

Temperature Dependent Decline in Soil Methane Oxidizing Bacterial Population in Tropical Dry Deciduous Forest Ecosystems

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Abstract: Culturable methanotrophic bacteria (CMB) were studied in the soils of forest and savanna of tropical dry deciduous forest ecosystems employing most probable number (MPN) technique. The spatiotemporal study was conducted at the six sites differing in the soil physicochemical properties and vegetational cover. CMB population was high in the moist sites compared to the dry sites and in sub soil below 10 cm depth. The top soil population ranged between 7.0×10^4 to $7.1 \times 10^6 \text{ g}^{-1}$ dry soil. The population declined exponentially with depth. In temporal study at bimonthly interval for two consecutive years, high CMB population was in winter that declined 10 - 20 fold in rain. The CMB population variation was explained solely by temperature (59%), temperature and soil nitrate (76%), and temperature soil nitrate and moisture (77.4%) in the step wise regression analysis.

Index Terms Dry tropical forest, Methanotrophs; MOB, NH_4^+ -N; NO_3^- -N; Soil moisture; spatiotemporal variation,

1 INTRODUCTION

Methane (CH_4) produced by various natural and anthropogenic sources (~ 600 Tg) is mainly destructed chemically due to reactions of atmospheric radical (~540 Tg y^{-1}) and biologically oxidized in aerobic soils due to bacterial activity (~ 30 Tg) [1]. The CH_4 sink of the soils is due to the activity of methanotrophic bacteria (Bykova et al 2006). The soil CH_4 sink is very critical in the sense that it is nearly equal to the net annual addition of methane to the atmosphere. The decrease in the soil methane sink is likely to increase the net methane addition to the atmosphere. These bacteria utilize atmospheric or endogenous CH_4 for their carbon and energy need, depending upon the availability and play an important role in global CH_4 budget by regulating CH_4 exchange between land and atmosphere. The methanotrophic bacteria have been divided into three groups, type II (Methylosinus, Methylocystis, Methylocella, Methylocapsa), type I (Methylomonas, Methylobacter, Methylochromium, Methylosphaera, Methylosarcina, Methylothermus) and type X (Methylococcus and Methylocaldum) based on cell morphology, intracellular membrane, resting stage and genetic characters [2].

It is difficult to culture all the members in a common nutrient medium. For evaluating diversity of methanotrophs in an ecosystem variously supplemented nutrient medium could be used [3]. The nitrate mineral salt as a growth medium for methanotrophs has been reviewed. Methanotrophic bacteria has been enumerated in ricefield soils by plating and MPN techniques [4]. The CMB population was well correlated with the CH_4 oxidation rate in landfill covers. The methane sink activity has been shown to vary between 0.36 to 0.57 $\text{mg m}^{-2} \text{h}^{-1}$ in this tropical dry deciduous ecosystem [5]. The information's regarding the methanotrophic bacterial population are lacking from such ecosystems. The CMB population could be affected by edaphic parameters, vegetation, soil nutrient or climatic conditions. The present study was conducted to study the influence of environmental variation on the methane oxidizing bacterial population in the tropical dry deciduous ecosystem.

2. MATERIAL AND METHODS

2.1. Site Description

Three spatially separated sites, Hathinala, Kotawa and Barkachha (Lat. $24^\circ 17' - 25^\circ 10'$ Long. $82^\circ 45' - 83^\circ 6'$, altitude 110 - 355 m) selected for the study, are located in Mirzapur and Sonbhadra districts of Uttar Pradesh, India. Hathinala and Barkachha each have two moist and dry sub sites based on soil moisture regimes while hill top and hill base are two sub sites at Kotawa. The Hathinala and Kotawa have forests while Barkachha exhibits characteristics of savanna. The soils are residual, ultisol, sandy / sandy loam texture and reddish / reddish-brown in colour, derived from Kaimoor sand stone (Dhandroul orthoquartzite), the main parent rock.

2.2. Climate

The area is seasonally dry tropical having a typical monsoonal character and a year could be divided in six bi-month such as winter (Dec.- Jan.), spring (Feb.- Mar.), summer (Apr.- May.), early rainy (Jun.-Jul.), rainy (Aug. - Sept.), autumn (Oct. - Nov.). During study ambient air temperature varied between 5 and 49°C , and total annual rainfall was 1339 and 977mm, respectively (Fig.1). The major precipitation (~ 85%) occurs due to south-west monsoon from mid-June to mid-September after break of nine months in an annual cycle.

