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

Thesis

SUBMITTED TO
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
LUCKNOW

BABASAHEB
BHIMRAO
AMBEDKAR
UNIVERSITY
LUCKNOW
प्रज्ञा शील करुणा
ESTABLISHED 1996

FOR THE DEGREE OF

Doctor of Philosophy

IN

ENVIRONMENTAL MICROBIOLOGY

Submitted By

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(A Central University, NAAC Accreditation ‘A’ Grade)
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UTTAR PRADESH, INDIA

2018

This Attempt is Dedicated to

My Parents

Whose inimitable love and affection stands before me as a constant source of energy & inspiration in every walk of my life.........

STUDENT DECLARATION

This is to certify that the material embodies in the present Ph.D. work entitled **"Impact of Land Use Changes on Methanotrophic Bacterial Abundance and Soil Microbial Biomass"** is original research work done by me. It has not been submitted in part or full for any other diploma or degree in any other University. In this Thesis, matter written, data presented and plagiarism, if any, is the sole responsibility of the student Mr. Shashank Tiwari. If any allegations/query/question arises regarding the thesis, I will be solely responsible and answerable.

I also declare that the thesis is essentially free from all kinds of plagiarism.

Shashank Tiwari
Research Scholar

CERTIFICATE

This is to certify that the thesis entitled "**Impact of Land Use Changes on Methanotrophic Bacterial Abundance and Soil Microbial Biomass**" submitted by **Mr. Shashank Tiwari** is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other university.

The Thesis submitted to Babasaheb Bhimrao Ambedkar University satisfies all the requirements as stipulated in the *Doctor of Philosophy (PhD) regulations - 1999 as amended in 2008/2010/2013* and it is fit for submission and evaluation for the award of the degree of Doctor of Philosophy of the University.

Date: 08/10/2018

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ACKNOWLEDGEMENTS

I bow in the reverence to 'the Almighty' for blessing me with strong will power, patience and confidence that helped me to complete the present work.

The present work in the manuscript accomplished under the sympathetic attitude, expert guidance, intellectual reviews/criticism, constant passion, fatherly behaviour and enlightened supervision of **Dr. Jay Shankar Singh**, Assistant Professor, Department of Environmental Microbiology, Babasaheb Bhimrao Ambedkar University, Lucknow. I am highly grateful to Dr. Singh for his consistent inspiration, constant encouragement and all round support throughout the course of doctoral work. I feel so richly blessed to have him as my supervisor.

I wish to express my profound gratitude and sincere thanks to **Prof. Rajesh Kumar**, Head, Department of Environmental Microbiology, BBAU Lucknow for providing infrastructural facilities during the study period.

I am highly thankful to Prof. Ram Chandra, Dean, Prof. Naveen K Arora, Dr. V.S. Baghel, Dr. R.N. Bharagava, Dr. Pankaj Kumar Arora, Dr. Digvijay Verma, Dr. Harish Chandra, Dr. Ravi Gupta, Assistant Professor, Department of Environmental Microbiology, Babasaheb Bhimrao Ambedkar University, Lucknow their help, healthy suggestions and valuable criticism to complete the manuscript successfully and Dr. Monali C. Rahalkar, Bioenergy Group, Agharkar Research Institute, Pune for providing the known methanotrophic culture for DNA. I also wish to thank Professor Surendra Singh, Department of Botany, BHU for his help in analyses of soil samples for soil microbial biomass-C, -N and -P and CCA analyses. I also avail this opportunity to thank non-teaching staff of the department for their continuous support during the research work.

I would like to extend my deepest thanks and regards to whole BBAU fraternity for providing Internet facility, and a well-equipped library and university grants commission (UGC) for the financial assistance without which this work would not have been possible.

I would like to express my sincere regards to all my lab mates and friends for their kind suggestions and encouragements during the course of research work. I am also thankful to Dr. Atul Upadhyay, Mr. Ranjan Singh, Mr. Ranjeet Sharma, Mr. Anupam Pathak, Mr. Prabhat Mishra and Mr. Aman Deep for their support and encouragement.

No word is present in the whole literature to convey my emotions and gratitude to my parents **Shri Parmanand Tiwari** and **Smt. Mithlesh Tiwari** and sisters **Diksha Tiwari**, **Nishtha Tiwari** for their moral support, love, inspiration and blessing to make this dream come true.

