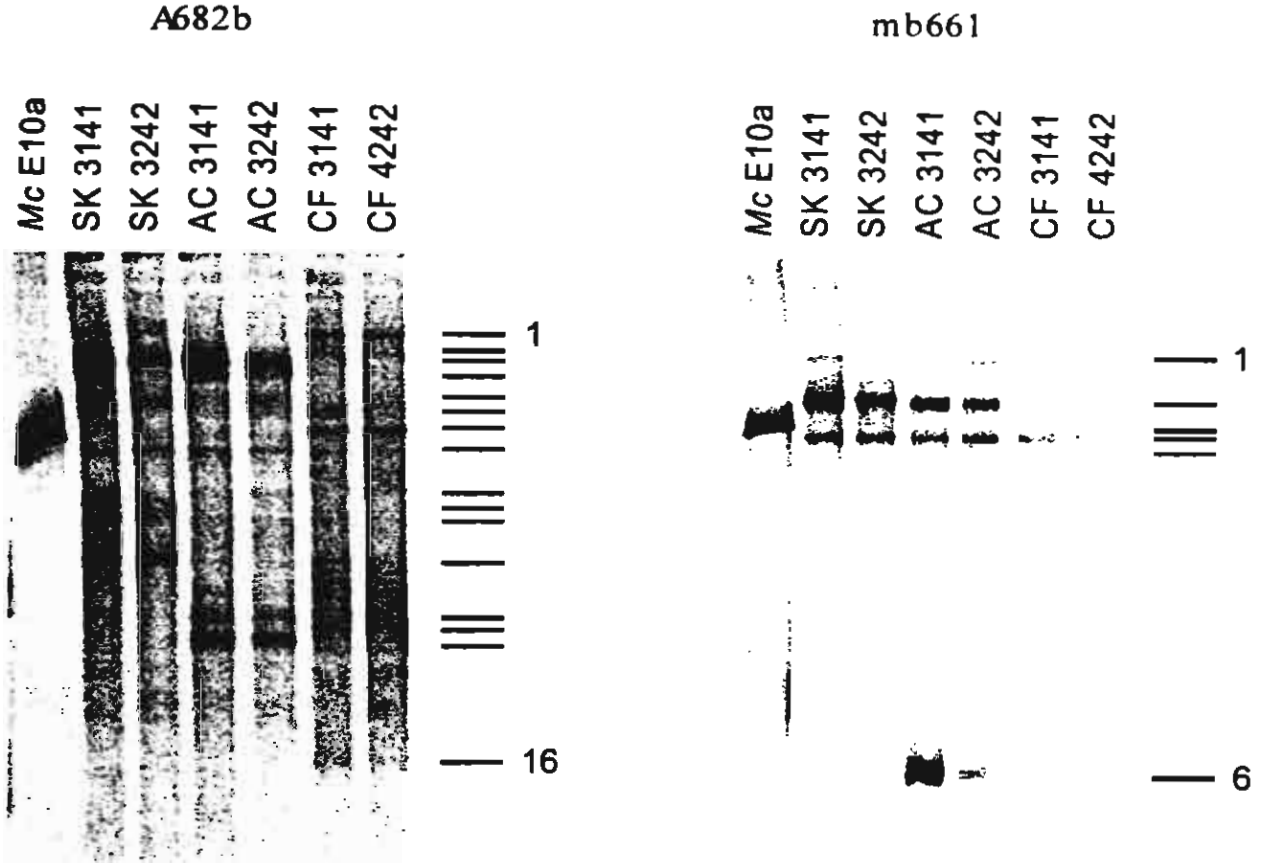
These data demonstrate clearly that land use changes are not only connected to changes in the methane uptake capacity of soils, but also to changes in the community composition of methane-oxidizing bacteria. The change in the community composition may be an important factor leading to the general decrease in the methane uptake rate of farmland soils. The methanotrophic communities of replicate samples within one sampling

site were highly similar, although methane uptake rates varied over space and time. However, several other factors influence the methane uptake rate of a soil. The water content is an important factor, since it heavily influences gas diffusion in the soil (Keller et al., 1993). Whether and how the community of MB responds to changes in the water content of a soil, remains to be studied.

The community composition of the reforested site AC represents a kind of intermediate state between the communities at sites SK and CF. Since it is unknown how the community composition and methane uptake activity of the reforested site AC looked before the site was reforested, we cannot conclude whether the community we see today has redeveloped or whether the community structure was not as much changed after deforestation. During this period this site was a farmland like site CF, so it is likely that the MB community was different at this time.

Table 4. Detected taxa of methanothrophic bacteria in soils taken form different land use.

	SK 3141 and SK 3242		AC 3141 and AC3242		CF 3141 and CF 3242	
	A682b	mb661	A682b	mb661	A682b	mb661
Methylocystis sp.			+	+	+	
USC α	+		+			
related to USC α		+		+		
Methylobacter sp.		+		+	+	+
related to Gammaproteobacteria.		+		+		+
PmoA / AmoA	+				+	
nitrifier (*amoA*)					+	



A682 bands (top down):

1 no PmoA or AmoA (CF)
2 PmoA / AmoA and *Methylocystis* (CF)
3 PmoA / AmoA (SK) and USC α (SK + AC)
4 no PmoA or AmoA (AC)
5 USC α (SK)
6 nitrifier, USC α and related to Gammaproteobacteria (CF, 2 bands)
7 PmoA / AmoA and *Methylocystis* (CF)
8 USC α (SK) and *Methylocystis* (AC)
9 PmoA / AmoA (SK)
10 PmoA / AmoA (SK)
11 PmoA / AmoA and USC α (SK)
12 USC α (SK), PmoA / AmoA, *Methylocystis* and nitrifier (CF)
13 *Methylocystis* (CF, 2 bands)
14 nitrifier (AC)
15 *Methylocystis* (AC)
16 *Methylocystis* (AC)

mb661 bands (top down):

1 *Methylobacter* (SK, AC, CF)
2 related to USC α (SK, AC)
3 related to Gammaproteobacteria (SK)
4 related to Gammaproteobacteria (SK, AC, CF)
5 related to Gammaproteobacteria (CF)
6 *Methylocystis* (AC)

Fig. 27 DGGE bands obtained with the primer A6682 and mb661.

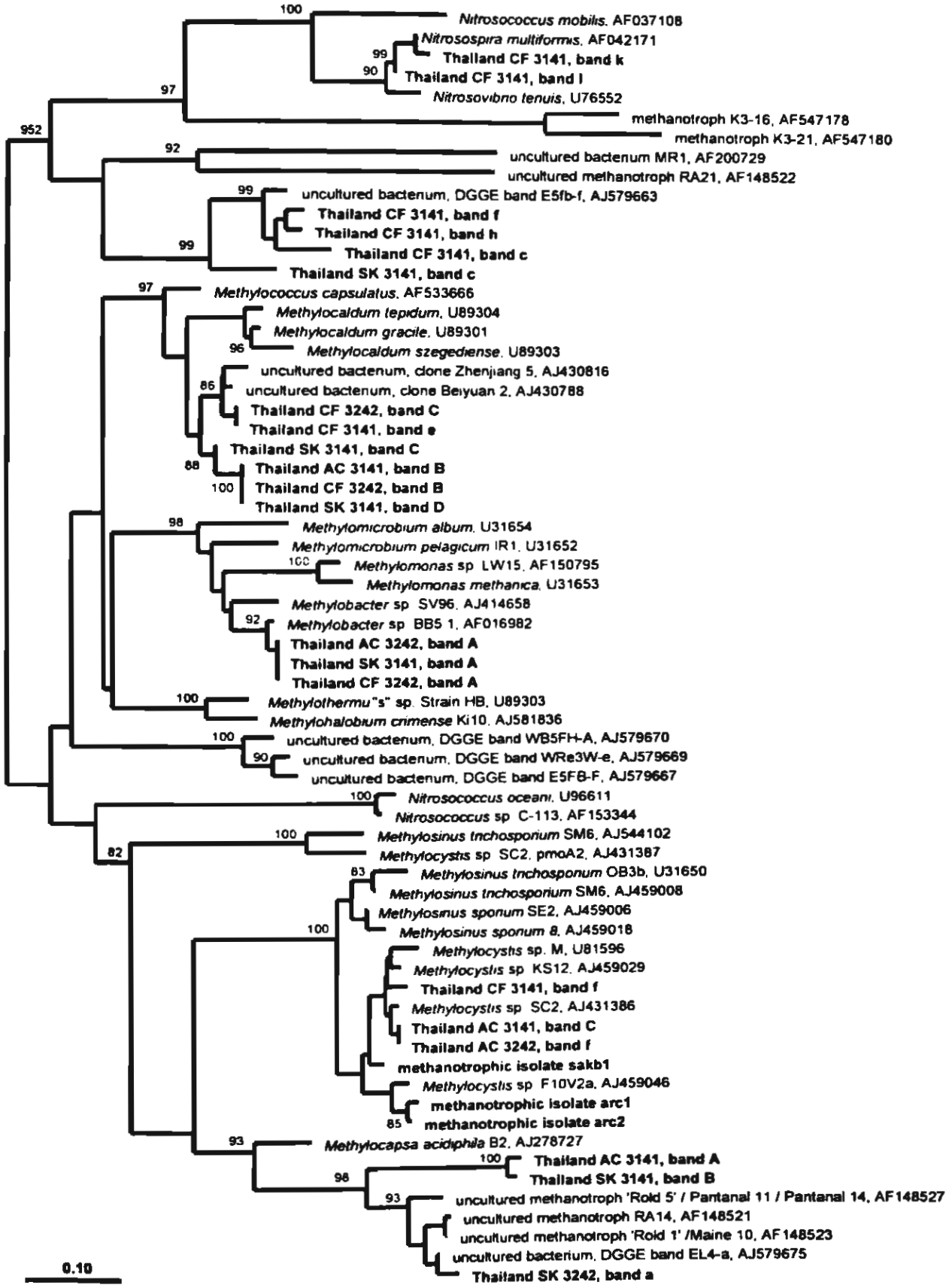


Fig. 28 Phylogenic tree constructed from amino acid sequences of partial pmoA retrived from native forest, forest-replanted (*A. magium*) and corn cultivated soils and public-domain PmoA and AmoA sequences. The scale bar indicates the estimated numbers of base change per nucleotide sequence position.

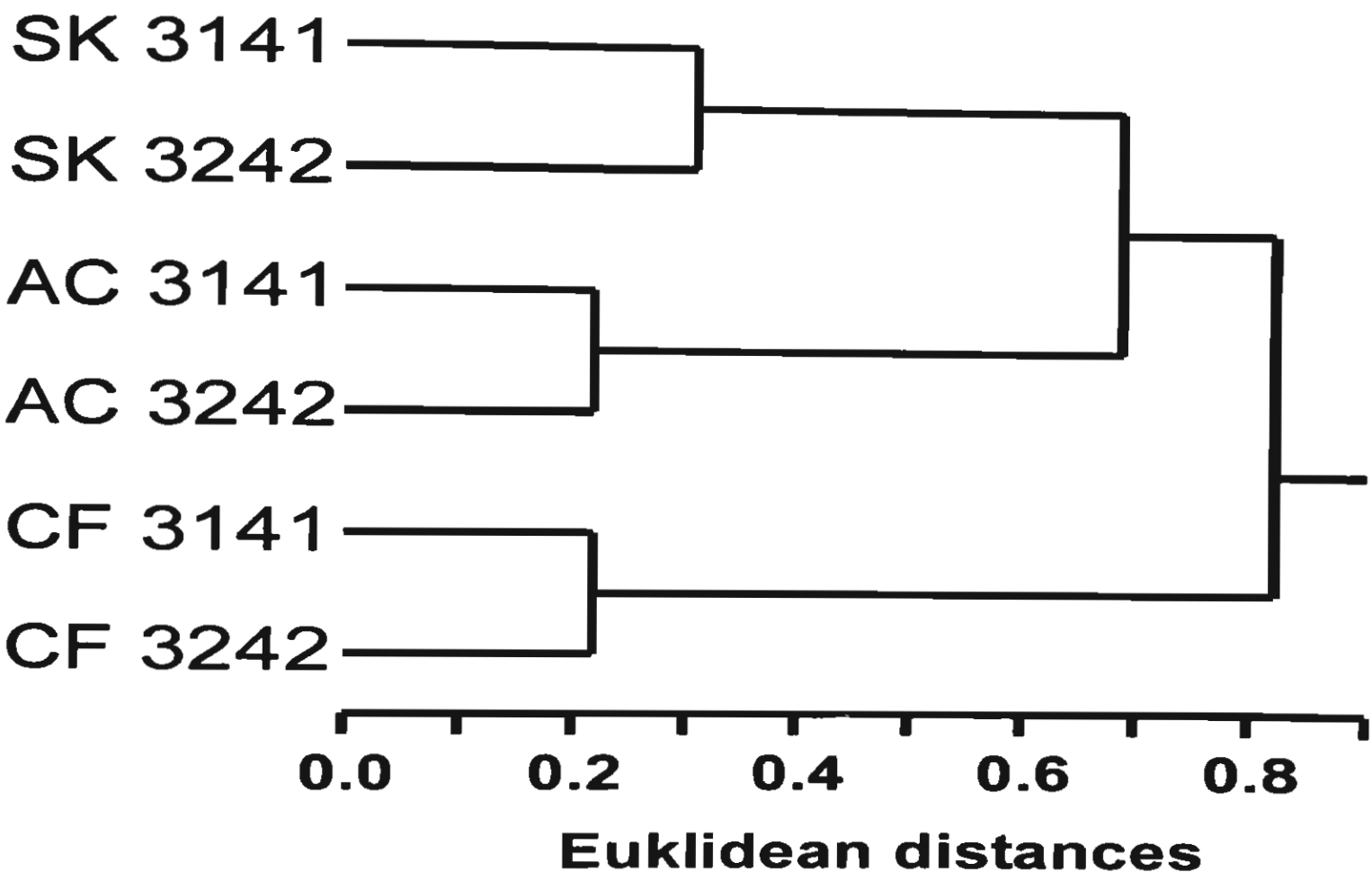


Fig. 29 Cluster analysis based on the presence or absence of 21 bands of DGGE gels from PCR products obtained with primer systems A189-GC/A682 and A189-GC/mb661. Jaccard similarity coefficients were calculated and a hierarchical tree was constructed based on average linking and Euclidean distances.

CHAPTER 4

GENERAL DISCUSSION AND CONCLUSIONS

4.1 Effects of land use on methane oxidation

The results obtained in this study clearly show that capacity of methane oxidation is strongly affected by land use type. Although characteristics of soil such as soil texture, bulk density and pH affects methane oxidation, but land use could mask the effects of such intrinsic soil properties. For example, soil pH (mild acidic) and soil texture (Sandy soil) in CF soil is supposed to be more suitable for methane oxidation than forest soils (strongly acidic with high clay content). However, methane oxidation rates in both forest soils are significantly higher than in CF soils. Thus, effects of intrinsic soil properties on methane oxidation may be comparable only among the same land use type, such as in forest of SK and AC soils. The reason for such differences in methanotrophic activity upon changing land use can be traced back to the fundamental unit responsible for dynamics of methane in the ecosystems; the activity and composition microbial community.

