

Impact of Land Use Changes on Methanotrophic Bacterial Abundance and Soil Microbial Biomass

**SUMMARY OF
Thesis**

SUBMITTED TO
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
LUCKNOW

BABASAHEB
BHIMRAO
AMBEDKAR
UNIVERSITY



प्रज्ञा शील करुणा
ESTABLISHED 1996

FOR THE DEGREE OF
Doctor of Philosophy
IN
ENVIRONMENTAL MICROBIOLOGY

Submitted By
Shashank Tiwari

(Enrolment No- 307/11)

Under the Supervision of
Dr. Jay Shankar Singh

Assistant Professor

DEPARTMENT OF ENVIRONMENTAL MICROBIOLOGY
(SCHOOL FOR ENVIRONMENTAL SCIENCES)
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY

(A Central University, NAAC Accreditation 'A' Grade)
VIDYA VIHAR, RAEBARELI ROAD, LUCKNOW-226 025
UTTAR PRADESH, INDIA

2018

Land use changes

Land use changes (LUC), considered as ecosystem destruction practices i.e. conversion of natural forests to farming lands. The LUC, one of the main environmental disturbances, greatly impacting to the global climate change, land degradation, disturbances in ecosystem services, loss of microbial species diversity and many more bad impacts (Maharjan et al., 2017; Leeuwen et al., 2017). The anthropogenic LUC mediated interventions, may be considered as one of the major key ecological drivers that strongly alter the soil characteristics and consequently, functioning of several terrestrial ecosystems (Pabst et al., 2013; Cao et al., 2017; Tian et al., 2017; Luneberg et al., 2018) particularly the nutrient poor soils of tropical regions (Tilman et al., 2001; Kumar and Ghoshal, 2014; Singh and Gupta, 2018). The study on land uses of any area, having a wide range of environmental characteristics (e.g. soil pH, temperature, moisture, etc.) may provide a baseline data for the soil disturbances. If, the selected sites varied in terms of dominant vegetation cover, physiography and anthropogenic activity, a wide variation in soil properties might be expected across different land uses. A greater tree density at natural forest site compared to agriculture land and other disturbed sites, may receive greater amount of plant residues in the form of litter and fine roots and consequently, an higher amount of total-C, -N and -P, soil moisture, WHC, inorganic nutrients, etc. might be expected. However, the detail experimental evidences for above arguments are lacking for soil physico-chemical properties variations impacted by land use covers in Vindhyan regions. Therefore, in this research work an attempt has been made to find out the impact of land use covers on soil physico-chemical conditions in dry tropical soils of Vindhyan region.

Methanotrophs

The dry tropical upland soils of Vindhyan region contributed to highest CH₄ consumption on a global scale (Singh, 2011). The soil CH₄ consumption, mostly accomplished by methanotrophic bacteria, widely distributed in different types of terrestrial soils however, knowledge about these microbes across different land use covers/types is still not very clear. The conversion of natural ecosystems into agro-ecosystems alters the below-ground soil conditions that may be a major cause for the microbial population loss including methanotrophic community structure (Bossio et

al., 2005; Flynn et al., 2009). Thus, the land use practices can alter the strength of soil CH₄ sink activity of methanotrophs too (Mosier et al., 1991; Dorr et al., 2010). It is proposed that land use changes can alter the soil physico-chemical soil properties which in turn may impact the methanotrophs abundance and *pmoA* gene copies in soils. However, no studies have been conducted to assess the impact of land uses on abundance of soil methanotrophs and *pmoA* gene copies of in dry tropical regions of Vindhyan uplands. To find out some answers of the above raised queries a field experiment, selecting different land use types, was conducted in upland region of Vindhyan plateau. It is hypothesized that variations in soil properties due to land use changes would correspond to the variation in soil methanotrophs abundance and their *pmoA* gene copies at Vindhyan upland soils.

Soil microbial biomass

The soil microbial biomass SMB, widely considered as the index of soil fertility, may be directly correlated with the disturbances in soil conditions due to land use/cover changes. Few studies in this regard, owing to land uses, have been carried out to demonstrate the disturbances in soil organic matter inputs/removal in a variety of forms, can significantly impact the soil microbial community composition, their biomass and activities (Singh and Gupta, 2018). In recent years, the effects of urbanization along a rural–urban gradient on soil microbial biomass and physico-chemical properties have been studied in India (Rai et al., 2018). However, the variations in quantity of SMB–C, –N and –P due to land use changes remains poorly understood in dry tropical regions of Vindhyan uplands. Therefore, a better understanding to the bad impacts of land use practices may provide further insight into soil microbial communities and SMB mediated restoration of soil fertility of dry tropical regions supported by nutrient poor soils. Very little is known about the effects of changes in land use/cover on SMB levels, especially by considering natural forest, savanna and agriculture ecosystems on comparative ground. Currently, it is also not clear how land cover changes may impact the SMB levels across different soil depths in a given forest or agro-ecosystem. The land use changes/conversion may lead to several unfavorable modifications to soil and environmental variables, which can indirectly or directly affect the soil microbial diversity, abundance and their biomass.