In the last but not least, I would also like to sincerely acknowledge all those persons and institutions, who was directly or indirectly involved and helped me in overcoming the difficulties faced by me during research work.

Thanks to all.

Date: (**Shashank Tiwari**)

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LIST OF ABBREVIATIONS & SYMBOLS

@	At
%	Percentage
ANOVA	Analysis of Variance
BD	Bulk density
BLAST	Basic local alignment search tool
FTIR	Fourier transform infrared spectroscopy
EC	Electrical conductivity
C	Carbon
$CHCl_3$	Chloroform
CCA	Canonical correspondence analysis
CH_4	Methane
CO_2	Carbon dioxide
cm	Centimeter
CEC	Cation exchange capacity
d.f.	Degrees of freedom
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
Fig	Figure
g	Gram
GHG	Greenhouse gas
ha	Hectare
hrs	Hours
IPCC	Intergovernmental Panel on Climate change
K	Potassium
kg	Kilogram
LSD	Least significant difference
LUC	Land use changes
m	Meter
M	Mole
µg	Microgram

MB-C	Microbial biomass carbon
MB-N	Microbial biomass nitrogen
MB-P	Microbial biomass phosphorus
mg	Milligram
mL	Milliliter
mm	Millimeter
mo	Month
MOB	Methane oxidizing bacteria
MPN	Most probable number
MMOs	Methane monooxygenase enzyme
Mt	Metric tones
N	Nitrogen
NMS	Nitrate mineral salt
NH_4^+	Ammonium
NH_2OH	Hydroxylamine
nm	Nanometer
No	Number
NO_2^-	Nitrite
NO_3^-	Nitrate
O_3	Ozone
P	Phosphorus
qPCR	Quantitative polymerase chain reaction
RT-PCR	Real time polymerase chain reaction
RNA	Ribonucleic acid
pmoA	Particulate methane monooxygenase gene
pMMO	Particulate methane monooxygenase enzyme
ppb	Parts per billion
ppm	Parts per million
S	Sulphur
SE	Standard Error
SM	Soil moisture
SMB	Soil microbial biomass
sMMO	Soluble particulate methane monooxygenase enzyme

sp (Singular)	Species
spp (Pleural)	Species
Tg	Teragram
WHC	Water holding capacity
Yr^{-1}	Per year
μL	Micro liter
USA	United States of America

CHAPTER 1

Introduction

Chapter 1

INTRODUCTION

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 LUC has been also reported to soil microbial diversity decline (HainesYoung and Potschin, 2009) and loss of soil microbial biomass in the soil of many tropical forest ecosystems (Lima et al. 2014; Qi et al., 2018). The land use management practices also influences the functional roles of soil microorganisms due to differences in quality and quantity of organic matter inputs because of variations in dominant vegetation covers (Steenwerth et al., 2002; Jin et al., 2010). The conversion of natural forest covers, savanna and grasslands into agriculture land may lead to negative impacts on soil functioning (Raiesi, 2017), soil fertility, microbial community structure (Li et al., 2015), ecosystem functioning (Singh and Gupta, 2016) and ultimate the human heaths in many ways.

The LUC mediated habitat destructions and soil degradation as well as other human perturbations strongly influences the sustainability of soil and ecological balances (Pabst et al., 2013). Understanding the responses of LUC alterations may be crucial to tropical forest ecosystems, known for its great contribution to high biodiversity, global climate and biogeochemical cycles, especially to C turnover and sequestration (Malhi and Phillips, 2004; Katovai et al., 2012). But in recent years, due to intense disturbances in environmental and climatic conditions, the productivity and functioning of tropical forest ecosystems are greatly impacted compared ecosystems of colder regions (Zech et al., 1997). Therefore, tropical forest ecosystems has been given special emphasis in several studies concerning the anthropogenic mediated disturbances and C budgets (Houghton and Goodale, 2004; Don et al., 2011). The organic-C contents in both moist and dry soils in tropical ecosystems altered due to LUC (Ogle et al., 2005), led to an average decrease in C stocks by 25–30% (Don et al., 2011). Several studies, conducted on soil microbial biomass, in tropical ecosystems, showed that land use management practices, such as tillage, also influences the soil microbial activity (Calderon et al., 2000) and sustainability of agricultural soils (Sanginga et al., 1992). Singh and Yadava (2006) investigated the dynamic of microbial biomass in soils in north-east India and found a decrease in soil microbial biomass (about 16%) due conversion from grassland into an agro-ecosystem within a short span of time (only after 4 years). It is assumed that the increasing demand for agricultural land may directly accelerates deforestation (conversion of forest to agricultural ecosystems) activity which could decrease up to 75% in both soil organic-C and microbial biomass (Lemenih et al., 2005; Houghton and Goodale, 2004; Waldrop et al., 2000). Although, deforestation is a common practice for the conversion of natural forest ecosystems to agricultural lands, afforestation has become a much discussed topic in recent years (Singh and Singh, 2012). So, there are mixed response of LUC in terms of beneficial (Afforestation) and harmful (deforestation) in prospects of agriculture, soil and environment health.

