Changes in *pmoA* gene containing methanotrophic population and methane oxidation potential of dry deciduous tropical forest soils

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In natural ecosystems such as forests topographical gradients, species composition variability and seasonality, are the potential drivers of methane (CH_4) metabolism, and thus, of the population size and activities of methane oxidizing bacteria (MOB). To test the hypothesis that topography, tree species and seasonal variability influence MOB population and soil methane oxidation potential (MOP), we conducted two consecutive years of study selecting three sites in a dry deciduous tropical forest in Chandauli district of eastern Uttar Pradesh, India. The qPCR results showed a large variation in MOB population size (copies g^{-1} dws), ranging from 1.0×10^6 to 2.1×10^7 and 9.0×10^5 to 2.2×10^7 during 2016 and 2017 respectively. The distribution of MOB population revealed the trend: hilltop > middle > hillbase with its maxima in the winter and minima in the rainy season. Laboratory incubation study revealed a similar trend in soil MOP (ng CH_4 g^{-1} h^{-1} dws), it varied from 10.6 to 20.6 and 10.5 to 20.7 during 2016 and 2017 respectively. The outer canopy soils showed lower MOB population and MOP compared to under canopy soils of tree species *Butea monosperma*, *Madhuca indica* and *Tectona grandis* during both years of study. The topography, season and tree species significantly influenced the MOB population size and MOP. Soil MOP showed a highly significant correlation ($r = 0.89$; $p < 0.01$) with MOB population, and a negative correlation was found with soil moisture ($r = 0.76$; $p < 0.01$). The results indicate that the dry deciduous tropical forest soils are potential sinks of atmospheric CH_4 wherein, the MOB population characteristically responds to topographical changes, tree species and seasonal shifts driving collectively the overall MOP of forest soils.

Keywords: Methanotrophs, topography, season, tree species, tropical dry deciduous forest.

METHANE (CH_4), an important greenhouse gas, contributes to about 20% of the global warming effects[1]. Since

the beginning of the industrial era (1750) to 2011, CH_4 concentration has increased from 715 to 1803 ppb; with 1% annual increase over a century[2]. The largest sink (about 90% of the total CH_4) is its photochemical oxidation mediated by reactions with hydroxyl (OH^-) radicals[3]. Other significant sinks include diffusion into the stratosphere, and microbial oxidation. Although total CH_4 sink strength in terrestrial ecosystems is driven by microbial oxidation in the order 33.5 ± 0.6 Tg CH_4/y (ref. 4), role of the latter cannot be underestimated as these are important contributors to attenuate CH_4 flux at the oxic–anoxic interfaces[5].

Methane oxidation in aerobic soils is mediated by methanotrophs (methane oxidizing bacteria; MOB), a subgroup of methylotrophs. These bacteria utilize CH_4 as the sole carbon and energy source[6,7]. Among terrestrial ecosystems, methanotrophic community inhabiting forest soils has been identified as a major contributor to atmospheric CH_4 oxidation[8]. Atmospheric CH_4 oxidation studies are mainly confined to temperate forest soils[9–11], whereas similar reports in tropical and subtropical forest soils, are very limited[12–14]. Some earlier studies suggested that CH_4 oxidation potential (MOP) of soils depends on the diffusive gas transport[8], soil nitrogen content[15,16], land use[17], soil temperature[18], pH[19], salinity[20], season[21], topographic positions[22] and tree species[23]. The CH_4 uptake rate studied in temperate forests reported higher values during summer compared to winter[21,24]. In earlier studies, soil moisture and temperature were the key regulatory factors that drive CH_4 oxidation in soil. High soil moisture creates barrier for oxygen diffusion into the soil, required by aerobic methanotrophic bacteria, whereas increasing temperature up to optimum level (25–35°C) enhanced the methanotrophic activity[25]. Methanotrophic population and their activity are also influenced by soil physical properties[26]. Thus it can be assumed that multivariate factors are linked to the regulation of CH_4 oxidation in the natural ecosystem, however, the pattern of MOB population and their activity are not well documented. Most studies in forest soils are, hence focused on the activity and diversity of methanotrophs. For tropical

Table 1. Physico-chemical characteristics of soils (mean ± SE)