Three main groups of microorganisms involve in the dynamics of methane in a given ecosystem. These include methane-oxidizing bacteria (methanotrophs), methane-producing bacteria (methanogens) and nitrifying bacteria. Interactions among these groups of bacteria determine whether the ecosystem under consideration is the net sink or source of methane. In upland soil with high water draining characteristics, methanotrophs become dominant and such ecosystem usually acts as the net atmospheric methane sink. However, fertilizing the soil under crop cultivation usually lead to the dominant of nitrifying bacteria and thus resulting in loss of methanotrophic characteristics. Such ecosystem could become either sink or source of methane depending on the environment factors. In the poor-drained soil, methanogenesis prevails and the system could become the net sources of atmospheric methane. Which bacterial group will become dominant is, thus, largely affected by the pattern of land use and land management.

Changes of some soil physical and chemical properties upon changing land use such as water permeability may induce anaerobic condition thereby methanogenesis develop. This is especially obvious in the raining season in CF soil. It is apparent that high water content was found mainly in CF soil especially in the subsurface. Such

conditions lead to methane production and the soil become the net source of atmospheric methane; namely methanogenesis become the dominant process in determining methane dynamics at that time.

Within the similar land use type such as between SK and AC soils, the results show that methanotrophic activity is similar. However, here the effects of soil characteristics become obvious. More sand fraction in AC soil relatively to SK soil (thus higher water permeability) may result in a consistent characteristics of net methane sink at this site. However, during the dry months (Jan-April) SK soil was the most active methane oxidation and methane oxidation in AC soil was less than SK by 10-36%. However, when the rain season began, AC took over oxidation and in some months SK turns into net methane source (June and July). Presumably, the high clay content of SK soil with poor water permeability allowed anoxic conditions to be developed within the soil profile. From the result of kinetic study it is reveal that if concentration of methane is too high, SK site cannot oxidize it. Mthanogenic activity may have been started in such condition, thereby produced high about of methane that exceeded the oxidizing capacity of SK soil. Thus, while SK is active methane sink during the summer months, disturbances and changes in environment conditions could easily change its characteristic as either source or sink. This indicates how delicate the natural forest soil is regarding to dynamics of methane. On the other hand, experiencing both human disturbances and recovering on the way to return to natural forest, the re-grown AC forest may have higher buffer capacity to encounter various levels of methane concentrations. It is thus assumed that such soil may have ability to oxidize both methane at low and high levels. It is noted that although AC soil shows the active atmospheric methane oxidation, but its kinetic characteristics are far different from SK soil. Thus, AC still inherits soil characteristics of cultivated soil once it had been about 16 years ago. Biphasic characteristics of reaction kinetics shown in Fig. 25 indicate the presence of both high and low affinity of methanotrophic communities. Thus, affinity of methanotrophs to methane changes according to environmental conditions. In the other words, threshold concentration of methanotrophic bacteria is changed by land use change.

It is interesting to note that in forest soils the active layer of methane oxidation lies below the surface. High concentration of inorganic nitrogen compounds in the topsoil may be the reason. However, rate of methane oxidation in the subsoil would be limited by diffusion of both methane and oxygen. It is thus assumed that methanotrophs in the subsoil are substrate limited and oxidation should increase upon exposing to high

concentration of methane. This is true for AC soil but surprisingly rate of methane oxidation in SK soil does not increase at elevated concentration of methane. Methanotrophs present in subsoil may well adapt to low methane concentration, making them good at consuming low methane concentration and easily saturated with methane at high concentration as observed in the forest soils.

4.2 Role of forest in Thailand as methane sink

Assuming that the annual average of methane oxidation obtained in the present study is valid and a rough representative for methane oxidation capacity of forest soil in Thailand, total atmospheric methane consumption by forest areas in Thailand can be estimated. To do this, the average oxidation rate from both SK and AC sites is multiplied with the total area of forest in Thailand in 1985 and 1995. This estimation indicates that total methane uptake by forest areas in Thailand (natural and regrown forests) is estimated at 61.12 Gg yr^{-1} in 1995. About 10% reduction in total methane uptake due to decrease in forest area between 1985 and 1995 (Table 5) was observed. Although the estimated amount of methane oxidation by forest soils makes up a small portion of total emission in Thailand in 1994 (which is 3.17 Tg), methane uptake by forest could cancel out the amount emitted from forestry section such as by biomass burning (which is estimated at 59.57 Tg in 1994, Ministry of Science, Technology and Environment, 2000). The estimated uptake by Thai forest, in addition, is more than two times higher than the amount of methane emitted by waste section (35.22 Gg in 1994). Thus, methane oxidation by forest soil alone could help improve the net methane released by Thailand. It is noted that this is only a preliminary estimate and accuracy of such estimate cannot be assessed due to small number of measurements and large temporal and spatial variations of methane emission. To improve accuracy and reliability of methane sink estimate by biological oxidation, more studies on relationship between environmental factors and methane oxidation rate, variations of oxidation in different types of forests and other aspects are necessary.

Table 5 Estimate of methane uptake by forest soil in Thailand in 1985 and 1995 using the averaged uptake rate of natural and re-grown forest obtained in the present study.

Year	Forest area ($\times 10^6$ ha)	Methane uptake ($GgCH_4\ yr^{-1}$)*
1985[1]	15.09	66.75
1995	13.83	61.17
-natural forest[1]	13.15	58.16
-reforestation (1997)[2]	0.68	3.01

[1]Royal Forest Department, 1997
[2] Luangjame, 1997 (cited in Thailand's National Greenhouse Gas Inventory 1994)
* Using the average uptake rate of 1.21 $mgCH_4\ m^{-2}\ day^{-1}$

4.3 Conclusions

The results reveal that forest soils in Thailand have high potential to oxidize atmospheric methane. The oxidation rates fall within the range reported earlier and generally higher than those observed in European forests (Table 1). The oxidation varied according to seasons, land use types and sampling locations. The main findings can be concluded as followed.

1. High rate of methane oxidation was found during the summer months. On the other hand, low oxidation rate was found during the rainy months. Natural forest soils show the high oxidation capacity in summer but sometimes it becomes net carbon sources in wet season. In corn cultivated soil, however, only occasionally that net methane consumption was observed. From these results it can be concluded that (1) undisturbed forest has the highest atmospheric methane oxidation capacity, (2) reforestation to *Acadia* recovers the methane oxidation capacity of soils, and (3) land-use change to agriculture (corn plantation in this case) results in loss of the methane oxidation capacity of soil.

2. Methane oxidation in all three sampling sites is associated with large seasonal variations. Similarly, variations in oxidation rates among different chambers during each sampling time were also large, especially at CF site where both net emission and oxidation were observed.

3. Along the soil profile of forest soils, the most active oxidation layer in most cases is between 15-40 cm. Results of kinetic study reveal that soil at SK has highest affinity for atmospheric methane, followed by soil at AC and CF sites, respectively.

4. The phylogenic tree illustrating composition of methanotrophic community was constructed based on the partial sequences of amino acid of *pmoA* gene (encoding the a subunit of pMMO which is present in all methanotrophs) and of *amoA* gene (encoding the subunit of AMO). The PmoA sequences related to the nitrifier *Nitrosospira muliformis* was only detected in CF soil. The PmoA sequences of methanotrophs those closely related to the genera *Methylocyctis*, *Methylobacter*, *Methylococcus*, and distantly related to *Methylocapsa*, were detected. In addition, the results show that there is a clear difference in methanotrophic community according to land use type. For example, *Methylocystis* was detected in only CF and AC soils but not SK soil. The USC α (a novel sequence cluster usually called the "fiorest sequence cluseter") is present only in SK and AC soil samples. All trees indicate for higher similarity between SK and AC than between SK and AC to CF.

5. Some chemical and physical characteristic of soil such as nitrogen content, soil pH along with soil profile, seasonal and spatial variations in soil moisture along soil profile and during the study period, soil temperatures at 2 and 5 cm and air temperature at all sampling sites were measured. High content of nitrate and ammonium was observed in the top layers of both forest soils and their concentrations in the soil below 20 cm were low. Soil pH under forest (native and replanted) exhibited the highly acidic properties, with the pH in the surface soil as low as 2.4. Contrarily, pH of corn-cultivated soil was higher than forest soil but still in the acidic region. In addition, different land use types significantly affect the water regime and temperature characteristics of soils.

REFERENCES

1. Adamsen A.P.S. and King G.M. 1993. Methane consumption in temperate and subarctic forest soil: rates, vertical zonation, and responses to water and nitrogen. Appl. Environ. Microbiol., 59: 485-490.Ambus P. and Christensen S. 1995. Spatial and seasonal nitrous oxide and methane fluxes in Danish forest, grassland and agroecosystems. J. Environ. Qual., 24: 993-1001.
2. Ambus P. and Christensen S. 1995. Spatial and seasonal nitrous oxide and methane fluxes in Danish forest, grassland and agroecosystems. J. Environ. Qual., 24: 993-1001.
3. Auman A. J., S. Stolyar A. M. Costello and M. E. Lidstrom. 2000. Molecular characterization of methanotrophic isolates from freshwater lake sediment. Appl. Environ. Microbiol. 66:5259-5266.
4. Bender M. and Conrad R. 1994. Methane oxidation activity in various soils and freshwater sediments: occurrences, characteristics, vertical profiles, and distribution on grain size fractions. J. Geophys. Res., 99: 16531-16540.
5. Bender M. and Conrad R. 1995. Effects of methane concentrations and soil conditions on the induction of CH_4 oxidation activity. Soil Biol. Biochem., 27: 1517-1527.
6. Blake G. R. and K. H. Hartge. 1986. Bulk density. p. 363-375. *In:* A. Klute et al. (ed.) Methods of soil analysis: Part 1: Physical and Mineralogical Methods. Monograph Number 9 (Second Edition). ASA, Madison, WI.
7. Bradford M.A., Ineson P., Wookey P.A. and Lappin-Scott H.M. 2001. Role of methane oxidation, production and transportation in forest soil methane flux. Soil Biology & Biochemistry, 33: 1625-1631.
8. Born M., Doerr H. and Levin I. 1990. Methane consumption in aerated soils of the temperate zone. Tellus, 42B: 2-8
9. Bourne D. G., McDonald I. R. and. Murrell J. C. 2001. Comparison of *pmoA* PCR primer sets as tools for investigating methanotroph diversity in three Danish soils. Appl. Environ. Microbiol., 67:3802-3809.

10. Brumme R. and Borken W. 1999. Site variation in methane oxidation as affected by atmospheric deposition and type of temperate forest ecosystem. Global Biogeochem. Cycles, 13: 493-501.
11. Butterbach-Bahl K., Gasche R., Huber C.H., Kreutzer K. and Papen H. 1998. Impact of N-input by wet deposition on N-trace gas fluxes and CH4 oxidation in spruce forest ecosystem of the temperate zone in Europe. Atmos. Environ., 32: 559-564.
12. Castro M.S., Melillo J.M., Steudler P.A. and Chapman J.W. 1994. Soil moisture as a predictor of methane uptake by temperate forest soils. Can. J. Forest Res., 24: 1805-1810.
13. Castro M.S., Steudler P.A. and Melillo J.M. 1995. Factors controlling atmospheric methane consumption by forest soils. Global Biogechem. Cycles, 9: 1-10.
14. Conrad R. 1996. Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O and NO). Microbiol. Rev., 60: 609-640.
15. Costello A. M. and M. E. Lidstrom. 1999. Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments. Appl. Environ. Microbiol. 65:5066-5074.
16. Dlugokencky, E.J., Houweling, S., Bruhwiler, L., Masarie, K.A., Lang, P.M., Miller, J.B., Tans, P.P. 2003. Atmospheric methane levels off: Temporary pause or a new steady-state? Geophys. Res. Lett. 30: 1-4
17. Dobbie K.E. and Smith K.A. 1996. Comparison of methane oxidation rates in woodland, arable and set aside soils. Soil Biol. Biochem., 28: 1357-1365.
18. Dobbie K.E., Smith K.A. Prieme A., Christensen S., Degorska A. and Orlanski P. 1996. Effect of land use on the rate of methane uptake by surface soils in northern Europe. Atmos.Environ., 30: 1005-1011.
19. Dörr H., Katruff L. and Levin I. 1993. Soil texture parameterization of the methane uptake in aerated soils. Chemosphere, 26:697-713.
20. Dunfeild P., Knowles R., Dumont R. and Moore T.R. 1993. Methane production and consumption in temperate and subarctic peat soils: response to temperature and pH. Soil Biol. Biochem., 25: 321-326.
21. Duxbury J.M. 1994. The significant of agricultural sources of greenhouse gases. Fertil. Res., 38: 151-163.

22. Flessa H., Doesch P., Beese F., Koenig H., and Bouwman A.F. 1996. Inflence of cattle wastes on nitrous oxide and methane fluxes in pasture land. J. Environ. Qual., 25: 1366-1370.

23. Gee G. W. and Bauder J. W.. 1982. Particle-size analysis. p. 383-411. *In:* A. Klute (ed.) Methods of soil analysis: Part 1. Physical and mineralogical methods. ASA Monograph Number 9.

24. Goldman M.B., Groffman P.M., Pouyat R.V., McDonald M.J. and Pickett S.T.A. 1995. CH_4 uptake and N availability in forest soils along an urban to rural gradient. Soil Biol. Biochem., 27: 281-286.

25. Gulledge J. and Schimel J.P. 1998. Low-concentration kinetics of atmospheric CH4 oxidation in soil and mechanism of NH4+ inhibition. Appl. Environ. Microbiol., 64: 4291-4298.

26. Hanson R.S. and Hanson T. E. 1996. Methanotrophic bacteria. Microbiol. Rev., 60: 439-471.

27. Henckel T., Friedrich M. and Conrad R. 1999. Molecular analyses of the methane-oxidizing microbial community in rice field soil by targeting the genes of the 16S rRNA, particulate methane monooxygenase, and methanol dehydrogenase. Appl. Environ. Microbiol., 65: 1980-1990.

28. Henckel T., Jäckel U., Schnell S. and Conrad R. 2000. Molecular analyses of novel methanotrophic communities in forest soil that oxidize atmospheric methane. Appl. Environ. Microbiol., 66: 1801-1808.

29. Hoffmann T., Horz H.P., Kemnitz D., and Conrad R. 2002. Diversity of the particulate methane monooxygenase gene in methanotrophic samples from different rice field soils in China and the Philippines. Systematic and Applied Microbiology 25:267-274.

30. Holmes, A.J., Costello, A., Lidstrom, M.E., Murrell, J.C. 1995. Evidence that particulate methane monooxygenase and ammonia monooxygenase may be evolutionarily related. FEMS Microbiol. Lett. 132: 203-208

31. Holmes, A.J., Roslev P., McDonald I.R., Iversen N., Henriksen K. and Murrell J.C. 1999. Characterization of methanotrophic bacterial population in soils howing

atmospheric methane uptak. Applied and Environmental Microbiology, 65: 3312-3318.