Changes that occur in vegetation composition due to land use type conversion are responsible for most of the variation that occurs in chemical and physical soil properties (Lisboa et al., 2014). In turn, these changes tend to correlate with variation in the microbial community, linking the changes above and below the soil surface (Mitchell et al., 2010; Lisboa et al., 2012, 2014). Land use changes have marked effects on soil quality, ecological processes and soil functions due to the modification of soil physical, chemical and biological properties (Bending et al., 2002; Zhao et al., 2013). In recent years, great efforts have been made to elucidate the effects and mechanisms of land use change on soil ecosystems (Zhao et al., 2007; Jiao et al., 2010). It has been suggested that the conversion of forest into grassland and of forest or grassland into agriculture can significantly affect the dynamics of soil organic matter (SOM), soil C and N cycles, and thus altering soil quality and functions (Chen et al., 2000; Zeng et al., 2009).

Rapid urbanization expansion such as park, garden, road side plantation, lawns, etc. strongly influences the soil biochemical processes and soil qualities of the unsealed green space (Betts, 2007). The LUC in urban and rural area led to decreased content of organic matter, variation of soil microbial biomass and increase in soil pollutions to park, roadside tree and other types of land uses (Zhu and Carreiro, 1999; Wang et al., 2011). Therefore, better understanding the influence of use changes on ecosystems of urban and rural area with reference to soil microbial processes is essential for the management in sustainability and viability of these mane made ecosystems.

The growing demand for food and energy creates various environmental problems, mainly caused by indiscriminate increasing human population (Borlaug, 2002; Mazzetto et al., 2016). The intensive land use causes may negatively affect both the environmental stability and the agricultural productivity, ignoring the soil conservation practices (Cerri et al., 2004; Foley et al., 2005). The degradation rate of natural tropical forest, which covers 7% of the earth's surface, is ~15.4 is greatly impacting the global environmental services (Parrotta et al., 1997; Kumar and Ghoshal, 2014). The rapid growth rate of human population caused by urbanization in northern India requires additional farmlands for the production of crops. One option is to expand the farming lands by clearing the natural forest covers, which may be correlated to further increase in serious environmental problems such as soil erosion/desertification, eutrophication, green house gases emissions and other environmental threats to northern India. Differences in the quantity and quality of substrates (organic C and N) returns caused by varying plant residue types (litter and fine roots) and the associated nutrient dynamics can be crucial drivers to influence the soil productivity (Singh and Gupta, 2018). It is also suggested that shifts in plant species composition during land use changes can influence the microbial community composition dynamics basically by changing the soil organic nutrient status (Zhang et al., 2016). Therefore, in view of above it expected that, to satisfy the future human food demands, better land use management practices such as reduced inputs of synthetic chemicals, promotion of organic farming and bio-fertilizer application, restoration of degraded land, re-forestation, etc. may be eco-friendly options for the improvement of global soil and environmental quality.

Soil Physico-Chemical Properties

The soil, one of the main natural resources, comprises of minerals, soil organic matter microbes, water and air. The soil physico-chemical characteristics vary in space and time due to variations in topography, climate, physical weathering processes, vegetation cover, microbial activities and several other biotic and abiotic variables (Paudel and Sah, 2003). The soil development in particular area is governed by biological (vegetation, soil micro-and macro-flora) and non-biological (parent rocks, climate and topography), factors over time (Brady and Weil, 2002). The composition and nature of these components greatly influence the soil physical properties, including texture, structure, porosity and nutrient status. In turn, these soil properties may affect soil's ability to support an ecosystems functioning.