Characteristics	Experimental sites		
	Hilltop (Naugarh)	Middle (Jamsoti)	Hillbase (Chakia)
Coordinates	24°50′N, 83°15′E	24°57′N, 83°11′E	25°03′N, 83°13′E
Altitude (msl) (m)	300	150	90
Bulk density (g cm^{-3})	1.94 ± 0.09	1.90 ± 0.04	1.77 ± 0.03
Soil texture	Sand 25%, silt 54.63%, clay 20.37%	Sand 20%, silt 54%, clay 26%	Sand 16%, silt 56%, clay 28%
WHC (%)	40.55 ± 0.21	43.18 ± 0.41	45.59 ± 0.20
TN (mg kg^{-1})	823.66 ± 16.28	1230 ± 18.71	1560 ± 33.22
TOC (mg kg^{-1})	6220.66 ± 158.33	9803.33 ± 278.35	16311.67 ± 115.97
pH	6.99–5.89	6.76–5.21	7.53–6.06
EC (µS m^{-1})	21.73 ± 1.86	57.25 ± 2.58	74.96 ± 1.0

dry deciduous forest soils, the information on MOB population size seems equally important but has not been studied well.

Quantitative data in these lines can be used to relate potential changes in gene abundance and/or the level of gene expression associated with shifts in abiotic determinants[27]. Accordingly, systematic database on the magnitude of MOB population size may possibly add to furthering our understanding of the significance of MOB in the ecology of CH_4 oxidation in forest soils. Few reports are available on the population size of methanotrophs in forest soils of temperate regions[28,29]. Giri *et al.*[30] used most probable number (MPN) technique for counting MOB in tropical forest soils. This technique, however, has its own bias due to selective culture conditions. Overall, in tropical forest soils, information on MOB is almost scanty, particularly in the context of Indian tropical forests that constitute a major global share[31].

Understanding the key determinants of activity and MOB population size here, has relevance because the dry deciduous tropical forest of Vindhyan region constitutes ~53% of total forest area of India. This study was targeted to investigate the effect of topography, tree species and seasonal factors on MOP and MOB population size in the dry deciduous tropical forest soils of dry tropics.

Materials and methods

Study site

The experimental sites were located in the dry deciduous tropical forest of Vindhyan plateau situated in Chandauli district of eastern Uttar Pradesh, India. The climate of this region is tropical monsoonal, and receives about 82–85% rainfall during rainy season (late June–September) due to southwest monsoon. The whole year is divisible into three seasons: rainy (late June–September), winter (October–February) and summer (March–early June). The annual mean rainfall ranges from 400 to 900 mm, while mean monthly temperature varies between a minimum of 8°C in January to maximum 42°C in June. The average rainfall in rainy, winter and summer season during study period was 685.0, 3.0 and 2.0 mm respectively. The soil order of the study sites was Ultisols and red to brown in colour. The vegetation in the study area represents northern tropical mixed deciduous forest[32], with the abundance of woody plants such as *Butea monosperma*, *Boswellia serrata*, *Lagerstroemia parviflora*, *Tectona grandis*, *Madhuca indica*, *Acacia catechu*, *Ziziphus oenoplia*, *Ziziphus glaberrima*, etc. The under storey vegetation of the forest floor mainly comprised of *Lantana camara*, *Eragastris* sp., *Dicanthium* sp., *Desmostachya binnata*, *Oplismenus* sp., *Trifolium* sp., *Cassia tora*, *Phyllanthus urinaria*, *Sida acuta* and *Sida cordifolia*.

Experimental design and soil sampling

Soil samples were collected from three forest sites of Naugarh, Jamsoti and Chakia, representing upper, middle and lower elevation landscapes at a height of 300 m, 150 m and 90 m respectively (Table 1). At each site, three plots of 1 hectare (ha) area were randomly demarcated, based on the respective dominance of *Butea monosperma*, *Tectona grandis* and *Madhuca indica*. From each plot, soil samples were taken from 0–15 cm depth using a corer in triplicate at five randomly selected locations. Triplicates were mixed to form a composite sample ($n = 15$). Also, at each site, three plots, 1 ha each, were selected to represent off-canopy condition. Thus, a total of 30 composite soil samples (15 each for inside and outside canopy) were collected from each site in a month, and three times in a season (rainy: July, August, September; winter: November, December, January; summer: March, April, May), for two consecutive years (2016 and 2017). Soil sampling was avoided during February, June and October as they were identified as transition month of winter–summer, summer–rainy and rainy–winter seasons respectively. Soil samples were sieved (2 mm mesh) to remove fine roots, and were aliquoted into two: one part was stored at –20°C for qPCR analyses and the other at 4°C for CH_4 oxidation experiments and soil

physico-chemical studies. All analyses were done in triplicate.