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2.3. Vegetational cover

The potential vegetation of the study site is dry mixed deciduous forest with site specific dominance of different species such as Hathinala moist (*Shorea robusta*), Hathinala dry (*Ziziphus glaberrima*), Kotwa hill top (*Boswellia serrata*), Kotwa hill base (*Accacia catechu*), and savanna of Barkachha having scattered trees, bushes of *Ziziphus* sp. and tall grasses.

2.4. Soil sampling

For study of temporal variation in top soil, monoliths (5cm x 5cm x 5cm) were collected from three random plots (3m x 3m) from each site at bimonthly interval for two years from January 2003 to November 2004. Soil cores were collected in winter season from Hathinala, Kotawa and Barkachha to study distribution pattern of CMB in soil profile. Soil cores were sliced at the interval of 5 cm, mixed well and sieved through mesh (2 mm) before enumeration of CMB population.

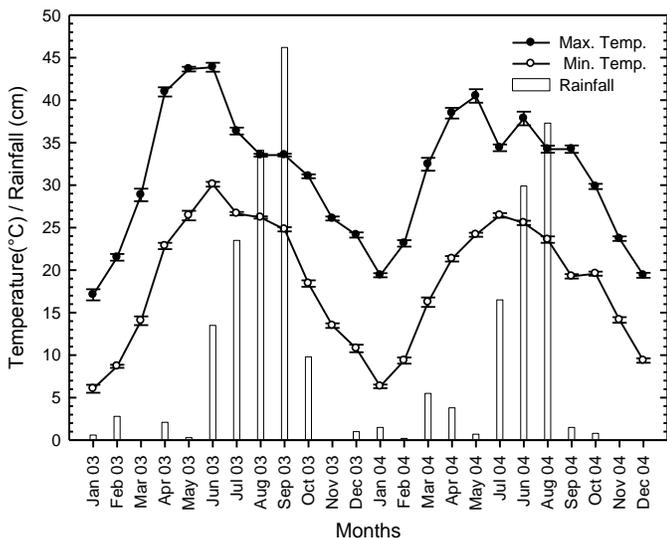


Fig.1. Meteorological data for study sites located in the Vindhyan region. (Data adapted from Forest Division, Renukoot, Sonbhadra, Uttar Pradesh, India)

2.5. Physicochemical analysis of soil

Soil texture was determined by passing the soil through the mesh of different size. In Brief, for analysis of particle size oven dried soil (250g, 105°C, 24h) was dissolved in water and passed through the mesh (75 µm) to filter the gravel and sand. Part of oven dried filtrate (50g) was suspended in 1l distilled water containing sodium hexa-metaphosphate (5%), thoroughly mixed and hydrometer reading was taken after different time interval (0.5, 5, 20, 40 min, 1, 2, 24h) to determine silt and clay fractions [8]. Organic carbon was analysed following Walkley [9] and total N by Kjeldohli method [10]. NH_4^+ -N was estimated in supernatant of soil extract in 2M KCl by endophenol method [11] and NO_3^- -N by phenol disulphonic acid method [10]. Bulk density of the soil was determined by weighting unit volume to 10 cm depth and water holding capacity by employing perforated circular brass boxes described by Piper [12]. Soil pH was determined in 1:2 soil-water ratio using micro pH meter equipped with electrode.

Soil moisture content was determined by drying at 105 °C for 24h.

2.6. CMB Population

A suspension was prepared by dissolving soil in sterile nitrate mineral salt medium in flask using gyratory shaker (60 rev min^{-1} , 4°C, 4h) and serial dilutions were prepared from four consecutive dilutions. Equal amount of suspension was inoculated in five tubes from each dilution. Turbidity in the tubes of three consecutive dilution was considered for the estimation of most probable number of bacteria in soil sample [6]. The Inoculated culture tubes were closed with sterile cotton plugs under aseptic conditions and incubated in the atmosbag (porosity 0.2 nm, opening 60-96 cm and volume 2890 liter) having 20% CH_4 in air at 25°C in the dark. Inoculated tubes incubated in synthetic air without CH_4 were used as control [7] After four weeks tubes were checked microscopically for bacterial growth and positive tubes were considered for CMB population determination by MPN using three consecutive dilution.

2.7. Statistical Analysis

Analysis of variance and stepwise regression of data was performed using statistical package SPSS 16.