32. Hütsch B.W, Webster C.P. and Powlson D.S. 1994. Methane oxidation as affected by land use, soil pH and nitrogen ferilization. Soil Biol.Biochem., 26: 1613-1622.
33. Hütsch B.W, Russel P. and Menkel K. 1996. CH4 oxidation in two temperate arable soils as affected by nitrate and ammonium application. Biol. Fertil. Soils, 23: 86-92.
34. Hütsch B.W. 2001. Methane oxidation in non-flooded soils as affected by crop production-invited paper. Europ. J. Agronomy, 14: 237-260.
35. Ishizuka S., Sakata T. and Ishizuka K. 2000. Methane oxidation in Japanese forest soils. Soil Biol. Biochem., 32: 769-777.
36. Iwamoto, T., K. Tani, K. Nakamura, Y. Suzuki, M. Kitagawa, M. Eguchi, and M. Nasu. 2000. Monitoring impact of in situ biostimulation treatment on groundwater bacterial community by DGGE. FEMS Microbiol. Ecol. 32:129-141.
37. Kassavalou A., Mosier A. R., Doran J.W. Drijber R.A., Lyon D.J. and Heinemeyer O. 1998. Fluxes of carbon dioxide, nitrous oxide, and methane in grass sod and winter wheat-fallow tillage management. J. Environ. Qual., 27: 1094-1104.
38. Keller M., Mitre M. E. and Stallard R.F. 1990. Consumption of atmospheric methane in soils of Central Panama: effects of agricultural development. Global Biogeochem. Cycles, 4: 21-27.
39. Keller M., Veldkamp E., Weitz A.M. and Reiners W.A. 1993. Effect of pasture age on soil trace-gas emissions from a deforested area of Costa Rica. Nature 365: 244-246
40. Keller M. and Reiners W.A. 1994. Soil-atmospheric exchange of nitrous oxide, nitric oxide and methane under secondary succession of pasture to forest in the Atlantic lowlands of Costa Rica. Global Biogeochem. Cycles, 8: 399-409.
41. King G.M. 1992. Ecological aspects of methane oxidation; a key determinant of global methane dynamics. Adv. Microb. Ecol., 12: 432-468.
42. Knief C., Lipski A. and Dunfield P. F. 2003. Diversity and activity of methanotrophic bacteria in different upland soils. Applied and Environmental Microbiology 69:6703-6714.

43. Koschorreck M. and Conrad R. 1993. Oxidation of atmospheric methane in oil: measurements in the field, in soil cores and in soil samples. Global Biogeochem. Cycles, 7: 109-121.

44. Lessard R., Rochette P., Topp E., Pattey E., Desjardins R.L. and Beaumont G. 1994. Methane and carbon dioxide fluxes from poor drained adjacent cultivated and forest sites. Can. J. Soil Sci., 74: 139-146.

45. Macdonald J.A., Skiba U., Sheppard L.J., Hargraves K.J., Smith K.A. and Fowler D. 1996. Soil environmental variables affecting the flux of methane from a ragne of forest, moorland and agricultural soils. Bigeochemistry, 34: 113-132.

46. McDonald I. R., and Murrell J. C.. 1997. The particulate methane monooxygenase gene *pmoA* and its use as a functional gene probe for methanotrophs. FEMS Microbiol. Lett. 156:205-210.

47. Ministry of Science, Technology and Environment, Thailand. 2000. Thailand's National Greenhouse Gas Inventory 1994. 118 pp.

48. Mosier A.R., Schimel D., Valentine D., Bronson K. and Parton W. 1991. Methane and nitrous oxide fluxes in native, fertilized and cultivated grasslands. Nature, 350: 330-332.

49. Mosier A.R. and Schimel D. 1991. Influence of agricultural nitrogen on atmospheric methane and nitrous oxide. Chem. Ind., 23: 874-877.

50. Ojima D.S., Valentine D.W., Mosier A.R., Parton W.J. and Schimel D.S. 1993. Effect of land use change on methane oxidation in temperate forest and grassland soils. Chemosphere, 26: 675-685.

51. Phopinit S. And Limtrakul K. 1999. Change of soil proporties after 10 years forest plantation. Silvicultural Research Report 1990, Division of Silviculture Research, Office of Forest Technique Advisor, Royal Forest Department, Thailand. pp. 80-102.

52. Reay D.S., Radakewski S., Murrell J.C., McNamara N. and Nedwell DB. 2001. Effects of land-use on the activity and diversity of methane oxidizing bacteria in forest soils. Soil Biology & Biochemistry. 33:1613-1623.

53. Reeburgh W.S., Hirsch A.I., Sansone F.J., Popp B.N. and Rust T.M. 1997. Carbon kinetic isotope effect accompanying microbial oxidation of methane in boreal forest soils. Geochim. Cosmochim. Acta, 61: 4761-4767.

54. Roslev P., Iversen N, and Henriksen K. 1997. Oxidation and assimilation of atmospheric methane by soil methane oxidizers. Applied and Environmental Microbiology 63:874-880.

55. Royal Forest Department. 1997. Forestry Statistics of Thailand 1997. Information Office, Royal Forest Department, 149 pp.

56. Ruser R., Flessa H., Schilling R., Steindl H., and Beese F. 1998. Soil compaction and fertilization effects on nitrous oxide and methane fluxes in potato fields. Soil Sci. Soc. Am. J., 62: 1587-1595.

57. Sakurai K., Tanaka S., Ishizuka S. and Kanzaki M. 1998. Difference in soil properties of dry evergreen and dry deciduous forests in the Sakaerat Environmental Research Station. *Tropics*, 8: 61-80.

58. Sakurai K., Puriyakorn B., Preechapanya P., Tanpibal V., Muangnil K., and Prachaiyo B. 1995. Inprovement of biological productivity in degraded lands in Thailand. III. Soil hardness measurement in the field. *Tropics*, 4: 151-172.

59. Singh J.S., Singh S., Raghubanshi A.S., Singh S., Kashyap A.K. and Reddy V.S. 1997. Effect of soil nitrogen, carbon, and moisture on methane uptake by dry tropical forest soils. Plant and Soil, 196: 115-121.

60. Situala B.K., Bakken L.R. and Abrahamsen G. 1995. CH4 uptake by temperate forest soils: effect of N input and soil acidification. Soil Biol. Biochem., 27: 871-880.

61. Steudler P.A., Bowden R.D., Melillo J.M. and Aber J.D. 1989. Influence of nitrogen fertilization on methane uptake in temperate forest soils. Nature, 341: 314-316.

62. Strunk O., Gross O., Reichel B., May M., Hermann S., Stuckman N., Nonhoff B., Lenke M., Ginhart A., Vilbig A., Ludwig T., Bode A., and Schleifer K. H.. 1998. ARB: a software environment for sequence data. Technische Universität München, Munich.

63. Van der Weenden T.J. Sherlock, R.R., Williams, P.H. and Cameron K.C. 1999. Nitrous oxide emission and methane oxidation by soil following cultivation of two different leguminous pastures. Biol. Fertil. Soils., 30: 52-60.

64. Wacharakitti S., Intrachanda S. and Mungkorndin S. 1980. Natural resources and land use studies of Sakaerat Environmental Research Station. Forest Research Bulletin 69. Kasetsart University.

65. Whalen S.C. and Reeburgh W.S. 1990. Consumption of atmospheric methane by tundra soils. Nature, 346: 160-162.

66. Willison T.W., Webster C.P., Goulding K.W.T. and Powlson D.S. 1994. Methane oxidation in temperate soils; effects of land use and the chemical forms of nitrogen fertilizer. Chemosphere 30: 546-549.

Appendix

Manuscripts for publication

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Abstract for Conference

Manuscript #1: Diversity of methanotrophic bacteria in tropical upland soils under different land use

Journal: Applied and Environmental Microbilogy

Manuscript #2: Kinetics of methane oxidation in tropical upland soils under different land use

Journal: FEMs Microbilogy Ecology

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Diversity of methanotrophic bacteria in tropical upland soils under different land use

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Running title: methanotrophic communities in tropical upland soils

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Abstract

Three upland soils from Thailand; a natural forest, a reforested site, and an agricultural field, were studied with regard to methane uptake and the community composition of methanotrophic bacteria (MB). Methane oxidation rates were lower in the agricultural soil than in the native forest or reforestedsites. Sites also differed in the community composition of MB, which was characterized by denaturing gradient gel electrophoresis (DGGE) of *pmoA* gene fragments (encoding for a subunit of particulate methane monooxygenase) and PCR-amplified from total soil DNA extracts. Cluster analysis based on the DGGE banding patterns indicated that the MB community of the forested and reforested sites were similar to each other but different from that of the farmland site. Sequence analysis of excised DGGE bands indicated that *Methylobacter* spp. and *Methylocystis* spp represented genera of cultivated MB at the sampling sites. Sequences of upland soil cluster α (USC α), that has been suggested to represent organisms involved in atmospheric methane consumption in diverse soils, were detected in the native forest and reforested site. Such sequences formed a separate branch related to USC α. Other sequences that indicated the uncultivated groups of potential MB were related to methanotrophic Gammaproteobacteria or an unknown sequence cluster that may represent either *pmoA* or *amoA* (coding for a subunit of the ammonia monooxygenase of ammonia oxidizing bacteria).

Introduction

The current atmospheric mixing ratio of the greenhouse gas methane (CH_4) is 1.75 ppmv [1]. An estimated 30 Tg of CH_4 $year^{-1}$ is consumed via microbiological oxidation in upland soils, accounting for about 6% of the global methane sink [2]. Atmospheric methane oxidation has been detected in many different upland soils; ranging from arctic and sub-arctic tundra soils, grasslands and arable soils of the temperate zone, to tropical forests, savannahs and even arid desert soils [3-6]. While most of studies have been performed to estimate the methane uptake capacity of upland soils of the temperate zone, only few data are available for tropical and subtropical soils [7-13]. These data indicate that methane uptake rates of tropical and subtropical forest soils are comparable to those in forest soils of the temperate zone, although higher oxidation rates have been reported for some tropical soils [14].

Among upland soils, forest soils are much more efficient methane sinks than are cultivated soils [15-17]. Changes in land use, especially cultivation of formerly undisturbed soils, reduce the sink strength for atmospheric methane by 60 – 90% [14, 18, 19]. Such reduction has been reported for tropical soils [8, 10], but the methane oxidation rate seems to recover much faster after abandonment of agricultural activities [11], compared to systems of the temperate zone. Recovery of the oxidation rate to predisturbance levels takes > 100 years for soils of the temperate zone [14].

Methane oxidation rate of tropical soils is subjected to strong seasonal variations. Minimum uptake rates were observed during the rainy season and maximum rates during the dry period in several sites in India [13]. Keller *et al.* [20] reported a 2 – 3 fold increase in the uptake rates of soils in Costa Rica during the dry season. The seasonal variation in methane uptake was related to the moisture content of the soils, a trend commonly observed because soil moisture controls the diffusion rate of methane and oxygen in the soil [21]. Even a net methane emission was observed for tropical pasture sites during the wet season [20].

Methane oxidation in upland soils is mediated by methanotrophic bacteria (MB). Seven recognized genera of MB belong to the group of type I methanotrophs (Gammaproteobacteria), while the group of type II methanotrophs consists of 4 genera of MB (Alphaproteobacteria). MB catalyze the first step in methane oxidation through activity of

the enzyme methane monooxygenase (MMO). With the exception of *Methylocella* [22-24], all MB possess the particulate form of this enzyme (pMMO). The *pmoA* gene, encoding the α subunit of the pMMO, is therefore used as a marker gene for methanotrophs and has often been targeted by cultivation-independent methods to characterize the community of MB in different upland soils. Sequences closely related to those of 7 different genera of MB have been detected by these methods in upland soils [25-31]. Additionally, some *pmoA* sequences closely related to cultivated MB have been detected, indicating that uncultivated MB are present in these soils. Uncultivated organisms that harbour *pmoA* sequences of upland soil cluster α (USC α) or upland soil cluster γ (USC γ) are most likely involved in atmospheric methane oxidation in upland soils [26, 28]. With exception of one soil sample from a rainforest in Brazil, the characterization of soil methanotrophic communities has been restricted to upland soils from the temperate zone. The only detected *pmoA* sequence type in this rainforest soil sample belonged to USC α [26].

In the present study, we compared the methane oxidation activity and methanotrophic community composition of different land-use, including a natural forest site, a reforestation site and a corn field. The methane oxidation activity and the community composition of these soils were analyzed and compared with other data available from temperate soils.

Materials and Methods

Sampling sites. Three adjacent sampling sites located in Amphur Wang Nham Keaw, Nakorn Ratchasrima province in Thailand. The forest site (SK) was a natural dry evergreen forest within the Sakerat Experimental Station. The dominant tree species were *Hopea ferrea*, *Pterocarpus marcrocarpus*, *Xylia xylocarpar*, *Dalbergia cochinchinensis*, *Lagerstroemia duppereana*, and *Shorea henryana*. The reforestation site (AC site) located about 5 km away from the SK site. It was planted with fast-growing, nitrogen-fixing tree species in 1988. Studies were performed on a plantation of *Acacia mangium*. Before reforestation, this site was used as a farmland (corn and cassava). The third sampling site was a cornfield (CF), situated adjacent to the restoration area (2 km away). It was deforested more than 40 years ago and corn has been continuously cultivated at this site during the last 16 years. Soil samples for molecular analysis were taken from 10 – 20 cm soil depth on 27th and 28th July 2003. Soils at both SK and AC sites are highly acidic (pH = 4.2, 1:1 in water) while pH at CF site was 5.6. Soil texture at SK site is Clay (clay content = 51%) while at both AC (clay content = 30%)

and CF (clay content = 21%) sites is Sandy Clay Loam. Soil preparation for corn plantation at CF site started in the mid of June, 2003 and seeds of corn were sown on July 10, 2003. On this date, chemical fertilizer (16-20-0, N-P_2O_5-K_2O) was applied at the rate of 125 kg ha^{-1}.

Methane oxidation activity. The net methane flux across the soil surface was determined in March and July 2003 by using the closed-static chamber method. Ten chambers were used at SK site, while 5 chambers were used at AC and CF sites, respectively. Chambers 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 collar made of stainless steel. The collar was inserted into between 10 – 15 cm soil-depth and remained there during the whole study period. During measurement, the about 1 cm of chamber top was inserted into a water-filled channel of the fixed collar. Six or ten gas samples of 25 – 30 ml were taken within 1 h by a 30-ml syringe through a silicon stopper in the chamber top. 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 (injection 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.

DNA extraction. DNA was extracted from 0.5 g of soil using the Fast DNA SPIN Kit (BIO 101, La Jolla, CA, USA), according to the manufacturer's instructions with modifications as described elsewhere [32]. DNA extracts were purified with polyvinylpolypyrrolidone and a QIAquick PCR Purification Kit™ (Qiagen, Hilden, Germany) [28].

PCR amplification and DGGE. A partial fragment of the *pmoA*-gene was PCR-amplified with primer A189 with GC-clamp [33] and primer A682 of Holmes *et al.* [34] and primer mb661 of Costello and Lidstrom [35]. Composition of the PCR reactions and the PCR program are described elsewhere [32]. A fragment of the *mmoX* gene encoding the active-site subunit of the soluble MMO was amplified with forward primer mmoXf945 with GC-clamp [32] and reverse primer mmoXB1401 of Auman *et al.* [36] using the PCR program of Knief *et al.* [32].

Mixed *pmoA*-PCR products were separated by DGGE. Visible bands were excised and reamplified. PCR products from excised DGGE bands were purified and sequenced exactly as described in Knief *et al.* [28].

Phylogenetic sequence analysis. Phylogenetic tree reconstructions were based on deduced amino acid sequences of partial *pmoA* and *amoA* sequences and were performed with the ARB software package [37]. Original tree construction included sequences of all DGGE bands, all available *pmoA* sequences in the GenBank database (December 2003), and selected public-domain *amoA* sequences. A selection was then made of 24 representative sequences from this study plus 38 public-domain sequences.

Isolation of mehanotrophic bacteria. Diluted nitrate mineral salts medium at slightly acid pH (5.8) was used to enrich for MB as described by Dunfield *et al.* [24] from soil samples of the forested sampling sites SK and AC.