OBJECTIVES

Since, LUC may also alter the soil properties and micro-flora by affecting the transport of soil nutrients in deeper soil horizon either through alteration of belowground input of organic matter or surface mixing processes. Abundance, diversity of CH₄ oxidizing bacteria, *pmoA* gene copies, and SMB levels at different soil depths, affected by soil physico-chemical soil properties due to different land uses are relatively unknown. Hence, there are strong reasons to investigate the land use changes on above selected soil parameters at different soil horizons (0-10, 10-20, and 20-30 cm soil depths). The objectives of the present research work are:

- 1. To isolate and identify the methanotrophic bacterial abundance and soil microbial biomass under different land use changes.**
- 2. To study the impact of land use changes on physico-chemical properties of soil.**
- 3. Correlation between land use changes and methanotrophic bacterial abundance and soil microbial biomass.**

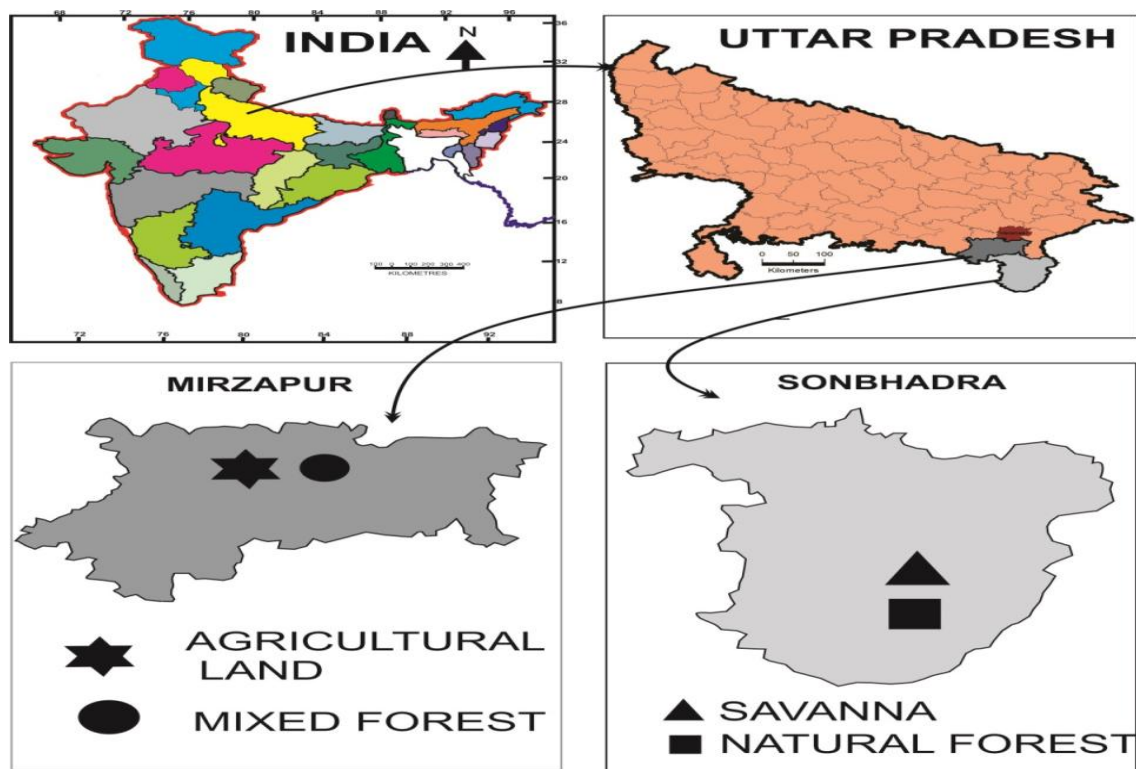


Fig. 1. Location of different land use types across Mirzapur and Sonbhadra districts of Uttar Pradesh, India.