Soil properties

Soil variables such as pH, texture, bulk density (BD) and water holding capacity (WHC) were determined using standard protocol described in Vishwakarma and Dubey[33]. In brief, the soil particle proportions were estimated using sieves of variable mesh sizes and hydrometer method[34]. The BD of the soil samples were determined by core method[35]. The perforated circular brass boxes were used to determine WHC of the soil samples as described by Piper[35]. The soil : water (1 : 2.5; w/v) suspensions were used to measure soil pH using pH meter equipped with a glass electrode (CyberScan pH 510, Eutec Instruments Pte Ltd). The electrical conductivity was measured in soil water suspension using digital conductivity meter (Systronics, India). Macrokjeldahl method was used for total soil N estimation[36]. For the measurements of organic carbon (C), dichromate oxidation procedure was followed[37]. Leaf litter was collected during the peak litter fall period (February to March) of the year 2017. The litter samples were oven dried, and powdered to determine C and N content as per the standard protocol.

Soil moisture and ammonium–N content

Soil moisture was determined by gravimetric method. Exchangeable NH_4^+–N was estimated in 2 M KCl extracts, and analysed spectrophotometrically by phenate method[38].

Methane oxidation potential

Laboratory incubation experiment was used to determine CH_4 oxidation in soils[33]. In brief, 10 g of soil sample was allowed to equilibrate for 24 h at 25°C, and then, transferred to gastight 135-ml Erlenmeyer flask, added with 50 μmol l^{-1} (~1150 ppmv) CH_4 (Sigma Aldrich; 99.9% pure), and incubated for 10 days in dark at 30°C. Further, for CH_4 analysis, headspace samples (100 μl) were withdrawn using a gas-tight glass syringe every 24 h up to 240 h. The gas samples were analysed in Agilent 7820A gas chromatograph attached with a flame ionization detector (FID), Porapak N column, and nitrogen as the carrier gas (flow rate 12 ml min^{-1}). The temperature for column, injector and detector was maintained as 180°C, 210°C and 250°C respectively. MOP is expressed as nano gram CH_4 per hour per gram of dry weight soil (dws).

Population size of MOB

Genomic DNA was extracted from the forest soil samples (0.5 g) using FastDNA Spin Kit for Soil (MP Bio-

medicals, LLC, Solon, Ohio, USA) by a bead beater (FastDNAprep, MP Bio, USA) for lysis of cells according to a manufacturer's protocol. Real-time PCR was performed using iCycler iQ5 thermocycler (Bio-Rad). Population of methanotrophs was quantified using specific primer pair A189f 5′GGNGACTGGGACTTCTGG3′ and mb661r 5′CCGGMGCAACGTCYTTACC3′ (refs 39, 40). The reaction mixture (20 μl) contained 10 μl of PowerUp™ SYBR™ Green Master Mix (Applied Biosystem), 0.4 μl of each primer (10 μM), 2 μl DNA template (50 ng/μl) and DNAase/RNase-free water to the final volume of 20 μl. For each sample, the reactions were performed in duplicate and for plasmid standards, samples were analysed in triplicate. PCR conditions for methanotrophs were optimized as three step cycling protocol with the initial 4 min denaturation (94°C), followed by 40 cycles of denaturation (94°C for 15 s), annealing at 56°C for 30 s and extension at 60°C for 30 s. Fluorescence data were acquired at 77.5°C after each consecutive cycle. After completion of 40 cycles, the melting curve analyses were performed by increasing the temperature from 60°C to 95°C and recording fluorescence for every 0.5°C increase. Plasmid DNA containing the target gene (methanotrophic bacteria specific *pmoA* gene) was taken as the standard DNA in PCR (real time) assay acquired by PCR-cloning using A189f and mb661r primer pairs. Plasmids were isolated using HiPurA (TM) Plasmid DNA Miniprep Purification Kit (Himedia), and DNA concentration was determined by Nanodrop 2000 spectrophotometer (Thermoscientific). Standard curves were prepared with triplicate 10-fold dilutions of plasmid DNA[29].