Nucleotide sequence accession numbers. Representative *pmoA* nucleotide sequences obtained during this study have been deposited in the GenBank, EMBL, and DDBJ nucleotide sequence databases under the accession numbers XXXXXXXX – XXXXXXXX.

Results

In situ atmospheric methane oxidation

We measured methane oxidation activity twice at each sampling site, one in March and another in July 2003, or at the same day when soil samples were taken. Net atmospheric methane consumption was observed for both dates at SK and AC sites. However, only in March that we observed net oxidation at CF site (Fig. 1). Large spatial variations typically associated with net methane flux at all sites and not all chambers showed net consumptions. Likewise, in some chambers neither net emission nor consumption was observed. In this study, thus, only in the chambers with significant changes in headspace methane concentration ($p<0.05$, fitted by linear regression) were taken into account for flux estimate. Only six chambers showed significant changes in headspace methane concentration over sampling time ($n=6$) in both months at SK site. For example, at SK site the net methane flux

ranged from -4.5 to -0.6 $mgCH_4\ m^{-2}\ day^{-1}$ in March and –0.5 to –1.2 $mgCH_4\ m^{-2}\ day^{-1}$ in July (negative flux indicates a net consumption while positive flux indicates a net emission). For AC site, net methane flux ranged from -0.9 to –1.8 $mgCH_4\ m^{-2}\ day^{-1}$ in March (n = 4) and +2.7 to –5.6 $mgCH_4\ m^{-2}\ day^{-1}$ (n = 3), and for CF site it ranged from –1.4 to +1.4 $mgCH_4\ m^{-2}\ day^{-1}$ (n=3) in March and +0.3 to +1.4 $mgCH_4\ m^{-2}\ day^{-1}$ in July. Fig. 1 shows the average values of net methane fluxes taken from these chambers.

Characterization of the methanotrophic communities.

Using cultivation-independent methods, the communities of MB were characterized in soil samples taken from 10 – 20 cm soil depth during the second half of the wet season. Duplicate soil samples were taken from each sampling site. The *pmoA* gene fragment, used as a functional marker for the characterization of the methanotrophic community, was successfully PCR-amplified from all soil DNA extracts. A gene fragment of the *mmoX* gene could not be amplified. Two different reverse primers were used for the amplification of the *pmoA* gene fragment. Primer A682 is the less specific of the two, since it was designed to amplify both *pmoA* and *amoA* gene fragments [34]. Primer mb661 was designed to amplify *pmoA* gene fragments specifically, excluding amplification of *amoA* gene fragments [35].

The recovery of PmoA sequences very closely related to sequences from isolates of *Methylocystis* and *Methylobacter* indicated that well-known cultivated genera of MB were present in the soil samples of the three sampling sites (> 98 % identity on amino acid level) (Table 1). Sequences from uncultivated bacteria of USC α were detected at SK and AC sites (98 % identity), but not in the soil samples from the cornfield (CF). Likewise, *pmoA* sequences in a closely related, but clearly distinct group from previously detected USC α sequences (Cluster 5) were detected in these two soil samples, but not at CF site. Cluster 5 sequences AC-A and SK-B, which are shown in the phylogenetic tree (Fig. 1), showed a maximum identity of 83 % to sequences of USC α, represented by DGGE band E L4-a in Figure 1. While USC α sequences were obtained only when using primer A682 in PCR reactions, this novel sequence type was detected only when using primer mb661. Another PmoA sequence type indicative for a second taxon of uncultivated MB was detected at all sampling sites. PmoA sequences of this "Cluster 3" formed two separate branches, which were related to sequences of methanotrophic Gammaproteobacteria. The amino acid identity of PmoA sequences of these branches was 95 – 98 % to the uncultivated bacteria represented

by "clone Zhenjiang 5" and "clone Beijyuan 2" and 91 – 92 % to the cultivated methanotrophs of the genera *Methylococcus* and *Methylocaldum*. Since the amino acid identity of these novel sequences was approximately equal to PmoA sequences from both of these genera, the phylogenetic relationship could not be resolved and is represented by a multifurcation in the tree (Fig. 1). Sequences with a phylogenetic position intermediate to those of clearly definable AmoA or PmoA sequence types were detected in soil samples SK and CF. These sequences of "Cluster 2" had 87 – 97 % identity to a sequence previously recovered by cultivation-independent methods from a temperate forest soil, represented by DGGE band E 5FB-f [28]. Sequences that were phylogenetically most closely related to *amoA* genes of ammonia-oxidizing bacteria were detected only in soil sample CF.

Isolation of MB from forested sites. The methanotrophic isolate sakb1 was isolated from site SK, while two isolates (arc1 and arc2) were obtained from site AC. Based on the partial *pmoA* gene sequence, these isolates were identified as *Methylocystis* spp. (Fig. 1). This result was confirmed by 16S rRNA sequence analysis and by the morphology of the isolates. The *pmoA* sequences of the isolates from site AC were not identical to the *pmoA* sequences that were detected in this soil by cultivation independent methods. At site SK *pmoA* sequences indicating the presence of *Methylocystis* spp. were not detected at all.

Relationship between sampling site and community composition of methane-oxidizing bacteria. The DGGE banding pattern of PCR products from both backward primers indicated clear differences in the community composition of methane-oxidizing bacteria at the different sampling sites. The analyzed duplicates from each sampling site were very similar to each other. This impression was confirmed by the results of a cluster analysis (SYSTAT version 9; SPSS Inc. Richmond, Calif.), which was performed based on the DGGE banding pattern (Fig. 2). The duplicates of all soil samples formed separate clusters. The MB community similarity between sites SK and AC was higher than the similarity of either to site CF.

Discussion

In recent years, several studies have examined the methane oxidation activity together with the structure of the methanotrophic community of diverse upland soils [26-29, 31]. With the exception of Brazilian rainforest soil [26], all data on methanotrophic community structure originate from upland soils of the temperate zone of the northern hemisphere. Because some

tropical sites have much higher rates of methane oxidation than temperate sites [12-14], it is interesting to know the MB community composition in these areas. This is the first study to analyze the methanotrophic community composition of a tropical soil under different land use.

The measured methane oxidation rates of the soils corresponded quite well to those published in several other studies for upland soils (up to 7 mg CH_4 m^{-2} d^{-1}), including tropical and subtropical forest soils [14], but were not as high as the rates reported for some tropical forest soils (up to 14 mg CH_4 m^{-2} d^{-1}) [12, 13]. In all land use types examined in this study, the methane oxidation rates were higher in March than in July. Our long interval of measurement (once a month) does not permit us to discuss the reason of such methane oxidation differences. However, we speculated that difference in soil moisture between these two months might have some influences on oxidation activity. Rainy season in the study site normally begins in May. Unusual for March, in 2003 we encountered the heavy rainfall before gas sampling date. As a result, when compares the moisture content of soil (at 6 cm depth) the results do not significantly difference (23±4% by vol in March and 20±3% in July for to SK site, 15±4% in March and 15±5% in July for AC site, and 22±2% in July (no data in March) for CF site, unpublished data). As described above, even within a single day large spatial variations among chambers were found. Thus, further study is needed to understand the correlation between environmental factors and methane oxidation activity in the field.

At CF site the methane oxidation activity was always lower than in the forested sites, and net methane production was observed in July. Thus, some parts of the soil profile might have been saturated with water to the level that methanogenesis developed and took over the net oxidation. In addition, field preparation and fertilization in July may inhibit methane oxidation and allow the net flux to shift to emission. The inhibitory effects of fertilizer especially nitrogen fertilizer are well understood [4, 7, 17].

In general, the oxidation capacity decreased in the order of forest site (SK), reforestation site (AC), and cornfield (CF). This agrees well with the general observation that the conversion of natural forest sites to farmland leads to a reduction of 60 – 90% of the atmospheric methane uptake rate, but that the activity recovers in reforested sites [10, 14, 18, 19]. It has been found that the oxidation rate of tropical soils recovers much faster after abandonment of agricultural activities, compared to temperate systems [11, 14]. This may explain why the methane

oxidation rate of the reforestation site AC was comparable to the rate in site SK, although site AC was reforested only 16 years ago.

Fragments of the *pmoA* gene were amplified from all soil DNA extracts, but *mmoX* genes were not recovered from the soil samples. This is in accordance with results from upland soils from Central Europe [28] and suggests two different explanations. Either the primer systems used are not very sensitive for the detection of *mmoX* genes, or the dominant MB in upland soils do not possess *mmoX* genes. The latter is reasonable considering that MB in upland soils have to live on very low substrate concentrations. Under these oligotrophic conditions it should be more preferable to use pMMO over sMMO, since it has a lower energy demand for the oxidation of methane to methanol. MB expressing pMMO have higher growth yields and show a higher affinity for methane [38].

The only known genera of MB detected in the Thai soil samples were those of *Methylocystis* and *Methylobacter*. While *Mehylocystis* has been detected in several different upland soils [25, 26, 28-30], *Methylobacter* has only been detected in an acidic heath-land soil [25]. Unusual sequences obtained from several DGGE bands indicated that different taxa of uncultivated putative methanotrophs were also present in the soil samples. Organisms harbouring *pmoA* sequences of USC α have been detected in several upland soils before, including acidic tropical forest soils [26]. They were present at sampling sites SK and AC, as were sequences that are represented by Cluster 5, which is closely related to USC α. Cluster 3 sequences represent a group of uncultivated type I methanotrophs related to *Methylocaldum* and *Methylococcus*. A further branch of PmoA sequences within this broad *Methylococcus-Methylocaldum* cluster is formed by sequences that were detected in different rice field soils (clone Beiyuan 2, clone Zhenijang 5) [39]. Thus, different uncultivated taxa of MB seem to exist, which have – on PmoA level – a phylogenetic position between *Methylocaldum* spp. and *Methylococcus* spp.

The detection of the third cluster representing uncultivated bacteria, Cluster 2, has as yet been restricted to upland soils. Since the sequences of this branch are only distantly related to PmoA sequences of recognized genera of MB, it cannot be excluded that these sequences code for the AmoA of uncultivated ammonia-oxidizing bacteria. However, the growth of the organisms harboring these sequences was possibly stimulated in sieved soil samples after incubation under elevated methane mixing ratios (Knief, unpublished data). In this study, only

Methylocystis spp. were isolated. These isolates probably did not represent the dominant *Methylocystis* spp. in the soil samples, since PmoA sequences identical to those of the isolates were not detected by cultivation-independent methods in the soil samples.

The different land-use of the sampling sites was reflected by different methane uptake rates and different soil pHs, especially for the cornfield soil. The characterization of the methanotrophic community indicated that the community composition reflected the land use change as well. The *pmoA* sequences of USC α and Cluster 5 were detected in samples from sites SK and AC, but not in the soil samples from the cornfield. The absence of USC α at site CF is in agreement with the results of other studies, in which the methanotrophic community of some farmland soils was analyzed and in which USC α sequences were not detected, although they were present in adjacent grassland and forest soils [28]. The pH shift in the farmland soil is probably not the reason for the disappearance of USC α sequences at this sampling site. The organisms harbouring USC α sequences, although they appear to occur more frequently in acidic than in pH-neutral soils, do occur in soils of a wide pH range (4.0 – 8.0) [28]. Further sequences that led to the different community compositions of the different sampling sites were those of *Methylocystis* spp., Cluster 2 and of ammonia-oxidizing bacteria (Table 1). Sequences of *Methylobacter* and Cluster 3 were detected at all sampling sites. A cluster analysis was used to compare the degree of similarity across sites among the communities of MB plus ammonia-oxidizing bacteria. The resulting tree confirmed the high similarity of replicate samples from one sampling site and differences between the different sampling sites (Fig. 2). The differences within the replicate samples from one site were due to faint DGGE bands visible in one sample but not in the other ones. Thus, *pmoA* sequences with abundance near the detection limit of the methods were mainly responsible for the dissimilarity of duplicate samples. The main purpose of this cluster analysis was the comparison of the different sampling sites. The community composition of SK and AC sites were more similar to each other than either was to CF site. This finding is in accordance with the methane uptake rates and pHs of the sampling sites, which also indicated a high degree of dissimilarity of site CF compared to sites SK and AC.

These data demonstrate clearly that land use changes are not only connected to changes in the methane uptake capacity of soils, but also to changes in the community composition of methane-oxidizing bacteria. The change in the community composition may be an important factor leading to the general decrease in the methane uptake rate of farmland soils. The

methanotrophic communities of replicate samples within one sampling site were highly similar, although methane uptake rates varied over space and time. However, several other factors influence the methane uptake rate of a soil. The water content is an important factor, since it heavily influences gas diffusion in the soil [21]. Whether and how the community of MB responds to changes in the water content of a soil, remains to be studied.

The community composition of the reforested site AC represents a kind of intermediate state between the communities at sites SK and CF. Since it is unknown how the community composition and methane uptake activity of the reforested site AC looked before the site was reforested, we cannot conclude whether the community we see today has redeveloped or whether the community structure was not as much changed after deforestation. During this period this site was a farmland like site CF, so it is likely that the MB community was different at this time.

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References

[1] Dlugokencky, E.J., Houweling, S., Bruhwiler, L., Masarie, K.A., Lang, P.M., Miller, J.B., Tans, P.P. (2003) Atmospheric methane levels off: Temporary pause or a new steady-state? Geophys. Res. Lett. 30: 1-4

[2] Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., Van Der Linden, P.J., Xiaosu, D. (2001) IPPC Climate Change 2001: The scientific basis - contribution of working group I to the third assessment report of the Intergovernmental Panel on Climate Change (IPCC). Cambridge UK: Cambridge University Press

[3] Keller, M., Goreau, T.J., Wofsy, S.C., Kaplan, W.A., Mcelroy, M.B. (1983) Production of nitrous oxide and consumption of methane by forest soils. Geophys. Res. Lett. 10: 1156-1159

[4] Mosier, A., Schimel, D., Valentine, D., Bronson, K., Parton, W. (1991) Methane and nitrous oxide fluxes in native, fertilized and cultivated grasslands. Nature 350: 330-332

[5] Seiler, W., Conrad, R., Scharffe, D. (1984) Field studies of methane emission from termite nests into the atmosphere and measurements of methane uptake by tropical soils. J. Atmos. Chem. 1: 171-186

[6] Whalen, R.T., Reeburgh, W.S. (1990) Consumption of atmospheric methane by tundra soils. Nature 346: 160-162

[7] Castro, M.S., Peterjohn, W.T., Melillo, J.M., Gholz, H.L., Lewis, D. (1994) Effects of nitrogen fertilization on the fluxes on N_2O, CH_4, and CO_2 from soils in a Florida slash pine plantation. Can. J. For. Res. 24: 9-13

[8] Goreau, T.J., De Mello, W.Z. (1988) Tropical deforestation: some effects on atmospheric chemistry. Ambio 17: 275-281

[9] Keller, M., Kaplan, W.A., Wofsy, S.C. (1986) Emissions of N_2O, CH_4 and CO_2 from tropical forest soils. J. Geophys. Res. 91: 11791-11802

[10] Keller, M., Mitre, M.E., Stallard, R.F. (1990) Consumption of atmospheric methane in tropical soils of Central Panama. Glob. Biogeochem. Cycles 4: 21-27

[11] Keller, M., Reiners, W.A. (1994) Soil-atmosphere exchange of nitrous oxide, nitric oxide, and methane under secondary succession of pasture to forest in the Atlantic lowlands of Costa Rica. Global Biogeochem. Cycl. 8: 399-409