Selected land uses/covers of the study area

Land uses types		Dominant vegetation cover	Tree density (plants ha ⁻¹)	Latitude	Longitude	Average altitude (m)
Mirzapur district	Agriculture land (1133 ha)	Crop cultivation (Wheat, Paddy, Pulses, Mustard, etc.) on rotation basis	-	25° 3' 19" N	82° 35' 45" E	100
	Mixed forest	<i>Boswellia serrata</i> , <i>Acacia nilotica</i> , <i>Areca catechu</i> , <i>Phyllanthus emblica</i> , <i>Dendrocalamus</i> , etc.	419	25° 0' 6" N	82° 37' 18" E	100
Sonbhadra district	Savanna (199 ha)	<i>Butea monosperma</i> , <i>Panicum</i> sp., <i>Andropogon</i> sp., etc.	310	24° 25' 55" N	83° 5' 30" E	299
	Natural forest (2555 ha)	<i>Hardwickia binnata</i> , <i>Shorea robusta</i> , <i>Acacia catechu</i> , <i>Terminalia tomentosa</i> , etc.	2112	24° 25' 57" N	83° 6' 27" E	355

MATERIALS AND METHODS

Analyses of soil physico-chemical properties

- Soil texture: Indian Standards, 1965
- Soil temperature: Digital soil thermometer (Luster Leaf 1625)
- pH (1: 2.5 soil to water ratio)
- Electrical conductivity (EC): 1: 5 soil to water ratio)
- Soil bulk density (BD) and water holding capacity (WHC): Piper (1944)
- Soil moisture content (%): Buresh (1991)
- Soil organic-C: Walkley (1947)
- The soil total-N and total-P: Jackson (1958)
- Soil inorganic-N (NH₄-N and NO₃-N) and -P: Eno (1960)

FTIR analysis of soil samples across different land use types

- Followed the Narain et al. (2012) & Tripathi et al. (2014)
- Samples were kept in Desiccators and dried in an oven at 105 °C for 48 h
- The samples were grounded with KBr in the ratio of 100:1 (100 mg KBr and 1 mg soil sample)
- spectra were obtained at 4,000–400 cm⁻¹ mid-IR range

Soil microbial biomass (SMB) analyses

- SMB -C, -N & -P were determined by chloroform (CHCl₃) fumigation-extraction method by Vance et al. (1985) and Zhang et al. (2014)

Quantification of methanotrophs abundance by MPN and *pmoA* gene methods and across different land use types

- Modified enrichment technique described as by Saitoh (2002) was used for the isolation and quantification of soil methanotrophs.
- Methanotrophic growth in Serum Vials (NMS medium) at 580 nm (Whittenburry et al., 1970).

- Biochemical characterisation of isolated methanotrophs with the help of HiMedia Gram negative kit KB002.
- Molecular analysis of methanotrophic community (Nano-pore sequencing: Third generation sequencing).
- Methanotrophs *pmoA* gene quantification by Kiziloa et al. (2014).

Statistics

Soil physico-chemical parameters, methanotrophs abundance and *pmoA* gene quantity were analyzed using one-way and two-way ANOVA to test the statistical differences between different land use types and soil depths. Duncan's post hoc test was used to separate the means at $P < 0.05$. All data analyses were performed in triplicate \pm SE.

RESULTS

Meteorology and dominant vegetation cover of different land use covers

The study area is characterized by wide variations in land use covers due to physiographic and intense anthropogenic disturbances. The vegetation cover at natural forest (commonly called as sal forest) site in Sonebhadra district is dense, dominated by *Shorea robusta* and *Hardwickia binnata*. However, the mixed forest in Mirzapur district has been reforested by deciduous tree vegetation with mixed species plantation having scattered patches of densely growing trees dominated by *Boswellia serrata*. The savanna sites in Sonebhadra district have scattered and low height/stunted growth vegetation dominated by *Butea monosperma*. The agriculture land in Mirzapur district was previously covered by grasses, thorny semi-arid bushes and sparse trees, but the trees were cleared manually during last decades of 20th century for cultivation purpose and the area is under rotational crop cultivation practices.

Soil physico-chemical properties

In present study the land use changes had significant effects on soil physico-chemical properties particularly in top soil (0-10 cm depth). The soil colour was almost brownish-black of all the selected regions, except the savanna site having brownish colour. Two-way ANOVA showed significant variation in soil physico-chemical properties due to land use types, soil depths (except for pH at 10-20 cm) and

their interactions (except for BD at 20-30 cm). The soil temperature of savanna land (46.0 °C) was highest followed by agriculture land (44 °C), mixed forest (43 °C) and natural forest (42 °C). The soil pH was significantly higher for the savanna site (8.9) in comparison with the natural forest > mixed forest > agriculture land. The soil BD under agriculture land/savanna was significantly higher than natural forest \approx mixed forest land covers. The soil organic-C, total-N and -P strongly decreased from natural forest/mixed forest to savanna/agriculture land. Soil C/N ratio was significantly different among the land use/covers, and savanna soil had the highest value. The soil of natural forest/mixed forest had significantly higher soil moisture content than all the other land uses.

Soil microbial biomass (SMB) - C, -N and -P

ANOVA showed significant variations in SMB-C, -N and -P values due to land use covers ($P < 0.001$), soil depths ($P < 0.001$) and land use types \times soil depths interaction ($P < 0.001$), indicating that effect of land use changes on different soil depths (0-10, 10-20 and 20-30 cm) were different.