Data were tested for PCR efficiency. The values for methanotrophs (slope: –3.729; R^2: 0.983; efficiency: 85.4%) were within the suggested range. Zhang and Fang[41] mentioned that in practice, for an appropriate standard curve, R^2 should be above 0.95, with slope from –3.0 to –3.9 corresponding to PCR efficiency of 80–115%.

Statistical analysis

For statistical treatments, data were subjected to multivariate analysis of variance (MANOVA) to record significant effect of year, topography, season and tree species on the observed variables. Simple Pearson's correlation coefficient analysis was applied to determine the relationship of parameters to each other. The statistical analyses were performed using IBM SPSS version 20.0 (ref. 42).

Results

Soil properties and leaf litter C and N

Results on soil characteristics are presented in Table 1. Soil texture consisted predominantly of silty type at higher

Table 2. Variations in soil moisture (%) and NH_4^+–N content ($\mu g\ g^{-1}$ dry weight soil) at hilltop (Naugarh), Middle (Jamsoti) and hillbase (Chakia) under the canopy of *B. monosperma*, *T. grandis*, *M. indica* and outer canopy during rainy, winter and summer season for two consecutive years (2016 and 2017) (mean ± SE)

| | | Tree species | | | | | | | |
| | | *B. monosperma* | | *T. grandis* | | *M. indica* | | Outer canopy | |
Site	Season	Moisture	NH_4^+–N	Moisture	NH_4^+–N	Moisture	NH_4^+–N	Moisture	NH_4^+–N
2016									
Hilltop (Naugarh)	Rainy	16.7 ± 0.75	0.19 ± 0.03	18.6 ± 1.39	0.16 ± 0.02	15.8 ± 2.02	0.17 ± 0.04	12.2 ± 0.57	0.12 ± 0.02
	Winter	5.1 ± 0.54	0.67 ± 0.01	8.7 ± 0.67	0.72 ± 0.01	7.9 ± 0.16	0.73 ± 0.02	6.0 ± 0.54	0.54 ± 0.00
	Summer	4.3 ± 0.36	0.75 ± 0.01	6.3 ± 0.35	0.73 ± 0.04	5.1 ± 0.48	0.79 ± 0.02	3.6 ± 0.37	0.64 ± 0.02
Middle (Jamsoti)	Rainy	17.4 ± 0.29	0.20 ± 0.02	19.7 ± 1.84	0.27 ± 0.02	17.5 ± 0.86	0.24 ± 0.05	14.8 ± 0.16	0.16 ± 0.02
	Winter	6.9 ± 0.26	0.67 ± 0.05	8.6 ± 0.28	0.71 ± 0.04	7.4 ± 0.17	0.63 ± 0.02	6.4 ± 0.65	0.57 ± 0.01
	Summer	4.9 ± 0.41	0.72 ± 0.04	7.9 ± 0.53	0.73 ± 0.05	5.4 ± 0.51	0.77 ± 0.02	3.9 ± 0.16	0.58 ± 0.00
Hillbase (Chakia)	Rainy	17.0 ± 0.12	0.76 ± 0.09	22.4 ± 0.97	0.73 ± 0.02	19.8 ± 0.33	0.49 ± 0.04	13.3 ± 0.48	0.43 ± 0.02
	Winter	7.6 ± 0.55	0.78 ± 0.02	9.5 ± 0.36	0.73 ± 0.08	8.8 ± 0.13	0.86 ± 0.01	6.0 ± 0.44	0.78 ± 0.03
	Summer	5.8 ± 0.32	0.77 ± 0.01	9.4 ± 0.23	1.05 ± 0.09	6.5 ± 0.29	0.80 ± 0.03	5.1 ± 0.43	0.76 ± 0.02
2017									
Hilltop (Naugarh)	Rainy	15.7 ± 0.08	0.14 ± 0.03	17.3 ± 0.25	0.11 ± 0.02	16.5 ± 1.40	0.13 ± 0.04	11.9 ± 0.84	0.07 ± 0.02
	Winter	5.5 ± 0.25	0.62 ± 0.01	7.2 ± 0.28	0.65 ± 0.02	5.6 ± 0.41	0.63 ± 0.02	4.6 ± 0.22	0.46 ± 0.01
	Summer	4.4 ± 0.39	0.68 ± 0.01	6.0 ± 0.39	0.71 ± 0.03	5.5 ± 0.45	0.74 ± 0.02	3.6 ± 0.69	0.55 ± 0.01
Middle (Jamsoti)	Rainy	17.9 ± 0.30	0.17 ± 0.02	21.0 ± 0.25	0.18 ± 0.03	21.0 ± 0.25	0.22 ± 0.05	14.6 ± 0.42	0.14 ± 0.02
	Winter	6.3 ± 0.22	0.69 ± 0.04	8.4 ± 0.78	0.65 ± 0.03	8.7 ± 0.78	0.59 ± 0.02	3.7 ± 0.19	0.52 ± 0.02
	Summer	5.6 ± 0.15	0.75 ± 0.04	6.1 ± 0.24	0.72 ± 0.04	6.1 ± 0.24	0.74 ± 0.02	3.8 ± 0.58	0.55 ± 0.01
Hillbase (Chakia)	Rainy	17.0 ± 0.18	0.74 ± 0.04	18.2 ± 0.15	0.68 ± 0.02	15.4 ± 0.35	0.61 ± 0.03	14.5 ± 0.41	0.62 ± 0.01
	Winter	9.1 ± 0.10	0.89 ± 0.00	9.8 ± 0.29	1.01 ± 0.01	7.8 ± 0.76	0.92 ± 0.04	5.9 ± 0.42	0.92 ± 0.02
	Summer	6.0 ± 0.10	0.94 ± 0.01	6.6 ± 0.11	0.98 ± 0.02	5.8 ± 0.33	1.02 ± 0.07	4.9 ± 0.24	0.86 ± 0.01