[12] Singh, A.K., Raghubanshi, A.S., Reddy, V.S., Singh, S., Kashyap, A.K. (1998) Methane flux from irrigated paddy and dryland rice fields, and from seasonally dry tropical forest and savanna soils of India. Soil Biol. Biochem. 30: 135-139

[13] Singh, J.S., Singh, S., Raghubanshi, A.S., Singh, S., Kashyap, A.K., Reddy, V.S. (1997) Effect of soil nitrogen, carbon and moisture on methane uptake by dry tropical forest soils. Plant and Soil 196: 115-121

[14] Smith, K.A., Dobbie, K.E., Ball, B.C., Bakken, L.R., Sitaula, B.K., Hansen, S., Brumme, R., Borken, W., Christensen, S., Prieme, A., Fowler, D., Macdonald, J.A., Skiba, U., Klemedtsson, L., Kasimir-Klemedtsson, A., Degorska, A., Orlanski, P. (2000) Oxidation of atmospheric methane in Northern European soils, comparison

with other ecosystems, and uncertainties in the global terrestrial sink. Global Change Biology 6: 791-803

[15] Ambus, P., Christensen, S. (1995) Spatial and seasonal nitrous-oxide and methane fluxes in Danish forest-ecosystems, grassland-ecosystems, and agroecosystems. J. Environ. Qual. 24: 993-1001

[16] Born, M., Dörr, H., Ingeborg, L. (1990) Methane consumption in aerated soils of the temperate zone. Tellus B 42: 2-8

[17] Hütsch, B.W., Webster, C.P., Powlson, D.S. (1994) Methane oxidation in soil as affected by land use, soil pH and N fertilization. Soil Biol. Biochem. 26: 1613-1622

[18] Mosier, A.R., Delgado, J.A., Cochran, V.L., Valentine, D., Parton, W.J. (1997) Impact of agriculture on soil consumption of atmospheric CH_4 and a comparison of CH_4 and N_2O flux in subarctic, temperate and tropical grasslands. Nutrient Cycling in Agroecosystems 49: 71-83

[19] Willison, T.W., Webster, C.P., Goulding, K.W.T., Powlson, D.S. (1995) Methane oxidation in temperate soils: effects of land use and the chemical form of nitrogen fertilizer. Chemosphere 30: 539-546

[20] Keller, M., Veldkamp, E., Weitz, A.M., Reiners, W.A. (1993) Effect of pasture age on soil trace-gas emissions from a deforested area of Costa Rica. Nature 365: 244-246

[21] Smith, K.A., Ball, T., Conen, F., Dobbie, K., Massheder, J., Rey, A. (2003) Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes. Eur. J. Soil Sci. 54: 779-791

[22] Dedysh, S.N., Liesack, W., Khmelenina, V.N., Suzina, N.E., Trotsenko, Y.A., Semrau, J.D., Bares, A.M., Panikov, N.S., Tiedje, J.M. (2000) *Methylocella palustris* gen. nov., sp. nov., a new methane-oxidizing acidophilic bacterium from peat bogs, representing a novel subtype of serine-pathway methanotrophs. Int. J. Syst. Evol. Microbiol. 50: 955-969

[23] Dedysh, S.N., Berestovskaya, Y.Y., Vasylieva, L.V., Belova, S.E., Khmelenina, V.N., Suzina, N.E., Trotsenko, Y.A., Liesack, W., Zavarzin, G.A. (2004) *Methylocella tundrae* sp. nov., a novel methanotrophic bacterium from acidic tundra peatlands. Int. J. Syst. Evol. Microbiol. 54: 151-156

[24] Dunfield, P.F., Khmelenina, V.N., Suzina, N.E., A., T.Y., Dedysh, S.N. (2003) *Methylocella silvestris* sp. nov. a novel methanotrophic bacterium isolated from an acidic forest cambisol. Int. J. Syst. Evol. Microbiol. 53: 1231-1239

[25] Bourne, D.G., Mcdonald, I.R., Murrell, J.C. (2001) Comparison of *pmoA* PCR primer sets as tools for investigating methanotroph diversity in three Danish soils. Appl. Environ. Microbiol. 67: 3802-3809

[26] Holmes, A.J., Roslev, P., Mcdonald, I.R., Iversen, N., Henriksen, K., Murrell, J.C. (1999) Characterization of methanotrophic bacterial populations in soils showing atmospheric methane uptake. Appl. Environ. Microbiol. 65: 3312-3318

[27] Jensen, S., Holmes, A.J., Olsen, R.A., Murrell, J.C. (2000) Detection of methane oxidizing bacteria in forest soil by monooxygenase PCR amplification. Microb. Ecol. 39: 282-289

[28] Knief, C., Lipski, A., Dunfield, P.F. (2003) Diversity and activity of methanotrophic bacteria in different upland soils. Appl. Environ. Microbiol. 69: 6703-6714

[29] Reay, D.S., Radajewski, S., Murrell, J.C., Mcnamara, N., Nedwell, D.B. (2001) Effects of land-use on the activity and diversity of methane oxidizing bacteria in forest soils. Soil Biol. Biochem. 33: 1613-1623

[30] Steinkamp, R., Zimmer, W., Papen, H. (2001) Improved method for detection of methanotrophic bacteria in forest soils by PCR. Curr. Microbiol. 42: 316-322

[31] Henckel, T., Jäckel, U., Schnell, S., Conrad, R. (2000) Molecular analyses of novel methanotrophic communities in forest soil that oxidize atmospheric methane. Appl. Environ. Microbiol. 66: 1801-1808

[32] Knief, C., Kolb, S., Bodelier, P.L.E., Lipski, A., Dunfield, P.F. (2004) Diversity and activity of methanotrophic bacteria in temporary flooded upland soils. in preparation

[33] Henckel, T., Friedrich, M., Conrad, R. (1999) Molecular analyses of the methane-oxidizing microbial community in rice field soil by targeting the genes of the 16S rRNA, particulate methane monooxygenase, and methanol dehydrogenase. Appl. Environ. Microbiol. 65: 1980-1990

[34] Holmes, A.J., Costello, A., Lidstrom, M.E., Murrell, J.C. (1995) Evidence that particulate methane monooxygenase and ammonia monooxygenase may be evolutionarily related. FEMS Microbiol. Lett. 132: 203-208

[35] Costello, A.M., Lidstrom, M.E. (1999) Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments. Appl. Environ. Microbiol. 65: 5066-5074

[36] Auman, A.J., Stolyar, S., Costello, A.M., Lidstrom, M.E. (2000) Molecular characterization of methanotrophic isolates from freshwater lake sediment. Appl. Environ. Microbiol. 66: 5259-5266

[37] Strunk, O., Gross, O., Reichel, B., May, M., Hermann, S., Stuckman, N., Nonhoff, B., Lenke, M., Ginhart, A., Vilbig, A., Ludwig, T., Bode, A., Schleifer, K.H. (1998) ARB: a software environment for sequence data. Technische Universität München, Munich.

[38] Hanson, R.S., Hanson, T.E. (1996) Methanotrophic bacteria. Microbiol. Rev. 60: 439-471

[39] Hoffmann, T., Horz, H.P., Kemnitz, D., Conrad, R. (2002) Diversity of the particulate methane monooxygenase gene in methanotrophic samples from different rice field soils in China and the Philippines. Syst. Appl. Microbiol. 25: 267-274

[40] Jones, D.T., Taylor, W.R., Thornton, J.M. (1992) The rapid generation of mutation data matrices from protein sequences. CABIOS 8: 275-282

Table 1: PmoA sequence types detected in the different sampling sites. Phylogenetic positions of the different taxa are given in Figure 1. (Designation of Clusters 2, 3, and 5 according to Knief *et al.* [32].), SK = natural forest site, AC = reforested site and CF = cornfield site.

Detected taxa	SK		AC		CF	
	A682b	mb661	A682b	mb661	A682b	mb661
Ammonia oxidizer (AmoA)					+	
Cluster 2 (PmoA/AmoA)	+				+	
Methylobacter		+		+	+	+
Cluster 3		+		+	+	+
Methylocystis			+	+	+	
USC α	+		+			
Cluster 5		+		+		

Legends

Figure 1 In situ net methane flux. The values are the average of measurements from 6, 4 and 3 chambers for SK, AC and CF sites, respectively.

Figure 2 Phylogenetic tree of deduced amino acid sequences of partial *pmoA* and *amoA* sequences. The tree includes representatives of different PmoA sequences that were detected at the different sampling sites. Sequences recovered from PCR products that were amplified using the A682 reverse primer are shown in small letters while capitals indicate sequences from PCR products that were obtained using the mb661 reverse primer. PmoA sequences of three methanotrophic isolates, obtained from soils SK and AC, are also included. Tree calculation was based on 130 amino acid positions using the treepuzzle algorithm with the Jones-Taylor-Thornton evolutionary model [40]. The position of recovered PmoA and AmoA sequences in this tree was confirmed by the topology of a maximum-likelihood and a neighbor-joining tree. Black circles indicate branches that were present in 90 % of 25,000 reconstructed treepuzzle trees, white circles indicate branches present in 80 % of the trees. AmoA sequences of ammonia-oxidizing bacteria were set as an outgroup. The bar represents 0.10 change per amino acid position.

Figure 3: Cluster analysis based on the presence or absence of 21 bands of DGGE gels from PCR products obtained with primer systems A189-GC/A682 and A189-GC/mb661. Jaccard similarity coefficients were calculated and a hierarchical tree was constructed based on average linking and Euclidean distances.

Figure 1

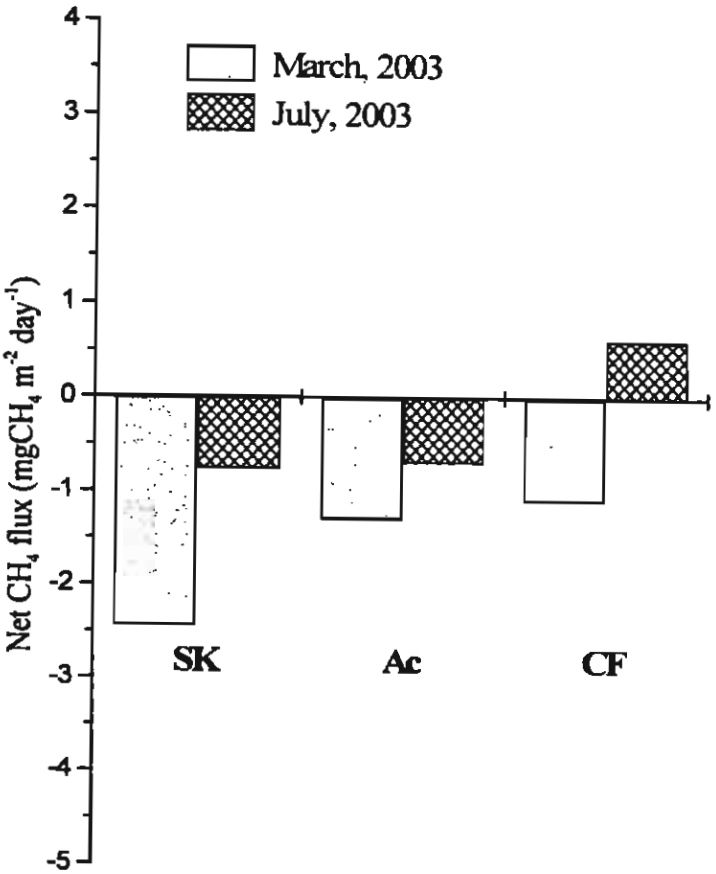

March, 2003
July, 2003
Net CH_4 flux ($mgCH_4$ m^{-2} day^{-1})
4
3
2
1
0
-1
-2
-3
-4
-5
SK
Ac
CF

Figure 2

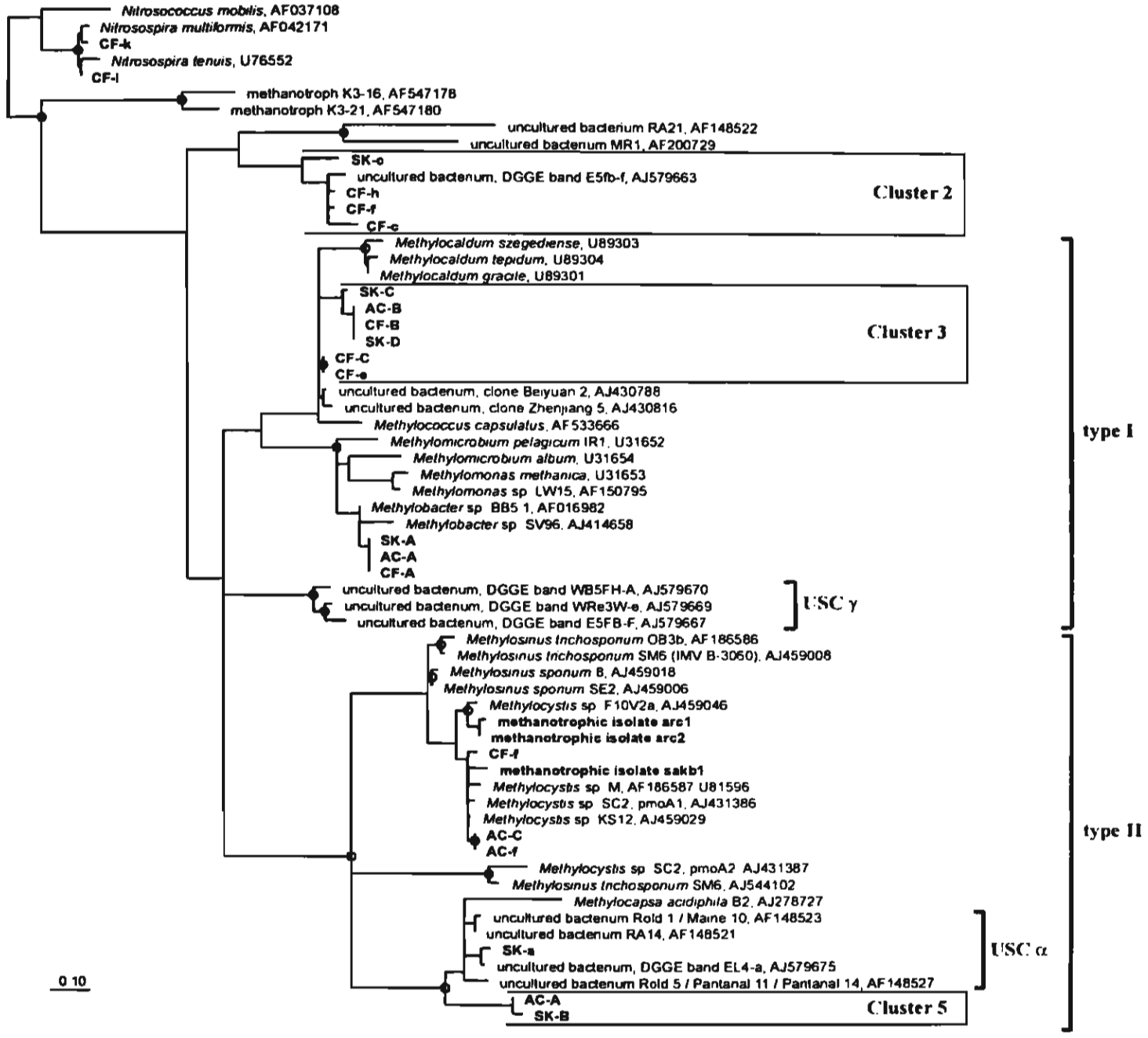
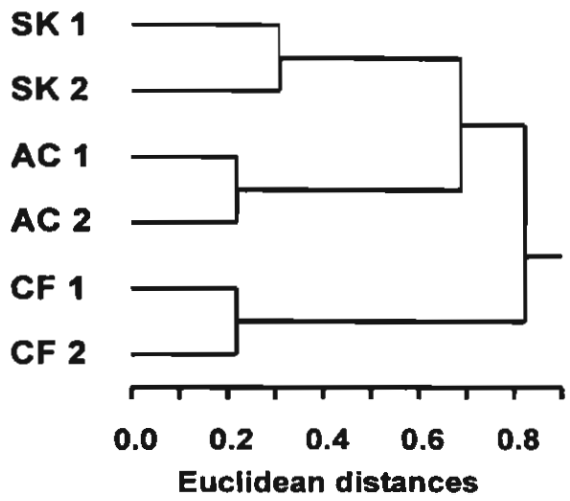