The level of SMB-C differed significantly among the land-use types and soil depths ranging from 297.65 to 768.25 $\mu\text{g g}^{-1}$ dry soil. Among the land-use types and soil depths, the level of SMB-C was highest for top soil layer (0-10 cm depth) (768.25 $\mu\text{g g}^{-1}$) in the natural forest soil, followed in decreasing order in mixed forest, savanna, and the minimum (297.65 $\mu\text{g g}^{-1}$) in the agriculture land at 20-30 cm soil depth. The trend for SMB-N was similar to that for SMB-C under different soil depths and land use types (ranged from 38.14 to 98.78 $\mu\text{g g}^{-1}$ dry soil). The SMB- P ranged from 22.45 in the agriculture land to 48.9 $\mu\text{g g}^{-1}$ in natural forest soil. Similar to SMB-C, SMB-N and -P was also highest for top soil horizon (0-10 cm depth) in natural forest followed by mixed forest, savanna and agriculture land.

The correlation analyses clearly showed that the soil physico-chemical parameters i.e. pH, soil moisture (SM), water holding capacity (WHC), soil temperature (Temp), total-N (TN), C/N ratio and organic-C (OC) are very closely linked and strongly associated with the SMB levels, indicating that these parameters are key soil factors affecting soil microbial biomass across land uses.

Quantification of soil methanotrophs

The methanotrophic colonies on Petri plates also evidenced for the highest number in natural forest soil followed by mixed forest, savanna and cropland. The cream and pink coloured bacterial colonies on Petri plates containing NMS medium confirm the growth of aerobic methanotrophs. During cultivation and incubation of methanotrophic bacterial abundance across different land uses, methanotrophs growth in soil of natural forest was observed higher than the soils of other sites.

The natural and mixed forest soil showed a statistically higher number of viable soil methanotrophic abundance as compared to the savanna and agriculture land. The results also demonstrated that methanotrophic population at all the land uses was significantly higher in top (0-10 cm) soil layer than the deeper soil profiles. ANOVA indicated significant difference in viable methanotrophs abundance due to land uses ($P < 0.001$), soil depths ($P < 0.001$) and land uses \times soil depths interaction ($P < 0.001$). The correlation analysis revealed significant ($P = < 0.01$) positive interaction between methanotrophs population with WHC, total-C, -N and -P, nitrate-N soil moisture content and C/N ratio and negative with BD and ammonium-N contents at different soil depths.

The number of *pmoA* gene copies in the soils of studied land uses were significantly greater for natural forest soil followed by mixed forest, savanna and agriculture land. For all land use sites, while considering the soil depth profile had a similar trend i.e. being maximum at 0-10 cm depth and lower at 20-30 cm soil depth profile for *pmoA* copies was noted. ANOVA showed significant ($P < 0.001$) variations in soil *pmoA* gene copies due to land use types ($F = 83.246$; $P = < 0.001$), soil profile depths ($F = 92.048$; $P = < 0.001$) and land uses \times depths interaction ($F = 5.916$; $P = < 0.001$).

DISCUSSION

Impact of land use covers on soil physico-chemical characteristics

The soil organic carbon content basically based on the balance between C incorporation and decomposition rates in the ecosystem (Saggar et al., 2001; Huang and Song, 2010; Xiangmin, et al., 2014). Several studies have demonstrated that soil organic carbon content rapidly reduces when natural forest is converted into agriculture land (Yang et al., 2009; Pandey et al., 2010). Transformation of natural forest into agricultural land converts the vegetation cover. The vegetation cover has

the potential to affect soil properties, including species-specific impacts on the quality and quantity of plant litters (Talkner et al., 2009; Wang et al., 2010). The yearly entry of C into agricultural soils is often poorer than natural forest ecosystems (Huang and Song, 2010). Therefore, in comparison to natural and mixed forest the level of soil organic carbon is lost rapidly in agricultural and savanna soils. In addition, Wang et al. (2007) showed that the poor quality of slow decomposing conifer litter reduces organic matter input, leads to the decrease in soil organic carbon compared with natural forest. Moreover, cultivated soils often damage soil aggregates, thereby increasing organic matter exposure and faster soil organic carbon breakdown (Xiangmin et al., 2014).