3. RESULT

3.1. Physicochemical characteristics of soils

The studied soils differed with respect to the texture. They were either silty loam or sandy loam in nature. Different soil parameters of the studied sites have been summarized in Table 1. Total C was high at Hathinala moist site (30.4 mg g^{-1}) and low at Kotawa hill base site (6.5 mg g^{-1}). Total nitrogen was highest at Hathinala moist site (2.3 mg g^{-1}) and lowest at Kotawa hill base site (0.8 mg g^{-1}). Water holding capacity of the soils was positively correlated with the total C ($r = 0.92$, $P < 0.001$) and total N ($r = 0.70$, $P = 0.001$).

TABLE 1

SOIL PHYSICOCHEMICAL PROPERTIES OF SELECTED SITES LOCATED IN THE VINDHYAN REGION

Sites	Hathinala		Kotawa		Barkachha	
	Moist	Dry	Hill top	Hill base	Moist	Dry
Soil properties	Moist	Dry	Hill top	Hill base	Moist	Dry
Texture	Silt loam	Sand loam	Silt loam	Sand loam	Silt loam	Silt loam
Bulk density (g cm^{-3})	1.18 ± 0.01^a	1.38 ± 0.01^b	1.28 ± 0.01^{ab}	1.66 ± 0.01^d	1.61 ± 0.01^d	1.49 ± 0.01^{cd}
WHC (%)	46.1 ± 0.5^e	44.8 ± 0.3^d	45.5 ± 0.3^{de}	38.4 ± 0.2^a	43.5 ± 0.2^c	40.0 ± 0.3^b
pH	5.7 ± 0.1^b	6.0 ± 0.1^c	5.4 ± 0.1^a	6.8 ± 0.1^d	7.3 ± 0.1^e	7.8 ± 0.1^f
Total C (mg g^{-1})	30.4 ± 1.2^e	29.9 ± 1.1^e	23.5 ± 0.6^d	6.5 ± 0.2^a	16.1 ± 0.9^c	9.4 ± 0.3^b
Total N (mg g^{-1})	2.3 ± 0.1^d	1.4 ± 0.1^c	1.3 ± 0.1^{bc}	0.8 ± 0.1^a	1.2 ± 0.1^b	1.2 ± 0.1^{bc}

Note: Table entries represents mean of $N=3$, ± 1 SE. Superscript denotes homogeneous subsets based on Duncan test; WHC, water holding capacity

3.2. Spatial Variation

3.2.1 Site specific variations

All the selected sites varied significantly in the mean soil moisture ($df = 5$, $F = 1145.81$, $P < 0.001$), NH_4^+ -N ($df = 5$, $F = 333.87$, $P < 0.001$), NO_3^- -N ($df = 5$, $F = 293.59$, $P < 0.001$) and CMB population ($df = 5$, $F = 293.59$, $P < 0.001$). The biennial mean data for these studied parameters has been presented in Table 2.

Study Sites	Soil moisture (mg g ⁻¹)	NH_4^+ -N ($\mu\text{g g}^{-1}$)	NO_3^- -N ($\mu\text{g g}^{-1}$)	CMB population $\times 10^6$
Hath M	237 \pm 7	6.0 \pm 0.1	5.1 \pm 0.2	35.3 \pm 3.9
Hath D	182 \pm 9	5.0 \pm 0.2	3.6 \pm 0.2	16.1 \pm 2.3
KHT	204 \pm 7	4.8 \pm 0.2	3.8 \pm 0.1	19.2 \pm 2.1
KHB	130 \pm 7	3.0 \pm 0.2	2.4 \pm 0.1	5.1 \pm 0.7
Bar M	215 \pm 8	6.4 \pm 0.3	5.3 \pm 0.2	16.7 \pm 1.2
Bar D	133 \pm 5	3.6 \pm 0.2	2.9 \pm 0.2	9.8 \pm 1.1

Data represents mean and standard error of triplicates at bimonthly interval for two years 2003 and 2004, N=36

3.2.2 Distribution in Soil Profile

On comparing the soil profiles of the three sites, CMB population was high at Hathinala ($4.5 - 6.6 \times 10^6$), intermediate at Kotawa ($3.3 - 4.6 \times 10^6$) and low at Barkachha ($1.9 - 2.3 \times 10^6$) throughout the depth (Fig 2). CMB population exhibited a trend of increase till depth of 5cm (Kotawa and Barkachha) or 10cm (Hathinala) followed by a similar decline at all the sites and followed an exponential relation that can be arranged as $\text{CMB}_{\text{Hath}} = 20.93e^{-0.143}(\text{Depth})$, $r^2 = 0.87$; $\text{CMB}_{\text{Kot}} = 10.58e^{-0.156}(\text{Depth})$, $r^2 = 0.93$, and $\text{CMB}_{\text{Bar}} = 4.24e^{-0.166}(\text{Depth})$, $r^2 = 0.98$.