Nitrosococcus mobilis, AF037108
Nitrosospira multiformis, AF042171
CF-k
Nitrosospira tenuis, U76552
CF-l
methanotroph K3-16, AF547178
methanotroph K3-21, AF547180
uncultured bacterium RA21, AF148522
uncultured bacterium MR1, AF200729
SK-c
uncultured bacterium, DGGE band E5fb-f, AJ579663
CF-h
CF-f
CF-c
Cluster 2
Methylocaldum szegediense, U89303
Methylocaldum tepidum, U89304
Methylocaldum gracile, U89301
SK-C
AC-B
CF-B
SK-D
Cluster 3
CF-C
CF-e
uncultured bacterium, clone Beiyuan 2, AJ430788
uncultured bacterium, clone Zhenjiang 5, AJ430816
Methylococcus capsulatus, AF533666
Methylomicrobium pelagicum IR1, U31652
Methylomicrobium album, U31654
Methylomonas methanica, U31653
Methylomonas sp LW15, AF150795
Methylobacter sp BB5 1, AF016982
Methylobacter sp SV96, AJ414658
SK-A
AC-A
CF-A
uncultured bacterium, DGGE band WB5FH-A, AJ579670
uncultured bacterium, DGGE band WRe3W-e, AJ579669
uncultured bacterium, DGGE band E5FB-F, AJ579667
USC γ
type I
Methylosinus trichosporium OB3b, AF186586
Methylosinus trichosporium SM6 (IMV B-3060), AJ459008
Methylosinus sporium 8, AJ459018
Methylosinus sporium SE2, AJ459006
Methylocystis sp F10V2a, AJ459046
methanotrophic isolate arc1
methanotrophic isolate arc2
CF-f
methanotrophic isolate sakb1
Methylocystis sp M, AF186587 U81596
Methylocystis sp SC2, pmoA1, AJ431386
Methylocystis sp KS12, AJ459029
AC-C
AC-f
Methylocystis sp SC2, pmoA2 AJ431387
Methylosinus trichosporium SM6, AJ544102
Methylocapsa acidiphila B2, AJ278727
uncultured bacterium Rold 1 / Maine 10, AF148523
uncultured bacterium RA14, AF148521
SK-a
uncultured bacterium, DGGE band EL4-a, AJ579675
uncultured bacterium Rold 5 / Pantanal 11 / Pantanal 14, AF148527
USC α
type II
AC-A
SK-B
Cluster 5
0.10

Figure 3



Draft:

Kinetics of methane oxidation in tropical upland soils under different land use

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Abstract

Three upland soils from Thailand; a natural forest (SK soil), a reforested site (AC soil), and an agricultural field (CF soil) were studied for their methane oxidation potential and kinetics of methane oxidation. In SK and AC soils a clear zonation for active methane oxidation layer was detected along the soil depth. The most active oxidation layers in SK and AC soils lied between 15 cm and 40 cm while in CF soil no clear active layer was observed. Stratification of active oxidation zones coincides with the trends of inorganic nitrogen content profile. In SK and AC soils, high concentration of inorganic nitrogen compounds (usually > 100 mg NO_3^- or $NH_4^+ \cdot$ kg $soil^{-1}$) was detected in the top 15-cm soil while there was no clear distribution trend found in CF soil. It was assumed that such high concentration of inorganic nitrogen in the topsoil inhibited the activity of methane oxidizing bacteria thus only in the subsoil that methanotrophs were active. Examining kinetic coefficients of these active layers revealed that soil at SK site had high affinity for methane (K_m of 52 ppmv) but rather low methanotrophic capacity (V_{max} of 0.82 nmol·g $soil^{-1} \cdot h^{-1}$). Soil at AC and CF 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 (Vmax about 10 nmol g $soil^{-1}$ hr^{-1}). These results indicate that land use type significantly affects depth distribution of methane oxidation and kinetics of methanotrophic community in tropical soils.

1. Introduction

Microbial uptake in upland soils represents the major terrestrial sink of atmospheric methane. This process consumes about 20-60 Tg of CH_4 annually, accounting for about 6% of the global methane sink [Houghton et al., 2001; Adamsen and King 1993; Reeburgh et al, 1993]. Atmospheric methane oxidation has been detected in many different upland soils; ranging from arctic and sub-arctic tundra soils, grasslands and arable soils of the temperate zone, to tropical forests, savannahs and even arid desert soils (Keller et al., 1983; Mosier et al., 1991; Seiler et al., 1984; Whalen and Reeburgh, 1990). While most of studies have estimated the methane uptake capacity of upland soils of the temperate zone, only few data are available for tropical and subtropical soils (Castro et al., 1994; Goreau et al., 1988, Keller et al., 1986, 1990, Keller and Reiners, 1994).

In many oxic soils, biphasic methane oxidation kinetics were observed and this is suggested to be attributed to the presence of two methanotrophic communities (Bender and Conrad 1992). The first group are the methanotrophs that have high capacity (high Vmax) and low affinity (high Km) for methane. They are capable of oxidizing methane at elevated concentrations (>1000 ppmv). The second group exhibits 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 in the second groups have been confirmed by culture-independence techniques (Holmes et al., 1999; Henkel et al., 2000 Knief et al., 2003). Recently, the methotrophs able to oxidize methane at atmospheric levels have been isolated from a humisol soil (Dunfield et al., 1995, Dunfield et al., 2003).

Among upland soils, forest soils are more efficient methane sinks than are cultivated soils (Ambus and Christensen, 1995; Born et al., 1990; Hütsch et al., 1994). Changes in land use, especially cultivation of formerly undisturbed soils, usually results in reduction of sink strength for atmospheric methane by 60 – 90% (Smith et al., 2003). This has been attributed to inhibition by nitrogen fertilizers applied during crop cultivation (Mosier et al., 1991, Hutsch 1994). Inorganic nitrogen, especially ammonium (NH_4^+) significantly inhibits 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 affect 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 tropic are as rapid as 2% $year^{-1}$ (Keller and Reiners, 1994). Such land use change leads to a reduction of methane oxidation and thus may have implicated the global methane by contribution to increasing atmospheric concentrations. However, recently reforestation in the abandoned lands has been promoted in many tropical countries. It is not know whether or not such activity could recover methanotrophic capacity of soil.

Here we report the kinetics of methane oxidation in soils taken from different land use in Thailand. Our main objective is to assess how oxidation kinetics of tropical upland soils are affected by converting natural forest into cultivation land, and by converting agricultural soil back into planted forests.

2. Materials and Methods

2.1 Soil sampling sites

Soil samples were taken from three adjacent sampling sites located in Amphur Wang Nham Keaw, Nakorn Ratchasrima province in Northeast Thailand. The forest site (SK) was a natural dry evergreen forest located in the Sakerat Environmental Research Station. The dominant tree species were *Hopea ferrea*, *Pterocarpus marcrocarpus*, *Xylia xylocarpar*, *Dalbergia cochinchinensis*, *Lagerstroemia duppereana*, and *Shorea henryana*. The reforestation site (AC) located about 5 km away from the SK site was planted with fast-growing, nitrogen-fixing tree species *Acacia mangium* in 1988. The third sampling site was a cornfield (CF), situated adjacent to the restoration area (2 km away). It was deforested more than 40 years ago and corn has been continuously cultivated at this site during the last 16 years. Soil texture at SK site is Clay (clay content = 51%) while at both AC (clay content = 30%) and CF (clay content = 21%) sites is Sandy Clay Loam. Soil preparation for corn plantation at CF site started in the mid of June, 2003 and seeds of corn were sown on July

10, 2003. On this date, chemical fertilizer (16-20-0, N-P_2O_5-K_2O) was applied at the rate of 125 kg ha^{-1}.

2.2 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. 30 g of soil samples were weighed into the vials and then they were incubated at room temperature (25-26 °C). Concentration of methane in the vial's headspace was 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 calcualated by multiplying the rate constant with the initial methane concentration.

The most active oxidation layer was then selected for further determination of kinetic coefficients. 30 g of 2-mm sieved soil 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 (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 CH_4 uptake were estimated from the plot between the initial CH_4 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 Solfware, Inc., Northampton, Maine).

2.3 Chemical analysis

A Shimadzu Gas Chromatograph (Model 14B) equipped with FID detector and "methanizer" was used to measure CH_4 in the present study. The GC operating conditions are: 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, Column; Unibead C packed column.

Inorganic nitrogen contents (NH_4^+, NO_3^-, NO_2^-) 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 Erlemeyer flask and 70 mL of deionized water was added. Extraction was carried out in triplicates for each soil layer. 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 use as a blank control. 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 Soil pH

Soil pH was measured in deionized water (soil : water = 1:1) after drying the soil samples at room temperature. Forest soils (both at SK and AC sites) were acidic, especially in surface layers (Fig. 1). The SK soil at surface had the pH of about 2.0 while at AC site the pH at surface was 3.5. However, pH values at both forest sites increased to about 4 at 10 cm and then stayed fairly constant at this value throughout a 50-cm profile. Contrarily, soil under corn plantation showed a different pH profile. In cornfield soil, the highest pH was found at surface (pH 6.2) and pH decreased to and stayed constant around 5.2 below 5 cm.

3.2 Concentration of inorganic nitrogen compounds

Inorganic N content (NO_2^-, NO_3^- and NH_4^+) of all soil was measured along the soil depth. Results are given in Fig. 2A-C. The top layer of natural forest soil (SK) showed the highest nitrate content (about 260 mg NO_3^- kg $soil^{-1}$, Fig. 2A). High level of nitrate was detected down the profile to 10-15 cm and below this layer nitrate content was below 25 mg kg $soil^{-1}$. On the other hand, nitrate content throughout soil profile at both AC and CF sites was fairly constant around 50-75 mg kg $soil^{-1}$. However, very low concentration of nitrate

was found below 30 cm in AC soil. No clear trend along the profile was observed for both AC and CF sites.

In contrast to nitrate, nitrite content along the soil profile and across the sampling sites was not different. Compared to nitrate, nitrite content was approximately more than 10 times lower (Fig. 2B). It was lower than 3 mg kg soil^{-1} below 10 cm in SK site and below 15 cm in AC site. NO_2^- was detected across the soil profile at CF site, although it concentration was generally lower than 10 mg kg soil^{-1}.

Ammonium content in all sites is given in Fig. 2C. Similar to the trends found in nitrate content, natural forest soil at SK site had the highest ammonium content. The highest concentration at SK was found in the top 10 cm surface layer (173 mg kg soil^{-1} at 0-5 cm depth). In both forest sites, ammonium concentration trended to decrease as soil depth increased and below 10 cm the typical concentration was below 50 mg kg soil^{-1}. In contrast to the forest site, a fairly constant amount of ammonium was observed throughout the soil profile at CF site. However, the concentration at CF site at all depths was below 50 mg kg soil^{-1} and it trended to increase in the deeper layers.

3.2 Methane oxidation rate along soil profile

Soils at different depths taken from all three sampling sites were studied in the laboratory. Soils from SK and AC sites were incubated under ambient methane concentration and then consumption was followed over times. Since soil from CF site shows low oxidation activity, incubation was carried out under 5 ppmv methane. Most of soil layers exhibited the methane oxidation activity. However, the most active methane oxidation layers for SK site was between 15 and 30 cm (Fig. 3A). For AC site clear oxidation activity was found only at 10-15 and 20-30 cm (Fig. 3B) and changes in concentration of methane in the incubation vials were erratic. Repeated experiments gave the similar results. Similarly, all layers of soil from CF site show oxidation activity (Fig. 3C). There was no oxidation observed at 5-10 cm. However, the most active layer was in the subsurface at 0-5 cm and 30-40 cm.

3.3 Kinetics of methane oxidation

Methane oxidation rate at each concentration was estimated by linear regression of CH_4 mixing ratios versus time. A plot of initial methane concentration and methane oxidation rate is given in Fig. 4. The initial methane concentrations used in the experiment were 5, 10, 20, 50, 100, 200, 300, 700, 800,1000 and 1800 ppmv. For SK site, the plot between consumption rate and substrate concentration followed an expected shape of enzymatic kinetics with initially increase oxidation rated paralleling increase concentration, followed by a rate that no longer shows concentration dependence. This is true even at low methane concentration. However, in AC and CF soil it seemed that at concentration below 50 ppmv, there was no clear correlation between oxidation rate and methane concentration as suggested by pseudo-first order kinetic model of Michaelis-Menten. Increase of oxidation rate in these two sites was observed at concentration above 50 ppmv (Fig. 4).

From Fig. 5, it is clear that at methane concentration below 250 ppmv, rate of methane oxidation in SK soil was higher than at both AC and CF site. Thus, soil at SK can oxidize methane at lowconcentration better than soils at AC and CF site. However, above about 200 ppmv, the oxidation rate becomes concentration independence at SK site. Thus, methane oxidation rate in SK soil will not increase significantly if its methane concentration is too high. In AC and CF sites oxidation rate still increased and then stabilized around 800 and 1000 ppmv, respectively. At ambient and 50 ppmv concentration, however, soil at AC site shows higher oxidation rate than at CF site, though the difference was not clear.

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 SK soil (K_m = 52 ppmv). Soil from AC and CF show lower methane affinity compared with SK site (Km of 724 ppmv and 155-2362 ppmv, respectively). The lowest Vmax was found in SK soil. However, there was no significant difference in Vmax between AC and CF soils.

4. Discussion

Results from previous studies indicate that methane oxidation in soils is affected by several factors. Among these, land use type and mode agricultural practices are among the most influencing ones (Hütsch, 2001). Conversion of natural forest to agriculture usually accompanies with partial loss of methanotrophic activity. Powlson et al. (1997) 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 (Powlson et al., 1997; Mosier et al., 1991; Hütsch, 1994). Among type of nitrogen compounds, ammonium seems to have the strongest effects. Although nitrite is directly toxic to methanotrophs, its low concentration and highly unstable in nature make it less important than ammonium. Inhibitory mechanisms on methane oxidation has been suggested to be through the competition between ammonium ions and methane for the binding site of methane monooxygenase, the key enzyme that catalizes the first step of methane oxidation by methanotrophs (Dunfield and Knowles, 1995). However, Gulledge and Shimel (1998) showed that besides the competitive mechanism, salting effects on methane oxidation accompanied with fertilization was also important. They 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, Adamsen and King, 1993).

Results in the present study agree well with the finding in the previous studies that when high concentration of inorganic nitrogen present (Fig. 2), low methane oxidation was observed. However, concentrations of all nitrogen species were higher in SK and AC soils than in CF soil and such levels of ammonium might be sufficiently high to inhibit methane oxidation. Nevertheless, relatively high oxidation rate was observed at both SK and AC

sites. Thus, nitrogen content alone in surface soil cannot explain the oxidation rate differences between forests (SK and AC) and agriculture soil (CF) in this case.