Impact of land use change on SMB–C, –N and –P

This results of this research work indicated strong support for our first proposed hypothesis that land use changes significantly influenced SMB–C, –N and –P quantity across different soil depths particularly in top soil layer (0-10 cm depth) in upland soils of dry tropical region of India. However, contrary to this study, SMB–C, –N and –P values in response to the land cover were not significant in temperate region of north Iran (Moghimian et al., 2017). These contradictory results, related to SMB values at different regions could be due to variations in land use covers, soil nutrient quantity and quality, dominant vegetation covers and environmental regimes (Wang et al., 2013). The soil organic matter contents such as C and N remained greater in top soil layer (0-10 cm) under natural and mixed forest compared to other land uses, possibly due to effect of greater returns of litters in the form of fine root biomass and aerial plant residues. However, soil organic matter contents and SMB decreases with deeper horizons (20-30 cm) possibly because of reduced plant residues inputs as suggested recently by Maharjan et al. (2017). They demonstrated that land use and management practices are the main cause for declining soil microbial C and N with soil depth due to declining C inputs (e.g. by plant residues) in sub-tropical soils. This indicates that land use and management practices affected SMB–C, –N and –P values only in the top soil horizon (0-10 cm depth), validating our first hypothesis. Leeuwen et al. (2017) also demonstrated that land use change had strong affect on the soil microbial biomass particularly in the upper (0-10 cm) soil horizons, due to differences in substrate quantity and quality. Several studies have shown that microbial biomass decreases with soil depth, which is likely linked to decreased resource (SOM) availability in the deeper soil horizons (Leeuwen et al., 2017).

A wide variations in values of SMB in this study showed that the organic substrate quantity and quality varied greatly under different kinds of land use change/cover. The SMB–C, –N and –P values were highest in soil of natural and mixed forest as compared with savanna and agriculture land use/cover. The dominant vegetation cover might be the reason for variations in SMB size across land uses because the differences in standing plant community covers can add to variations in quality and quantity of litters, which governs the soil organic matter and soil nutrients (Miki et al., 2010). Since the four selected sites of present study differed in terms of dominant vegetation cover therefore, differences in SMB–C, –N and –P contents among land-use types might be expected. In the agricultural and savanna land use types our studies, the cultivation of different seasonal crops are performed throughout the year, so the soil disturbances due to frequent tillage and cultivation might reduce the SMB levels. Peixoto et al. (2006) confirmed that disturbances in soil structures due to different tillage systems and land-use conversion as principal driving force to influence the community compositions of soil microbial communities and biomass.

The mixed forest of present study, which is a reforested land cover, had higher SMB-C, -N and -P as compared to agriculture and savanna land uses. Kardol and Wardle (2010) and Zhang et al. (2016) also found a greater averaged higher microbial biomass at afforested and reforested sites compared with cropland and uncultivated land. An increase in all types of SMB components are possibly attributed to increased litter input and soil organic C and N content in the afforested soils (Deng et al., 2014; Zhang et al., 2016). Differences in microbial community composition following land use change also have been attributed to differences in soil properties (Araujo et al., 2013; Garcia-Franco et al., 2015). Furthermore, conversion of natural dense forest to farming lands may leads to opening of the canopy cover and interference of physical environmental factors like light intensity, wind velocity and evaporation of soil moisture contents that may accelerate soil erosion and suppress the organic matter decomposition and consequently decline the soil microbial biomass (Singh and Ghoshal, 2014).

In this study the soil of savanna and agricultural land has lowest SMB–C, –N and –P values. The intense cattle grazing activity, conventional farming and crop rotation practices may continuously removes and reduce the incorporation of crop residues, resulting in lower organic matters in surface layers of savanna and farming land uses. This also could provide an un-favourable soil environment for microbial

communities, contributing to a reduced and un-stable microbial community structure in disturbed soil of savanna and agriculture land. Furthermore, the toxic effects of chemicalization and pesticides may also reduce the SMB quantity in agriculture farming compared natural forest experiencing insignificant anthropogenic disturbances.

Impact of land use change on soil methanotrophs abundance

The pink colour colonies and morphology of methanotrophs, isolated from mixed and natural forest soil suggest that these sites contain similar type of methanotrophic communities. While agro-ecosystem and savanna sites have cream colour bacterial colonies and therefore, it may be assumed that the methanotrophs isolated from these sites belong to similar type. The differences in methanotrophic population across different land uses could be due the variation in soil physico-chemical characteristics such as organic-C, organic and inorganic soil N and P, soil moisture, WHC and other soil drivers.

The result showed that compared to the savanna and agriculture soil the methanotrophic abundance and *pmoA* gene copies of natural and mixed forest soil was higher. Due to higher quantity of plant litter in the form of leaves and fine roots received from the dense vegetation cover at forest soil could add a larger amount of organic contents to the forest soil as compared to savanna and agriculture. Consequently, a greater microbial activity and population including methanotrophs might be expected in forest soil. The forest soil with higher amount of organic contents can contribute significantly to growth of methane-oxidizers in such well aerated soils (Meronigal and Guenther 2008; Shoemaker et al. 2014). Therefore, methanotrophic numbers in this study were observed greater in the soil of natural and mixed forest having well improved and aerated soil because of high amount of organic contents. Knief et al. (2005) also pointed out that methanotrophs activity related to methane consumption rates were lower at the agriculture site compared to native forest and reforested sites. The higher amount of ammonium-N contents in the soil of agro-ecosystem and savanna could suppress the number of methanotrophic community compared to mixed and natural forest soils. It is assumed that that incorporation of inorganic fertilizers in agriculture soils to enhance the crop productivity may reduce the methanotrophic population. Kravchenko et al. (2002), Seghers et al. (2003) also added that inorganic fertilizers are extensively documented

as one of the main reasons affecting CH₄ consuming activity of methanotrophs in cropland soils.