topography (Naugarh and Jamsoti), while silty clay loam type at hillbase (Chakia). Bulk density varied from 1.77 to 1.94 $g\ cm^{-3}$. The bulk density was highest at hilltop followed by middle and hillbase. The soil pH ranged from 5.21 to 7.53. The water holding capacity was maximum for hillbase followed by middle and hilltop. Total organic N and C ranged from 823.6 to 1536 and 6220.7 to 16311.0 $mg\ kg^{-1}$ respectively, with highest value at hillbase followed by middle and hilltop. The EC value ranged from 21.7 to 74.96 $\mu S\ m^{-1}$ along the topography. In leaf litter samples, C content was maximum (467.5 $mg\ g^{-1}$) in *T. grandis* followed by *M. indica* (433.07 $mg\ g^{-1}$) and *B. monosperma* (413.07 $mg\ g^{-1}$). The N content also followed the similar trend as C with the value 20.1, 10.3 and 5.1 $mg\ g^{-1}$ for *T. grandis, M. indica* and *B. monosperma* respectively.

Soil moisture and ammonium–N content

Soil moisture and NH_4^+–N content at each site during the study period (2016 and 2017) have been shown in Table 2. Soil moisture was significantly higher during rainy season, and it ranged from 11.9% to 22.4%, 3.7% to 9.8% and 3.6% to 9.4% during rainy, winter and summer season respectively, at all the sites throughout study period. Multivariate analysis of variance (MANOVA) indicated significant effect of year ($F_{1,214} = 33.51$; $p < 0.001$), topography ($F_{2,213} = 33.51$; $p < 0.001$), season ($F_{2,213} =$ 4091.04; $p < 0.001$) and tree species ($F_{3,212} = 223.80$; $p < 0.001$) on the soil moisture content.

At all the sites, soil NH_4^+–N content varied from 0.07 to 0.76, 0.46 to 1.01 and 0.55 to 1.05 $\mu g\ g^{-1}$ dws during rainy, winter and summer season respectively. The NH_4^+–N content of the soil showed pattern reverse to that of soil moisture being maximum during summer, followed by winter and rainy season at each study site. The significant effect of the topography ($F_{1,214} = 900.88$; $p < 0.001$), season ($F_{2,213} = 1864.24$; $p < 0.001$), tree species ($F_{3,212} = 82.34$; $p < 0.001$), except year ($F_{1,214} = 0.76$; $p > 0.001$) was observed on the soil NH_4^+–N content.