The conversion of natural forest into agriculture land disturbs most of the soil functioning due to disturbances between microbial-soil-plant interactions (Raiesi, 2017; Vimal et al., 2017). So, intensive forest clearing due anthropogenic involvement and cattle grazing at disturbed sites may reduce the input of organic-C via plant residues and therefore, a disturb soil physico-chemical conditions. Luneberg et al. (2018) also reported that land use change in dry lands, similarly to other ecosystems, alters the quantity and quality of organic matter, which ultimately led to the soil carbon stock loss. Singh et al. (2009) also showed that pH of forest soils was lower than savanna site, because of the enhanced nitrification rates at forest soils that may add higher H^+ ions from NH_4^+-N oxidation. In addition, Tripathi and Singh (2007) reported that conversion of forest ecosystem into savanna may slightly increase the soil pH level due to enhancement in salt concentration. Similar to the pH, the electrical conductivity also decreased due to soil disturbances and soil compactness (Chaudhari et al., 2013). Zhang et al. (1988) and Singh et al. (1989, 2009) have also reported that an increase in cultivation of land, increases the soil BD. The high organic content in the mixed forest in other study is also reported by Bui and Handerson (2003) and Fazhu et al. (2015). Singh et al. (2009) reported that soil total-N and -P concentrations and C/N ratios were also higher at the two forest sites compared to savanna and cropland. The well constrained C/N ratio along soil profile experiences the principles that the formation of spoil organic matter necessitates a certain amount of N in a relatively fixed ratio with C (Sterner and Elser, 2002).

Interestingly, although insignificant, soil C/N ratio in savanna and agriculture land tended to decrease with soil depth, possibly reflecting a greater degree of breakdown and older age of the humus stored in the deeper soil layer. The higher C/N ratio in natural forest soil than the agriculture land and savanna may be attributed to the addition of higher amount of organic matter due to greater density of plant species cover. But at disturbed sites the lower C/N ratio could be due to because the above ground biomass at agriculture land and savanna is usually periodically removed by harvest, fires and intense cattle grazing. Furthermore, a higher C/N ratio at natural forest site could be due to higher plant litter accumulation at upper soil layer can transform the organic matter into mineral soil due to the degradation of labile organic matter and the formation of humus. Singh et al. (2009) reported that the reason for reduction in moisture content level in the farming land as compared to natural forest ecosystem may be due to the least quantity of organic matter as well as aeration promoted drying. Further, the soil moisture content vary temporally and spatially due to different parameters such as soil texture, vegetation cover, litter density, root distribution and density (Griffiths et al., 2009; Xu et al., 2013; He et al., 2014; Ma et al., 2014). The composition and proportion of these components greatly influence soil physical properties, including texture, structure, and porosity, the fraction of pore space in a soil. In turn, these properties affect air and water movement in the soil, and thus the soil's ability to function.

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

Methane (CH_4), a potent greenhouse gas (GHG), contributing about 15 to 20% of global warming (Jang et al., 2006, IPCC, 2007, Dalal and Allen, 2008). Therefore, it is required to find out the more ways to increase the CH_4 sinks to reduce its atmospheric load. Besides, reaction with hydroxyl (OH^-) radicals in the troposphere and stratosphere, CH_4 oxidation in soils, mediated by methanotrophic bacteria consume about 10 and 40 Tg annually (King, 1997; Schlesinger, 1997; IPCC, 2001; Mohanty et al., 2007), comprises approximately 6-10% of the total global atmospheric CH_4 oxidation (IPCC, 2001). Thus, the most reasonable way to increase the sink or consumption of CH_4 is increasing the abundance/diversity/performance of the soil CH_4 consuming bacteria. Therefore, knowledge about the ecology and responses of the CH_4 consuming bacterial diversity/population in relation to land use changes in dry tropical soils are of particular interest.