At all the land use sites the methanotrophs abundance (*pmoA* genes copies) increased with increases of C/N ratios. Across the land uses and soil depths the highest *pmoA* genes copies was observed at natural forest with a C/N ratio of 25.25 at 0-10 cm depth. According to Stein et al. (2012), the decline in methanotrophs abundance in agricultural soils can be attributed to long-term inhibitory effects of N fertilizers. Long-term N amendment in soils changed the methanotrophic community structure, resulting in reduced CH₄ oxidation (Bodelier et al. 2000; Bodelier and Laanbroek 2004; Mohanty et al. 2006; Noll et al. 2008; Banger et al. 2012; Zheng et al. 2013). Application of nitrogen supplements to the agricultural soils particularly the ammonium-based N-fertilizers has been noted to influence the methanotrophs population (Bodelier and Laanbroek 2004). Sometimes the N-fertilizers showed inhibitory effects (Hutsch et al. 1994), and on the other times as stimulatory effects (Mohanty et al. 2006) or no effects (Delgado and Mosier 1996) on methanotrophic population. The addition of NH₄⁺-N inhibits the methanotrophs activity and growth in forest, grassland, arable and paddy soils (Cai and Yan 1999) because of the molecular analogy of NH₄⁺ with CH₄ molecules and consequently interfere the uptake of CH₄ by methanotrophs. The competitive inhibition of NH₄⁺ to CH₄ is also pointed out as main inhibitory effect for methanotrophs multiplication (Schimel et al. 1993). Zheng et al. (2008) revealed an inhibitory effect of soil N amendments on the methanotrophic population density and community composition in Chinese paddy soil. A negative relationship between methanotrophs population and soil NH₄⁺-N content confirmed the inhibitory effect of NH₄⁺-N treatments to the growth and multiplication of bacteria.

CONCLUSIONS

This study indicates that land uses are one of the important factors that govern the variations in soil physico-chemical properties and methanotrophs number in dry tropical region of Vindhyan plateau. Compared to agriculture land and savanna site, the minimum anthropogenic disturbances at mixed and natural forest sites showed higher amount of organic material due to return of leaf litter and fine root biomass due to greater tree density. Compared to deeper soil layers (10-20 and 20-30 cm), a greater microbial activities such as decay and decomposition at 0-10 cm soil depth may

resulted greater accumulation of inorganic nutrients (ammonium- and nitrate-N), WHC, organic-C and soil moisture contents.

Present study indicate that SMB levels decreases with soil depth, which is likely linked to decreased soil organic/nutrient matter, soil moisture availability and active soil micro-flora in the deeper soil horizons (20-30 cm). Thus, it is clear that land use changes caused adverse alterations in soil physico-chemical properties and SMB values. The present study explains that land use practices and certain types of dominant vegetation cover exert a profound influence on SMB values. In the deeper sub-soil horizon (20-30 cm depth), SMB-C, -N and -P values were almost similar among land use types, although organic matter contents were significantly higher under natural and mixed forest land covers. These results indicate that land use practices significantly influence the soil microbial activities in surface soil layers (0-10 cm depths), with less significant impacts in deeper sub-soil layers (20-30 cm depths.). Our data suggest that undisturbed soils of natural and mixed forest may be more productive/healthy compared to the soils of other land use covers and may be arranged in the order of natural forest < mixed forest < savanna < agriculture land. It is clear that different plant species via their quality and quantity of litter inputs, strongly affect the soil microbial community/biomass across different land uses and soil depths.

The favourable soil conditions (greater organic and inorganic nutrients, soil moisture and optimum pH) at mixed and natural forest sites exhibited higher number of CH₄ consuming bacterial abundance, *pmoA* gene copies and communities than the soils of agro-ecosystem and savanna site. It is suggested that disturbance in soil physico-chemical conditions due to forest cuttings by anthropogenic activity at agriculture and savanna sites may led to disturbances in soil moisture, WHC, organic matter and ultimately the low count of soil methanotrophs numbers. The results of this study provide ample evidence that the differences among upland soil properties due to land uses significantly influences the number of methanotrophs and, *pmoA* gene copies. The lower quantity of methanotrophs, *pmoA* gene copies and bacterial communities in the agricultural soil compared to natural forest soils indicates the strong role of soil disturbances due to tillage and cattle grazing to create a smaller bacterial population.