Methane oxidation potential

Methane oxidation potential of soils varied from 12.2 to 21.5, 9.9 to 21.0 and 11.20 to 20.8 $ng\ CH_4\ g^{-1}\ h^{-1}$ dws at Hilltop (Naugarh), Middle (Jamsoti) and Hillbase (Chakia) forest site respectively (Figure 1). The MANOVA demonstrated topography ($F_{2,213} = 74.12$; $p < 0.001$), season ($F_{2,213} = 1703.68$; $p < 0.001$) and tree species ($F_{3,212} = 102.34$; $p < 0.001$) were significant determinants of soil MOP. MOP varied along the topography being maximum at hilltop followed by middle and hillbase. Significant ($p < 0.001$) seasonal variability, with high CH_4 oxidation rates during dry periods (winter and summer) compared to wet season, was recorded. Tree species had significant ($p < 0.001$) effect on CH_4 oxidation with the maximum
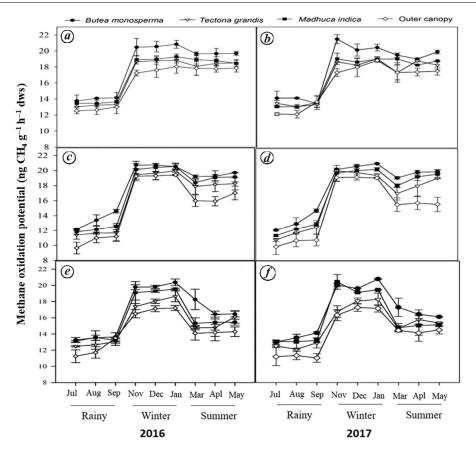
Figure 1. Variation in the CH_4 oxidation potential at hilltop (Naugarh) (*a* and *b*), middle (Jamsoti) (*c* and *d*) and hillbase (Chakia) (*e* and *f*) forest site respectively, during 2016 and 2017.

for *Butea monosperma* followed by *Madhuca indica*, *Tectona grandis*, and the outer canopy.

Quantification of MOB population

Variations in methanotrophic population size along the topography, in different seasons, and tree species for the year 2016 and 2017 are depicted in Figure 2. The population size of methanotrophs as estimated by the *pmoA* gene copy number varied between 7.0×10^5 and 3.0×10^7 copies g^{-1} dws. The MANOVA showed significant effect of topography ($F_{2,213} = 63.58$; $p < 0.001$), season ($F_{2,213} = 584.52$; $p < 0.001$) and tree species ($F_{3,212} = 41.5$; $p < 0.001$) on the population size of methanotrophs. The population size was smallest in soil samples from the outer canopy. The methanotrophic population size was maximum in soils from the under canopy of *Butea monosperma*, followed by *Madhuca indica* and *Tectona grandis* irrespective of the topography and season.

Discussion

Effect of topography

Methane oxidation range recorded here (9.9 to 21.5 ng $CH_4\ g^{-1}\ h^{-1}$ dws), is comparable with those observed for