It was observed that the distribution pattern of nitrogen content along the soil profile differed among three sites. In SK and AC 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 SK and AC site. On the other hand, soil at CF 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 CF 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 CF site. Such distribution of N and at level of concentration found may lead to a relatively uniform inhibition of methane oxidation throughout the CF soil profile (Fig. 3), while such inhibitory effects of N is not prevail in SK and AC soils due to their relatively low concentration in subsoil. As a result, net methane oxidation was observed in SK and AC 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. The reason for this is not clearly known. However, it is typical that oxidation in the organic layers of forest soils is usually lower than the mineral soil layers (Roslev et al., 1997; Bradford et al., 2001; Reay et al., 2001). This is due to the fact that mineralization in organic layers release inorganic nitrogen species that 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 is indeed due to high ammonium and nitrate contents (Fig. 2). In addition, the pH at the surface layers is very low in the present study (Fig. 1). Thus, The soil at the surface layers may be too acidic for methane oxidation.

Hütsch (2001) suggested that repeated application of ammonium could result in change in the kinetics of methatrophic bacteria with consequences for the threshold value (concentration below which no oxidation takes place). Although incubation was not long enough to allow estimate of threshold value, kinetic coefficients clearly indicated that no methane oxidation was observed over 200 ppmv in SK soil, and seemed that no oxidation

occurred below 50 ppmv in AC and CF soils. Nesbit and Breitenbeck (1992) reported 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 soil, 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 (1994) 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, it is reasonable to assume that methane oxidation in subsurface layer is limited by gas diffusion (oxygen and methane) from the atmosphere. Thus, methane oxidation of such soil is controlled mainly by substrate availability. Methanotrophic community in SK subsoil may have experienced and well adapted to such limitation. Therefore, SK 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 (Boeckx et al., 1997). On the other hand, low affinity for methane in CF soil may be due to the existence of different methanotrophic community which is classified as the first ground as mentioned above. Methanotrophic community in CF 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 AC soil come in the middle between SK and CF soil, as for the history of land use. This may indicate that soil at AC site is in the transient period from previously disrupted by human activity (agriculture) towards the natural conditions. Methanotrophic community in AC soil, thus, may be the mixture of those found in SK and AC sites, possibly means it could oxidize both

methane at low (as observed in SK site) and high methane concentration (as observed in CF site).

In conclusion, kinetics of methane oxidation is strongly depended 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. However, such forest plantation improves methane oxidation capacity substantially compared with agricultural lands.

References

Adamsen A.P.S. and King G.M. 1993. Methane consumption in temperate and subarctic forest soil: rates, vertical zon

Ambus P. and Christensen S. 1995. Spatial and seasonal nitrous oxide and methane fluxes in Danish forest, grassland and agroecosystems. J. Environ. Qual., 24: 993-1001.

Bender M. and Conrad R. 1992. Kinetics of methane oxidation in oxic soils exposed to ambient air or high methane mixing ratios. FEMS Microbiolo. Ecol., 101: 261-270.

Bender M. and Conrad R. 1994. Methane oxidation activity in various soils and freshwater sediments: occurrences, characteristics, vertical profiles, and distribution on grain size fractions. J. Geophys. Res., 99: 16531-16540.

Bradford M.A., Ineson P., Wookey P.A. and Lappin-Scott H.M. 2001. Role of methane oxidation, production and transportation in forest soil methane flux. Soil Biology & Biochemistry, 33: 1625-1631.

Boeckx, P., Van Cleemput, O. and Villaralvo, I. 1997. Methane oxidation in soils with different textures and land use. Nutri. Cycling Agroecosys., 49: 91-95.

Born M., Doerr H. and Levin I. 1990. Methane consumption in aerated soils of the temperate zone. Tellus, 42B: 2-8.

Castro, M.S., Peterjohn, W.T., Melillo, J.M., Gholz, H.L., Lewis, D. 1994. Effects of nitrogen fertilization on the fluxes on N_2O, CH_4, and CO_2 from soils in a Florida slash pine plantation. Can. J. For. Res. 24: 9-13.

Dunfield, P. and Knowles, R. 1995. Kinetics of inhibition of methane oxidation by nitrate, nitrite and ammonium in a humisol. Appl. Environ. Microbiol., 61: 3129-3135.

Dunfield, P.F., Khmelenina, V.N., Suzina, N.E., A., T.Y., Dedysh, S.N. (2003) *Methylocella silvestris* sp. nov. a novel methanotrophic bacterium isolated from an acidic forest cambisol. Int. J. Syst. Evol. Microbiol. 53: 1231-1239

Goreau, T.J., De Mello, W.Z. (1988) Tropical deforestation: some effects on atmospheric chemistry. Ambio 17: 275-281.

Gulledge J. and Schimel J.P. 1998. Low-concentration kinetics of atmospheric CH4 oxidation in soil and mechanism of NH4+ inhibition. Appl. Environ. Microbiol., 64: 4291-4298.

Henckel, T., Jäckel, U., Schnell, S., Conrad, R. (2000) Molecular analyses of novel methanotrophic communities in forest soil that oxidize atmospheric methane. Appl. Environ. Microbiol. 66: 1801-1808.

Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., Van Der Linden, P.J., Xiaosu, D. 2001. IPPC Climate Change 2001: The scientific basis - contribution of working group I to the third assessment report of the Intergovernmental Panel on Climate Change (IPCC). Cambridge UK: Cambridge University Press.

Hütsch B.W. 2001. Methane oxidation in non-flooded soils as affected by crop production-invited paper. Europ. J. Agronomy, 14: 237-260.

Hütstch, B.W. 1998. Tillage and land use effects on methane oxidation rates and their vertical profiles in soil. Biol. Fertil. Soils., 27: 284-292.

Hütsch B.W, Webster C.P. and Powlson D.S. 1994. Methane oxidation as affected by land use, soil pH and nitrogen ferilization. Soil Biol.Biochem., 26: 1613-1622.

Keller, M., Goreau, T.J., Wofsy, S.C., Kaplan, W.A., Mcelroy, M.B. (1983) Production of nitrous oxide and consumption of methane by forest soils. Geophys. Res. Lett. 10: 1156-1159.

Keller, M., Kaplan, W.A., Wofsy, S.C. 1986. Emissions of N_2O, CH_4 and CO_2 from tropical forest soils. J. Geophys. Res. 91: 11791-11802

Keller, M., Mitre, M.E., Stallard, R.F. 1990. Consumption of atmospheric methane in tropical soils of Central Panama. Glob. Biogeochem. Cycles 4: 21-27

Keller, M., Reiners, W.A. 1994. Soil-atmosphere exchange of nitrous oxide, nitric oxide, and methane under secondary succession of pasture to forest in the Atlantic lowlands of Costa Rica. Global Biogeochem. Cycl. 8: 399-409.

Knief C., Lipski A. and Dunfield P. F. 2003. Diversity and activity of methanotrophic bacteria in different upland soils. Applied and Environmental Microbiology 69:6703-6714.

Henckel, T., Jäckel, U., Schnell, S., Conrad, R. 2000. Molecular analyses of novel methanotrophic communities in forest soil that oxidize atmospheric methane. Appl. Environ. Microbiol. 66: 1801-1808

Holmes, A.J., Costello, A., Lidstrom, M.E., Murrell, J.C. (1995) Evidence that particulate methane monooxygenase and ammonia monooxygenase may be evolutionarily related. FEMS Microbiol. Lett. 132: 203-208

Hutsch, B.W. 1998. Tillage and land use effects on methane oxidation rates and their vertical profiles in soil. Biol. Fertil. Soils., 27: 284-292.

Mosier, A., Schimel, D., Valentine, D., Bronson, K., Parton, W. 1991. Methane and nitrous oxide fluxes in native, fertilized and cultivated grasslands. Nature 350: 330-332.

Nesbit, S.P. and Breitenbeck, G.A. 1992. A loboratory studyof factors influencing methane uptake by soils. Agric. Ecosyst. Environ., 41: 39-54.

Powlson, D.S, Goulding, K.W.T., Willison, T. W., Webster, C.P. and Hütsch, B.W. 1997. The effects of agriculture on methane oxidation in soil. Nutrient Cycling in Agroecosystems, 49: 59-70.

Reay D.S., Radakewski S., Murrell J.C., McNamara N. and Nedwell DB. 2001. Effects of land-use on the activity and diversity of methane oxidizing bacteria in forest soils. Soil Biology & Biochemistry. 33:1613-1623.

Reeburgh, W.S., Whalen, S.C. and Alperin, N.R. 1993. Role of methylotrophy in the global methane bugets. In: Murrell, J.C., Kelly, D.P. (Eds). Microbial Growth on C1 compounds. Kluwer, Dordrecht, pp. 1-14.

Roslev P., Iversen N, and Henriksen K. 1997. Oxidation and assimilation of atmospheric methane by soil methane oxidizers. Applied and Environmental Microbiology 63:874-880.

Seiler, W., Conrad, R., Scharffe, D. (1984) Field studies of methane emission from termite nests into the atmosphere and measurements of methane uptake by tropical soils. J. Atmos. Chem. 1: 171-186.

Singh, A.K., Raghubanshi, A.S., Reddy, V.S., Singh, S., Kashyap, A.K. (1998) Methane flux from irrigated paddy and dryland rice fields, and from seasonally dry tropical forest and savanna soils of India. Soil Biol. Biochem. 30: 135-139.

Singh, J.S., Singh, S., Raghubanshi, A.S., Singh, S., Kashyap, A.K., Reddy, V.S. (1997) Effect of soil nitrogen, carbon and moisture on methane uptake by dry tropical forest soils. Plant and Soil 196: 115-121.

Smith, K.A., Ball, T., Conen, F., Dobbie, K., Massheder, J., Rey, A. (2003) Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes. Eur. J. Soil Sci. 54: 779-791

Whalen, R.T., Reeburgh, W.S. 1990. Consumption of atmospheric methane by tundra soils. Nature 346: 160-162.

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

Site	V_{max} (nmol g^{-1} hr^{-1})	K_m (ppmv)
SK (10-20 cm)	0.82	52.02
AC (10-20 cm)	5.72	723.96
CF		
0-5 cm	10.66	1454.88
30-40 cm	9.97	2361.76

Figure Legends

Fig. 1 Soil pH along soil depths at SK, AC and CF sites (measured in H_2O)

Fig. 2 Nitrate (A), nitrite (B) and ammonium (C) contents along the soil profile in different land use.

Fig. 3 Oxidation rate of methane along the soil profile and in different land use types.

Fig. 4 A plot between initial methane concentration vs methane oxidation rate. Soil samples used in experiments at SK and AC sites were taken from 10-20, 5-10 cm depth, respectively, while at CF site two depths (0-5 and 30-40 cm) were examined.

Fig. 5 Exponential plot of relationship between oxidation rate and methane concentration below approximately 350 ppmv.

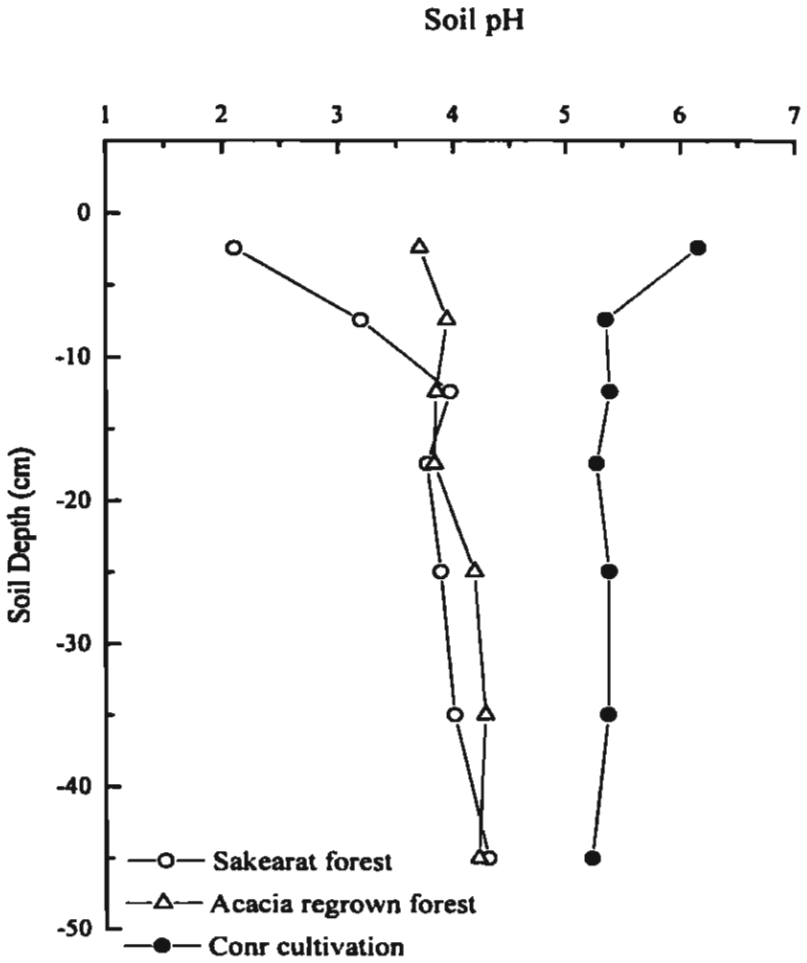
Soil pH
1
2
3
4
5
6
7
0
-10
-20
-30
-40
-50
Soil Depth (cm)
Sakearat forest
Acacia regrown forest
Conr cultivation