3.3. Temporal variation

3.3.1 Soil moisture

Variation in the soil moisture content of selected sites has been presented in Fig. 3.1 ABC. As expected high moisture was observed during rain (July) and decreased to lowest during summer (May) at all the sites. Highest soil moisture (29.1%) was recorded at Hathinala moist site and lowest (4.9%) at the Kotawa hill base. Soil moisture of the site was year ($df = 1$, $F = 37.9$, $P < 0.001$) and month ($df = 5$, $F = 1235.53$, $P < 0.001$) dependent.

3.3.2. NH_4^+ -N

The moist sites and hilltop contained higher NH_4^+ -N compared to their respective dry sites and hill base (Fig. 3.2ABC). In temporal study amount of NH_4^+ -N was highest in May and lowest during September, and ranged between 1.5 -10 $\mu\text{g g}^{-1}$ dry soils across the sites. There was significant temporal variation in NH_4^+ -N at all the six sites significantly differed with year ($df = 1$, $F = 184.0$, $P < 0.001$) and month ($df = 5$, $F = 166.4$, $P < 0.001$).

3.3.3. NO_3^- -N

On per gram dry soil basis, NO_3^- -N content across the sites and month ranged between 1.4 - 7.8 μg . Among the sites

maximum NO_3^- -N content was recorded at Barkachha moist site while lowest was at Kotawa hill base (Fig. 3.3 ABC). Similar to the NH_4^+ -N, NO_3^- -N content was also high in May and low in September. ANOVA indicated significant

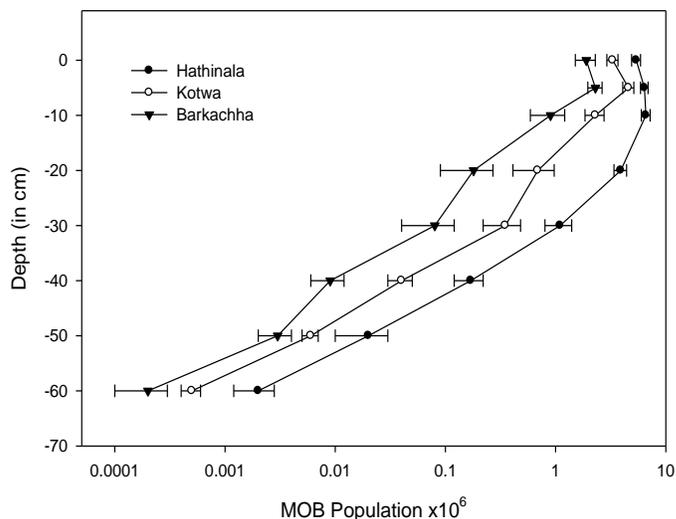


Fig.2. Distribution of culturable population of methanotrophs in soil profile at three sites, Hathinala; Kotawa and Barkachha. Bar at a point represents $\pm 1\text{SE}$.

difference in NO_3^- -N content depending on the year ($df = 1$, $F = 81.09$, $P < 0.001$) and month ($df = 5$, $F = 150.57$, $P < 0.001$).