soil of the temperate forest in Massachusetts[43], hardwood forest of South Korea[44], and the deciduous forest of Europe[29]. Topographical gradient showed significant ($p < 0.001$) effect on CH_4 oxidation being highest at hilltop followed by middle and hillbase site. Such a difference in CH_4 oxidation values could be correlated with high population size of methanotrophs at higher topography followed by middle and hillbase (Figure 2). So far, MOB population in terms of gene copy number is not available for data comparison from other regional scale studies. The data presented here, which form the first report from Indian tropics, can be used for comparing the values reported from other ecozones/ecosystems using comparable methodologies. Barcena *et al*.[28] reported MOB population between 10^6 and 10^7 copies g^{-1} dws in oak dominated temperate forest of Denmark. Malghani *et al*.[29] recorded methanotrophic population ranging from 4.3×10^7 to 7.5×10^7 copies g^{-1} dws in a temperate deciduous beech forest of Germany. We found strong correlation ($r = 0.89$; $p < 0.01$) between CH_4 oxidation and methanotrophic population irrespective of the topographic variation. Further, the hilltop site showed significant negative correlation ($r = -0.76$; $p < 0.01$) between MOP and the soil moisture content. The results of regression analysis of CH_4 oxidation and methanotrophic population to the soil physicochemical variables (BD, clay content,
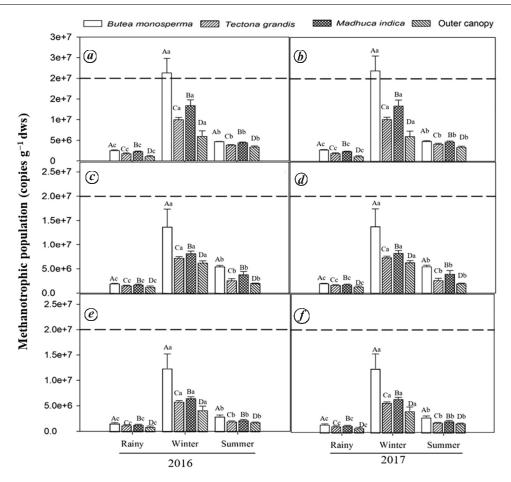
Figure 2. Population size of methanotrophs at hilltop (Naugarh) (*a* and *b*), middle (Jamsoti) (*c* and *d*), and hill-base (Chakia) (*e* and *f*) study site for two consecutive years. Different uppercases denote significant differences (at $p < 0.01$) between tree species in the same season and different lowercases denotes significant differences between seasons in the same tree species according to the Tukey's HSD test.

WHC, total organic carbon, total nitrogen and electric conductivity) are presented in Table 3. The clay content of the soil was found to be negatively correlated with the CH$_4$ oxidation and MOB population size ($R^2 = 0.98$; $p < 0.001$). The hilltop site with high coarse particles showed higher CH$_4$ oxidation compared to hillbase rich in clay content. This result is similar to that of Boeckx *et al.*[45] who reported higher CH$_4$ uptake rate in coarse soil compared to fine textured soils of Belgium forests. Fine particles of the soil regulate CH$_4$ uptake by regulating the diffusion of atmospheric CH$_4$ and oxygen in the soil[46]. In general, the site situated at the lower elevation, receives high erosional residues rich in soil organic matter, soluble salts, mineral colloids, etc. subject to natural downhill movement assisted by runoff. Subject to limited O$_2$ supply, the accumulation of organic substances creates anaerobic microhabitats and consequently limits methanotrophy[47]. The soil organic carbon and nitrogen were found to be negatively correlated with CH$_4$ oxidation and population size (Table 3). The overall results show that topographical variations with characteristically different soil properties are important determinants of population

size of CH$_4$ oxidizers, and consequently the MOP of the soil.

Effect of tree species

MOP and MOB population size, both varied significantly with tree species. The soil samples from the under canopy of *Butea monosperma* showed maximum CH$_4$ oxidation rate followed by those cultivated with *Madhuca indica*, *Tectona grandis* and outer canopies. The outer canopy soil with poor organic input was poor in CH$_4$ oxidation. The methanotrophic population at each outer canopy site, during every season was also poor. In a previous study, Reay *et al.*[23] reported the effect of vegetation type (alder, oak, Norway spruce, Scots pine and grassland) on high and low affinity MOP in Bowland forest, UK. They reported large reduction in CH$_4$ oxidation in alder soils than those from other vegetation types, and linked the process with the inhibitory effects of high nitrate concentrations. Tree species influence nitrogen capture and mineralization[48]. The litter composition strongly

Table 3. Result of regression analysis ($Y = a \pm bX$) of CH_4 oxidation potential (MOP) (ng CH_4 g^{-1} h^{-1} dws) and methanotrophic population size (Y, $\times 10^6$ copies g^{-1} dws) to different variables (bulk density, clay content, EC, WHC, total organic carbon, total nitrogen)