Based on the above discussions it is recommended to stop or minimize further degradation of remaining natural forest cover in the Vindhyan region. This research

recommends a plantation with suitable native broad-leaved species to restore the soil productivity/ health of degraded nutrient poor soils of dry tropical Vindhyan uplands of India. The agriculture management practices (use of biofertilizers in place of inorganic fertilizers), especially for the agricultural soils, could be employed to mitigate the negative impacts of land uses on soil methanotrophs and their CH₄ sink activity. The application of bio-fertilizers such as cyanobacteria/blue-green algae, diazotrophs, *Azolla* and mycorrhizae may reduce the amount of N fertilizer required for crop growth. Using such type of beneficial microbial bio-fertilizers to degraded agriculture soils may not only conserve the existing soil methanotrophs, but would also improve the abundance and diversity of soil methanotrophs. The restoration or re-vegetation of degraded forest lands at Vindhyan plateau can be an effective strategy for improving the soil physico-chemical conditions that may enhance the number of soil methanotrophic community to manage the atmospheric CH₄ load.

Significant References

- Kallistova, A.Y., Kevbrina, M.V., Nekrasova, V.K., Shnyrev, N.A., Einola, J.K.M., Kulomaa, M.S., Rintala, J.A., Nozhevnikova, A.N., 2007. Enumeration of methanotrophic bacteria in the cover soil of an aged municipal landfill. *Microb. Ecol.* 54, 637–645.
- Katovai, E., Burley, A.L., Mayfield, M.M., 2012. Understory plant species and functional diversity in the degraded wet tropical forests of Kolombangara Island, Solomon Islands. *Biol. Conserv.* 145 (1), 214–224.
- King, G.M., 1997. Responses of atmospheric methane consumption by soils to global climate change. *Glob. Change Biol.* 3, 351–362.
- Kizilova, A.K., Sukhacheva, M.V., Pimenov, N.V., Yurkov, A.M., Kravchenko, I.K., 2014. Methane oxidation activity and diversity of aerobic methanotrophs in pH-neutral and semi-neutral thermal springs of the Kunashir Island, Russian Far East. *Extremophiles* 18(2), 207–18.
- Singh, J.S., 2011. Methanotrophs: the potential biological sink to mitigate the global methane load. *Curr. Sci.* 100 (1), 29–30.
- Singh JS, Gupta VK (2016) Degraded land restoration in reinstating CH₄ sink. *Front. Microbiol.* 7, 923.

- Singh JS, Pandey VC, Singh DP, Singh RP 2010. Influence of pyrite and farmyard manure on population dynamics of soil methanotroph and rice yield in saline rain-fed paddy field. *Agric. Ecosyst. Environ.*, 139, 74–79.
- Singh, J.S., Raghubanshi, A.S., Singh, R.S., Srivastava, S.C., 1989. Microbial biomass acts as a source of plant nutrients in dry tropical forest and savanna. *Nature* 338, 499–500.
- Singh, J.S., Singh, D.P., Kashyap, A.K., 2009. A comparative account of the microbial biomass-N and N-mineralization of soils under natural forest, grassland and crop field from dry tropical region, India. *Plant Soil Environ.* 55 (6), 223–230.
- Singh, J.S., Singh, D.P., Kashyap, A.K., 2010. Microbial biomass C, N and P in disturbed dry tropical forest soils, India. *Pedosphere* 20(6), 780–788.
- Singh, S., Ghosal, N., Singh, K.P., 2007. Variations in microbial biomass and crop roots due to differing resource quality inputs in a tropical dry land agro ecosystem. *Soil Biol Biochem.* 39, 76–86.
- Singh, J. S., Singh, D. P., 2012. Reforestation: A potential approach to mitigate excess atmospheric CH₄ build-up. *Ecol. Manage. Restor.* 13(3), 245–248.
- Singh, J.S., Gupta, V.K., 2018. Soil microbial biomass: A key soil driver in management of ecosystem functioning. *Sci. Total Environ.* 634, 497–500.
- Singh, J.S., Gupta, V.K., 2018. Soil microbial biomass: A key soil driver in management of ecosystem functioning. *Sci. Total Environ.* 634, 497–500.
- Singh, L.I., Yadava, P.S., 2006. Spatial distribution of microbial biomass in relation to land-use in subtropical systems of north-east India. *Trop. Ecol.* 47 (1), 63–70.
- Steenwerth, K.L., Jackson, L.E., Calderon, F.J., Stromberg, M.R., Scow K.M., 2002. Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. *Biol. Biochem.* 35, 489–500.
- Steinkamp. R., Butterbach-Bahl, K., Papen, H., 2001. Methane oxidation by soils of an N limited and N fertilized spruce forest in the Black Forest, Germany. *Soil Biol. Biochem.* 33, 145–153.