3.3.4. CMB Population

CMB population on per gram dry soil basis was relatively higher in the moist sites compared to the dry sites (Fig 3.4 ABC). The mean biennial population of CMB was maximum at Hathinala moist site ($9.4 \pm 0.84 \times 10^6$), and minimum at the Kotawa hill base site ($7.0 \pm 0.02 \times 10^4$). At the hill forest site Kotawa, population was higher at the hill top ($1.78 \pm 0.2 \times 10^6$) compared to the hill base ($5.0 \pm 0.5 \times 10^5 \text{ g}^{-1}$). On temporal basis, population was high in winter (January) and low during rain (July). In the savanna site Barkachha population distribution was similar to the Hathinala forest sites i.e. CMB population was high at moist site (3.03 to 0.93×10^6) compared to the dry site (2.1 to 0.17×10^6). There was significant temporal variation in CMB population at all the six sites. The decline in CMB population was also low on moist sites (6 - 10 times) compared to dry sites (15- 16 times). ANOVA indicated significant difference in CMB population month ($df = 5$, $F = 150.57$, $P < 0.001$) and year wise ($df = 1$, $F = .001$, $P < 0.98$). Only two factors interactions, site \times year ($df = 5$, $F = 3.84$, $P = 0.003$) and site \times month ($df = 25$, $F = 23.55$, $P < 0.001$) were significant. CMB population was significantly correlated with soil moisture ($r = 0.26$, $p < 0.01$), NH_4^+ -N ($r = 0.29$, $P < 0.001$) and NO_3^- -N ($r = 0.39$, $P < 0.001$). There was a correlation between mean biennial CMB population of the site and its physicochemical properties. The population was positively correlated with the soil water holding capacity ($r = 0.81$, $P < 0.02$), total C ($r = 0.79$, $P < 0.03$) and total N ($r = 0.95$, $P < 0.002$), and negatively with bulk density ($r = -0.80$, $P < 0.02$). Stepwise regression analysis depended on the temperature ($\text{CMB} = 46.92 - 1.21x \text{ mean temp}$), temperature and nitrate ($\text{CMB} = 29.82 - 1.36 \text{ mean temp} + 5.39 \text{ Nitrate}$) and

temperature nitrate and soil moisture (CMB= 21.83 – 1.32 mean temperature + 5.13 Nitrate + 0.43 soil moisture). The % variability explained by these factors was 59, 76 and 77%, respectively.

4. DISCUSSION

The presently studied terrestrial ecosystems in the tropical dry deciduous forest and savanna exhibited a very unique pattern of CMB population variation in the top soil mainly influenced by the climatic and edaphic variables. In the soil profile, high CMB population was recorded in sub soils which decreased exponentially with depth at all the three sites (Fig.2). The CMB population was higher at all the sites during winter that declined in the summer and was minimum during the rain. The variation was site specific. The variation was more prominent in the dry sites compared to the moist sites. The variation in the population was between 6 to 16 fold. Such variation were studied in the rice field soil in the top 2-3 cm and 3-5 folds variation were recorded in the methanotrophs. The MPN of methanotrophs ranges in between 7.50×10^4 - 5.85×10^6 per gram dry soils in the presently studied ecosystems. The range was similar to the other terrestrial ecosystem across the world (0.7×10^5 to 3.5×10^7) reported by other workers [13, 14]. We observed low CMB population in the top soil and high population in the subsoil of the presently studied sites. The results could be due to the excessive accumulation of NH_4^+ -N in the forest and limited number of the methanotrophs in the

top soils [15]. The methanotrophs are aerobic in nature, the limiting oxygen will affect the population. The high sub soil population (30 cm depth) was attributed to ample availability O_2 and the upward diffusing endogenously produced CH_4 [16]. The coarse textured soil and erosional surface of KHB had low C and N content. At this site we enumerated lower CMB population ($5.0 \times 10^5 \text{ g}^{-1}$ dry soil) compared to KHT and HathM mainly due to the physical protection to organic matter. The soil habitat vary in moisture, nutrients, oxygen and pH with time in annual cycle and affect the methanotrophic population. The soil moisture is a strong determinant for methanotrophs in desert [17] and arable soils [18]. The high population of CMB population corresponded to 31-56% of WHC (20-25% w/w) that was well in the range (20-40% of WHC) where high atmospheric CH_4 consumption has been recorded by Gullledge and Schimel [19]. The mean soil moisture decreased from its maximum value during rain (23%) to the low value during extreme summer (7.5%). The CMB population was high during winter across the sites and minimum during rains. The relative abundance of Type II MB declined in high temperature and precipitation and low pore water methane concentration [20]. The decline in the CMB population with rain could be due to redistribution of loosely adsorbed bacterial population on soil particles through water filled pores or increased protozoa population in the top forest soils and their selective bacterial grazing [21].