Y	X	a	b	R^2	Significance level
MOP	Bulk density	1.66	0.01	0.17	NS
MOP	Clay	41.07	−0.90	0.98	***
MOP	EC	133.33	−4.52	0.59	*
MOP	WHC	46.17	−0.17	0.09	NS
MOP	Total organic carbon	29665.68	−1039.10	0.88	***
MOP	Total nitrogen	2589.35	−76.63	0.97	***
Methanotrophic population	Bulk density	0.03	1.75	0.26	NS
Methanotrophic population	Clay	32.47	−1.61	0.94	*
Methanotrophic population	EC	86.31	−7.35	0.46	***
Methanotrophic population	WHC	45.15	−0.43	0.18	NS
Methanotrophic population	Total organic carbon	20180.80	−1971.20	0.95	***
Methanotrophic population	Total nitrogen	1868.57	−140.88	0.98	***

MOP; Methane oxidation potential; NS, Not significant; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 4. Pearson correlation coefficient of parameter studied during two consecutive years at all the sites studied

Parameters	Moisture	Ammonium–N	MOP	*pmoA* gene copy no.
Moisture	1			
Ammonium-N	−0.61*	1		
MOP	−0.76*	0.32*	1	
pmoA gene copy no.	−0.41*	0.28*	0.90*	1

*Significant at $p < 0.01$; $n = 216$.

influences microbial colonization. The organic C and N content of the leaf litter of the tree species considered in this study showed significant variations. Fresh leaf litter of *Tectona grandis*, rich in organic C and N, might release N to the soil in proportion high enough to interfere with CH_4 oxidation through regulation of methanotrophic activity. Soil moisture also varied significantly ($p < 0.05$) with tree species, being highest for the under canopy soil of *T. grandis* followed by *M. indica* and *B. monosperma* (Table 2). The CH_4 oxidation rate and MOB population size were negatively correlated with the soil moisture (Table 4). Therefore, variation in soil moisture content could lead to changes in CH_4 oxidation across under canopy soils of different plant.

Effect of season

The MANOVA revealed significant ($p < 0.001$) seasonal variation in CH_4 oxidation. Seasonal variations in CH_4 oxidation were well linked with soil moisture. During rainy season, with maximum soil moisture, CH_4 oxidation declined sharply (Figure 1). Previous reports also indicated decrease in CH_4 oxidation rates[21] with increasing soil moisture. The population size of methanotrophs was high during winter, followed by summer and rainy season (Figure 2). In summer, the moisture content of the soil goes below 5%, which adversely affects the microbial population. Although soil moisture content is negatively correlated with CH_4 oxidation, it may show positive correlation below 8% (ref. 49). Ammonium–N was maximum in summer, followed by winter and rainy (Table 2). High ammonium–N content in the soil might be attributed to low nitrification during summer. In rainy season, high demand for nutrients by the growing vegetation leads to decline in NH_4^+–N (ref. 50). While in most cases, CH_4 oxidation of in forest soils is inhibited by N additions[51,52], NH_4^+–N in our study, showed significant but poor positive correlation with CH_4 oxidation (Table 3). Methane oxidation is inhibited in case inorganic N was substantially high. Positive effects of N on CH_4 oxidation have been reported in rice field[53] and forest soils[54].

Inter-annual variation

The CH_4 oxidation and methanotrophic population did not show significant annual variation although, for soil moisture it was significant. The inter-annual variation in soil moisture might be due to relatively poor rainfall during 2017. Although methanotrophic population showed negative relationship with the soil moisture, no significant changes were recorded annually.

Conclusions

The estimates of MOP of dry tropical forest soils provide a reference for comparing other ecosystems in terms of process and the associated microbial communities. Our analyses document the extent of variation in MOP and MOB population distribution in relation to ecosystem variables linked with topography, tree species and seasonality. The soils of all the forest sites showed CH_4 oxidizing potential, although it varied significantly with the topography, season and tree species. The soil MOP showed strong positive correlation with the MOB population size. Soil moisture emerged as the important driver

regulating CH_4 oxidation largely by regulating the diffusion of CH_4 and O_2. The study unravels the drivers of CH_4 cycle in the seasonally dry tropical forest, and will certainly help in exploring regional contributors to global CH_4 cycle. The study merits adding new dimensions furthering the need for assessment of molecular diversity of MOB, establishing the causal linkages, and enhancing sink efficiency of forest soils.

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