Fig. 1

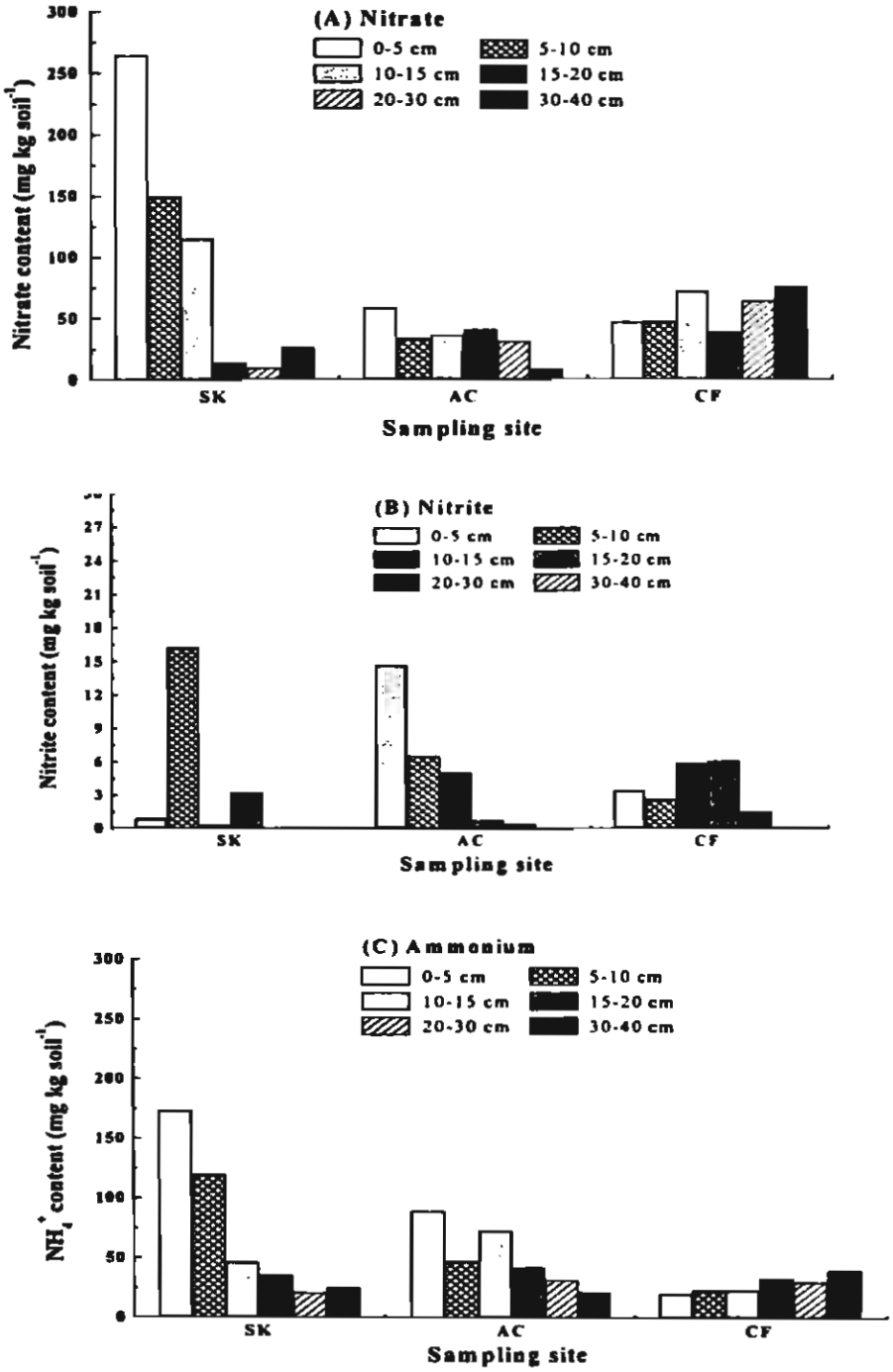
(A) Nitrate
0-5 cm
5-10 cm
10-15 cm
15-20 cm
20-30 cm
30-40 cm
Nitrate content (mg kg soil-1)
300
250
200
150
100
50
0
SK
AC
CF
Sampling site
(B) Nitrite
0-5 cm
5-10 cm
10-15 cm
15-20 cm
20-30 cm
30-40 cm
Nitrite content (mg kg soil-1)
27
24
21
18
15
12
9
6
3
0
SK
AC
CF
Sampling site
(C) Ammonium
0-5 cm
5-10 cm
10-15 cm
15-20 cm
20-30 cm
30-40 cm
NH4+ content (mg kg soil-1)
300
250
200
150
100
50
0
SK
AC
CF
Sampling site

Fig. 2

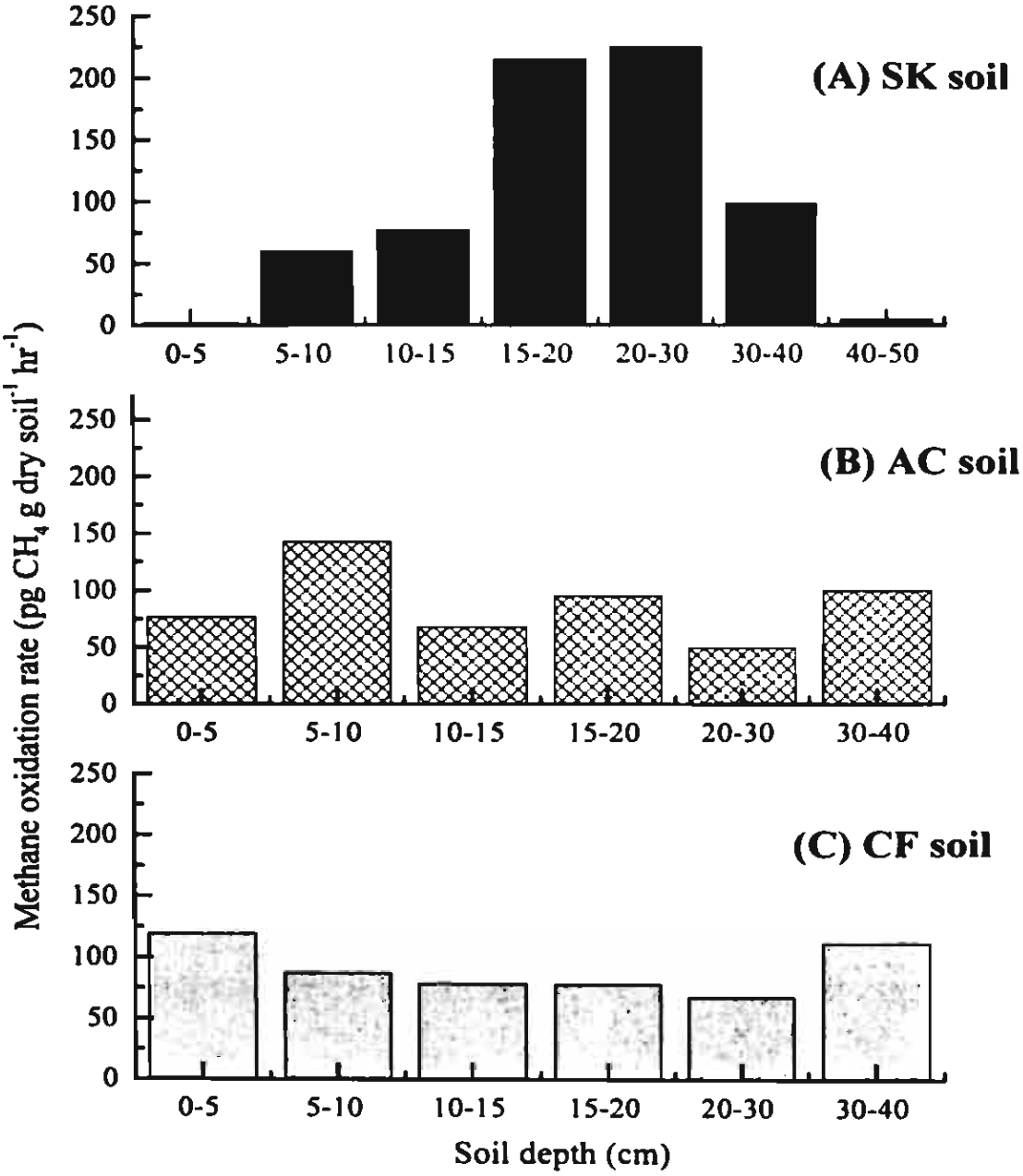
(A) SK soil
(B) AC soil
(C) CF soil
Methane oxidation rate (pg CH_4 g dry soil^{-1} hr^{-1})
Soil depth (cm)
0-5
5-10
10-15
15-20
20-30
30-40
40-50
0
50
100
150
200
250

Fig. 3

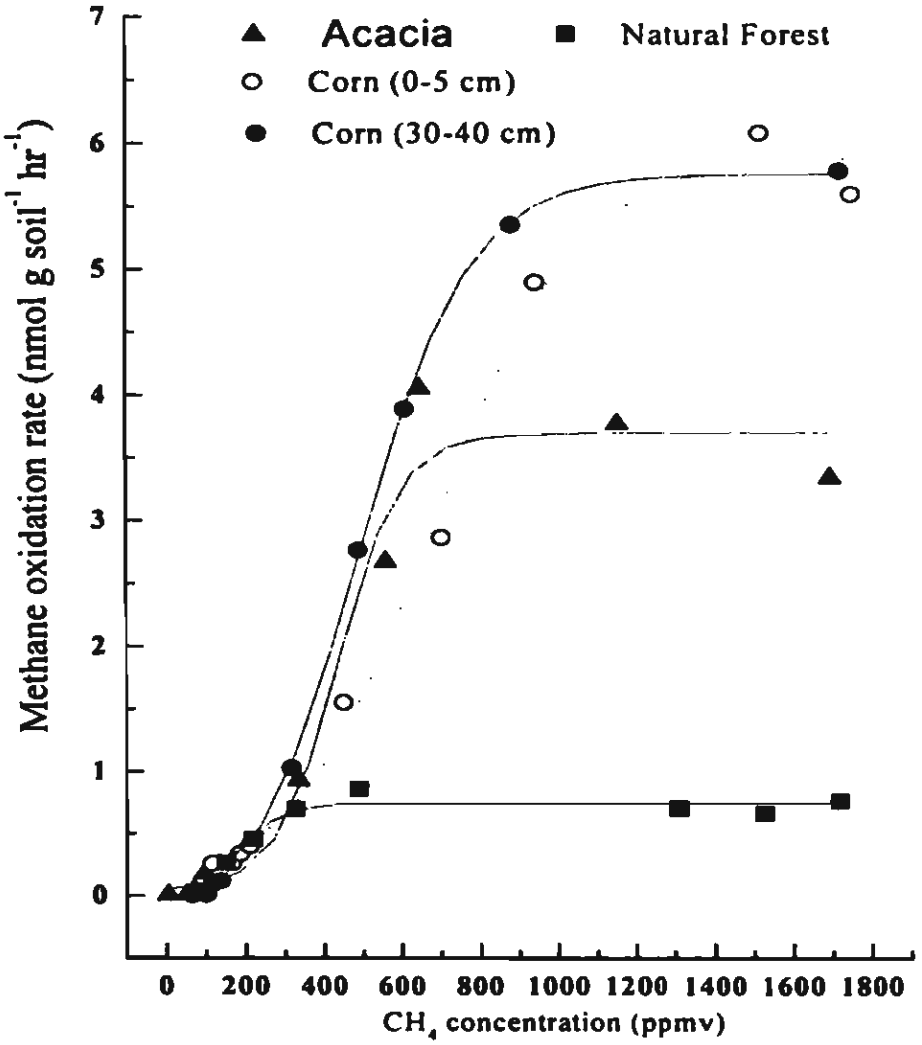
Acacia
Natural Forest
Corn (0-5 cm)
Corn (30-40 cm)
Methane oxidation rate (nmol g soil^{-1} hr^{-1})
CH_4 concentration (ppmv)
0
1
2
3
4
5
6
7
0
200
400
600
800
1000
1200
1400
1600
1800

Fig. 4

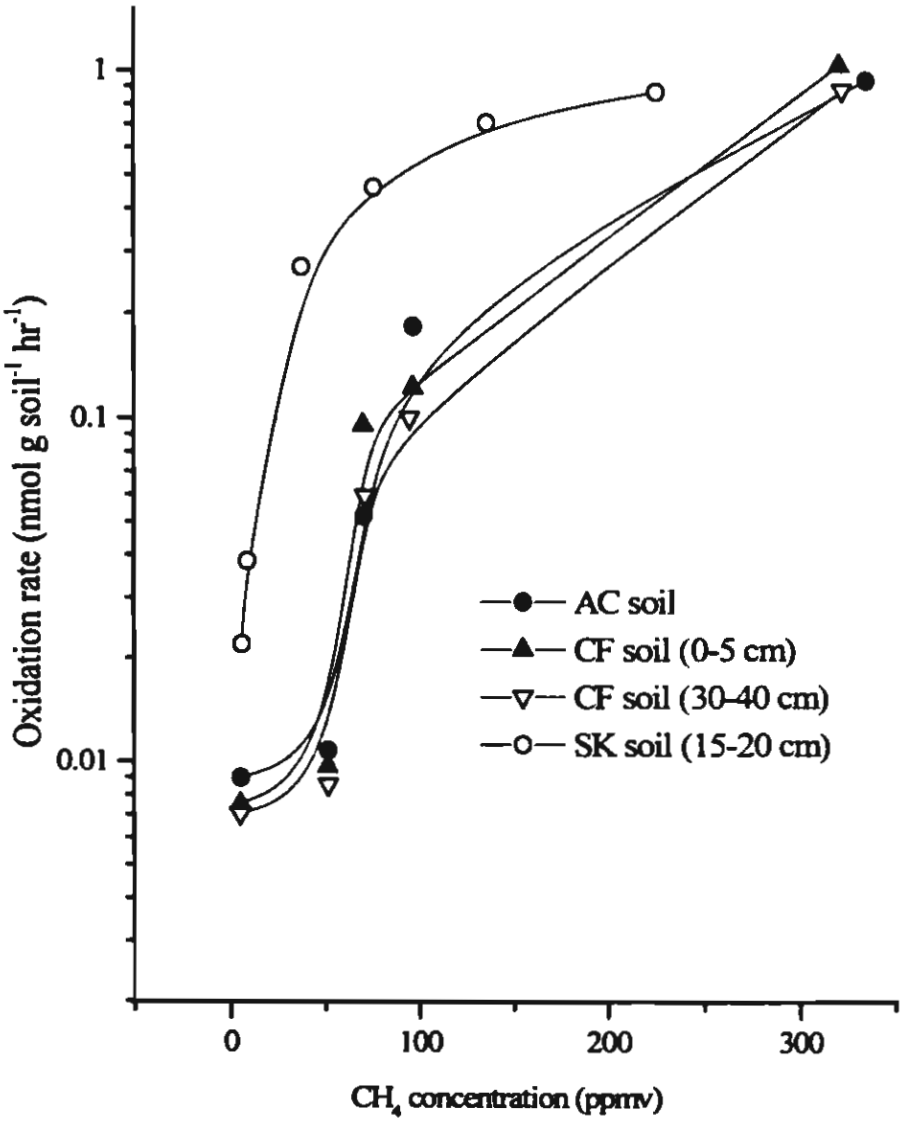
Oxidation rate (nmol g soil^{-1} hr^{-1})
1
0.1
0.01
0
100
200
300
CH_4 concentration (ppmv)
AC soil
CF soil (0-5 cm)
CF soil (30-40 cm)
SK soil (15-20 cm)

Fig. 5

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Effects of Land Use on Methane Oxidation in Tropical Upland Soils

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Abstract

Methane is an important greenhouse gas and methane oxidation in soil represents a significant sink, accounting for approximately 6% of the global methane sink. Land use is one of influencing factors that contributes to variations of methane oxidation by soils. In this study we investigated methane oxidation activity in different land use in Thailand. Three different land uses included dry evergreen forest, reforestation (a 16-year old *Acacia mangium*), and cultivation (corn field) that were located in Nakorn Ratchasima province Thailand were selected. Methane oxidation rates were measured during January – December 2003 by using a close chamber technique. Results reveal that methane oxidation in forest soil and reforestation soils were significantly higher than cornfield soil. The average methane oxidation from forest soil, reforestation soil, and cornfield soil were 1.50 ± 0.89, 1.17 ± 0.48, and -12.87 ± 48.65 mg $CH_4/m^2/d$ respectively (negative sing designates net methane emission). Soil chemical and physical property were also observed in all of land uses. Methane oxidation rates were higher during wet season, indicating that soil moisture is one of influencing factors on methane oxidation by soils. High soil moisture might limit gas diffusion (oxygen and methane) from atmosphere, thus, reduce soil oxidation rate in soil. In addition, application of nitrogen fertilizer during corn cultivation may also cause the lower oxidation rate than other sites. Inorganic nitrogen compounds especially ammonia is known for their inhibitory effects on methane oxidation, presumably through competition with methane for the binding site of methane oxidation-catalyzing enzyme. The results indicate that methane oxidation is strongly affected by land use types and high oxidation rate is usually found in undisturbed-natural forest soils. Reforestation, however, help recover methane oxidation activity.

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Other output:

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