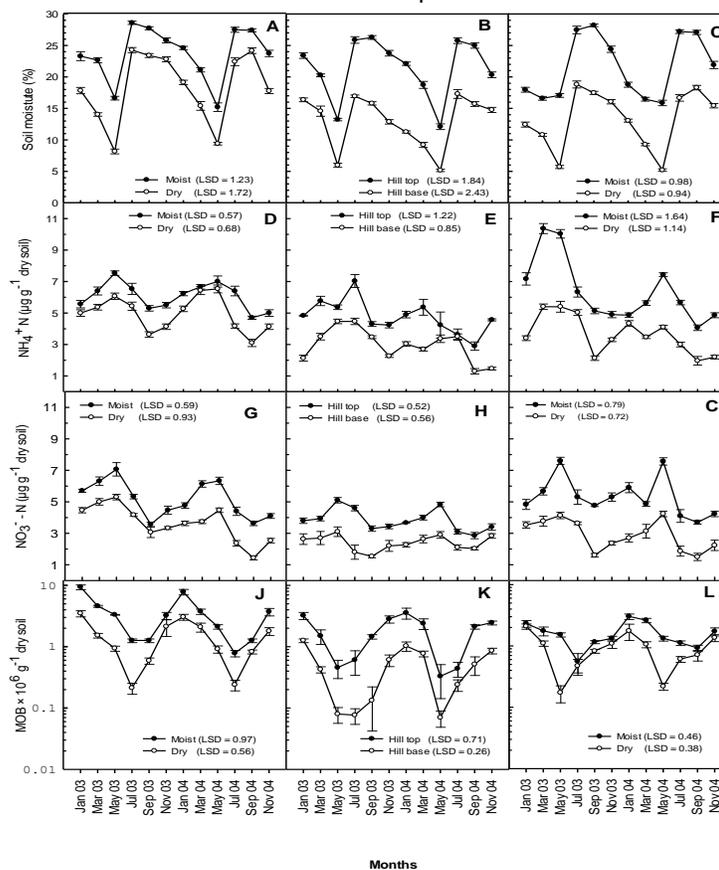


Fig.3. Temporal variation in soil moisture (ABC), ammonium (DEF), nitrate (GHI) and methane oxidizing bacteria (JKL) at three site Hathinala (ADGJ), Kotawa, (BEHK), Barkachha(CFIL) and their respective subsites during 2003 - 2004. Error bar at each point represents ± 1 SE.

The mineral-N of the soil depends on the mineralization and nitrification pattern and quality of overstory vegetational litter input. The mineral N of soil remains high during dry period and low during wet period due to respective low and high vegetational demand [22]. The observed high NH_4^+ -N at Barkachha savanna compared to forest. The result was similar to the Bottomley et al. [23] reporting high nitrification in meadow compared to forest. The NH_4^+ have three ecologically distinct pattern of influencing CH_4 oxidation, immediate inhibition due to substrate competition, delayed inhibition due to suppressed growth of methanotrophs and no inhibition due to development of NH_4^+ resistance in methanotrophs [24]. The observed positive correlation between CMB and NH_4^+ -N ($r = 0.29$, $P < 0.001$) could be related to suppressed growth or NH_4^+ resistance in some methanotrophic genera. The soil NO_3^- -N affect Type I and type II the methanotrophs, differentially. It inhibits growth of Type II and not Type I [25]. The NO_3^- -N can act as source of electron for methanotrophs under adverse CH_4 limiting conditions [26]. In the stepwise regression analysis of presently studied data, NO_3^- -N explaining about 11% variability in the CMB population in these soils supports this view. Under adverse condition, strictly aerobic methanotrophs *Methylophaga* JAM1 acquired narG gene for nitrate reduction by horizontal gene transfer and grow under denitrifying conditions by reducing nitrate into nitrite. It was a direct evidence of the adaptation of methanotrophs to an oxygen-limited environment [27]. The methanotrophs are affected by temperature in various habitats like land fill cover [28] and in forest [6]. However, temperature extremes are supposed to be crucial for methanotrophs survival of sensitive population and hence CH_4 consumptions [29, 30]. We enumerated higher number of methanotrophs in winter when temperature ranged between 15-20°C which favor growth of both type I and type II methanotrophs. Earlier higher average number of type I and type II methanotrophs have been reported when soil temperature varied between 11.5 to 22.5°C [6, 28]. In the stepwise regression analysis mean monthly temperature alone explained 59% variability in CMB population. The present study conclude that the important variable are atmospheric temperature (59%), nitrate (11%) and soil moisture contributed (1%) to a total of 77% variability explanation of soil methanotrophic population in this tropical dry deciduous ecosystem. Most probably, temperature gradient in annual cycle was responsible for synchronized population variation trend at spatially separated sites. The dry and moist sites are likely to differ in their dominant methanotrophs and community structure analysis are necessary for better understanding the ecology of type I and type II methanotrophs [31] and their contributions to methane sink in these selected ecosystems.

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