List of Publications**Research/Review articles**

1. **Shashank Tiwari**, Chhatarpal Singh, Pradeep K Rai, Jay Shankar Singh* & Vijai K Gupta (2018). Land use changes decline soil microbial biomass level in dry tropical uplands. *Journal of Environmental Management* (Elsevier). (Under review).
2. **Shashank Tiwari**, Chhatarpal Singh, Jay Shankar Singh* (2018). Land Use Changes: A Key Ecological Driver Regulating Methanotrophs Abundance in Upland Soils. *Energy, Ecology and Environment* (DOI: <https://doi.org/10.1007/s40974-018-0103-1>).
3. CP Singh, **Shashank Tiwari**, VK Gupta and Jay Shankar Singh* (2018). The effect of rice husk biochar on soil nutrient status, microbial biomass and paddy productivity of nutrient poor agriculture soils. *Catena* 171: 485–493. (**Impact Factor =3.256**).
4. Chhatarpal Singh, **Shashank Tiwari**, Jay Shankar Singh* (2017). Impact of Rice Husk Biochar on Nitrogen Mineralization and Methanotrophs Community Dynamics in Paddy Soil. *International Journal of Pure Applied and Bioscience* 5 (5): 428-435.
5. Chhatarpal Singh, **Shashank Tiwari**, Jay Shankar Singh* (2017). Application of Biochar in Soil Fertility and Environmental Management: A review. *Bulletin of Environment, Pharmacology and Life Sciences* 6 (12): 07-14.
6. **Shashank Tiwari**, Jay Shankar Singh, DP Singh (2015). Methanotrophs and CH₄ sink: Effect of human activity and ecological perturbations. *Climate Change and Environmental Sustainability* 3: 35-50.

Book Chapters

1. Suman Upadhyaya, **Shashank Tiwari**, N.K. Arora and D.P. Singh. Microbial protein: A valuable component for future food security. In: *Microbes in Environmental Management* (Eds: Singh JS and Singh DP), Studium Press, USA, pp. 259-279.
2. Siddharth Boudh, **Shashank Tiwari** and Jay Shankar Singh (2017). Microbial Mediated Lindane Bioremediation. In: *Agro-environmental Sustainability: Managing Environmental Pollution (Vol-II)* (Eds: Singh JS and Seneviratne G), Springer, Netherland, pp. 213-233.
3. Chhatarpal Singh, **Shashank Tiwari**, Siddharth Boudh and Jay Shankar Singh (2017). Biochar application in management of paddy crop production and

methane mitigation. In: Agro-Environmental Sustainability (Volume II: Managing Environmental Pollution) (Eds: Singh JS and Seneviratne, G) Springer, pp. 123-145.

4. Chhatarpal Singh, **Shashank Tiwari** and Jay Shankar Singh* (2018). Biochar: A sustainable tool in bioremediation of soil pollutants. In: Bioremediation of Industrial Waste for Environmental Safety Volume II: Biological agents and methods for industrial waste management (Eds: Saxena G and Bharagava RN) Springer. In press.

Magazine articles

1. **Shashank Tiwari**, Chhatarpal Singh and Jay Shankar Singh*(2017). Balancing methane level in the atmosphere. DREAM 2047 (Magazine-Vigyan Prasar, Govt. of India).
2. शशांक तिवारी, छत्रपाल सिंह, जय शंकर सिंह * (2017). वायुमण्डल में मीथेन के स्तर का संतुलन। ड्रीम 2047 (Magazine-Vigyan Prasar, Govt. of India).
3. शशांक तिवारी, छत्रपाल सिंह, जय शंकर सिंह * (2017). मीथेनशोषक (मेथनोट्रोफ) जीवाणुओं का पर्यावरणीय योगदान। विज्ञान प्रगति।(NISCAIR)
4. **Shashank Tiwari**, GRK, Chhatarpal Singh, Siddharth Boudh and Jay Shankar Singh*. Methanotrophs: A Bioweapon to Destroy Methane. Microbiology World (e-magazine), 3(3), Jan-Feb, 2016 (ISSN: 2350-8774).
5. छत्रपाल सिंह, शशांक तिवारी, जय शंकर सिंह. कृषि उत्पादन में बायोचर का महत्व। कृषिसेवा, Sep 27, 2017.
6. छत्रपाल सिंह, शशांक तिवारी, जय शंकर सिंह धान की खेती में बायोचर के रूप में अपशिष्ट का योगदान। खेती दुनिया, Sep 30, 2017.
7. छत्रपाल सिंह, शशांक तिवारी, जय शंकर सिंह (2018). पर्यावरण प्रबन्धन में बायोचर की भूमिका। विज्ञान प्रगति (NISCAIR) Accepted.

Signature of Supervisor

Signature of HOD