The *Bacillus methanicum* was the first methanotroph isolated in 1906 (Whittenbury et al., 1970). However, Whittenbury and colleagues isolated and characterized over 100 new methanotrophs (Whittenbury et al., 1970). For the classification point of view, 18 cultivated genera of methanotrophic γ-proteobacteria, which may exceed up to 20 if the "*Candidatus Crenothrix polyspora*" and "*Candidatus Clonothrix fusca*" are also added and 5 genera of α-proteobacteria have been explored representing approximately 60 species (Knief, 2015). The methanotrophic communities documented till date are clustered as *Proteobacteria* and *Verrucomicrobia* (Dumont et al., 2011). The methanotrophs are commonly referred as type-I includes *Methylomonas, Methylosphaera, Methylomicrobium, Methylosarcina, Methylobacter, Methylocaldum, Methylococcus, Methylohalobius* and *Methylosom* belongs to the family Methylococcaceae and fall under the range of γ-Proteobacteria (Tiwari et al., 2015). However, type-II includes *Methylocystis, Methylocella, Methylocapsa* and *Methylosinus* (Tiwari et al., 2015) exist in the group of α-Proteobacteria and include several genera in the family Methylocystaceae (Bowman, 2000) and the genera *Methylocapsa* (Dedysh et al., 2002) and *Methylocella* (Dedysh et al., 2000) in the family Beijerinckiaceae (Ho et al., 2013). The type-I methanotrophs can be further subdivided into two phylogenetic clades, the type-Ia, including primarily mesophilic methanotrophs, and type-Ib, also referred to as type-X includes some moderately thermophilic methanotrophs (Dumont et al., 2011). On the basis of culture independent studies the type-Ia and type-Ib may be extended as type-Ic, with the help of *pmoA* gene sequences of uncultivated taxan such as Methylothermaceae group of methanotrophs (Knief, 2016). Moreover, the *Nitrosococcus* and related uncultivated clades may be grouped as type-Id organisms based on *pmoA* studies (Knief, 2016). In addition, Knief (2016) states that *Verrucomicrobia* may be referred as type-III group of methanotrophs.

The oxidation of atmospheric CH_4 by methanotrophs is performed due to broad spectrum CH_4 monooxygenase (MMOs) enzyme (Singh et al., 2018). There are two distinct forms of this enzyme, the membrane-bound particulate CH_4 monooxygenase (pMMO) and the cytoplasmic soluble CH_4 monooxygenase (sMMO) (Hanson and Hanson, 1996). The particulate CH_4 monooxygenase (pMMO), a species of MMOs is found in all aerobic methanotrophs except *Methylocella* and *Methyloferula* (Kizilova et al., 2014). The pMMO is integrated into the inner membrane of the bacterial cell wall and is a copper containing protein (Zahn and Dispirito, 1996). The other sMMO is present within the cellular fluid (cytoplasm) and is therefore, called the soluble CH_4 monooxygenase. This enzyme differs bio-chemically from the pMMO by having a di-ion center at the active site for CH_4 oxidation (Rosenzweig et al., 1993). This enzyme is only expressed when copper deficiency prevents expression of pMMO. Methanotrophs expressing pMMO have higher growth yield, as pMMO is more specific and has a higher affinity for CH_4 than sMMO (Hanson and Hanson, 1996). The pMMO enzyme complex shares many similarities with the ammonia monooxygenase (AMO) enzyme complex found in ammonia-oxidising bacteria (Klotz and Norton, 1998). These similarities include a high degree of amino acid sequence identity, similar protein complex structures, and broadly similar substrate and inhibition profiles, while each play a crucial role in cell metabolism (Gilbert et al., 2000). Methanotrophs can oxidize both CH_4 and ammonia (NH_3); however, they can obtain energy only from the oxidation of CH_4 and ammonium (NH_4^+), respectively (Bedard and Knowles, 1989).

The dry tropical upland soils of Vindhyan region contributed to highest CH_4 consumption on a global scale (Singh, 2011). The soil CH_4 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. Further, a deeper insight into the methanotrophic diversity of soil is often limited by the inability to cultivate these soil microbes (Steinkamp et al., 2001). Methanotrophs or methane-oxidising bacteria (MOB) are a functional group of bacteria with proficiency of utilizing CH_4 as their sole carbon and electron source (He et al., 2012; Tiwari et al., 2015) and represent the only known biological sink for the potent greenhouse gas (GHG) CH_4 (Bodrossy et al., 2003; Tiwari et al., 2015). The MOB are ubiquitous in nature (Knief et al., 2003; Zheng et al., 2012) and has been reported from wetlands, oxic-anoxic interface and rhizospheric zone of rice field (Ferrando and Tarlera, 2009; Park and Lee, 2013), forest soil (Jang et al., 2006; Giri et al., 2007), landfill (Kallistova et al., 2007), sewage sludge (Ho et al., 2013; Park and Lee, 2013), faeces (Derrien et al., 2004), cow dung (Girija et al., 2013), cattle dung (Rastogi et al., 2009). These microbes are also exists in swamps, mud, ponds, rivers, streams, meadow soils, deciduous woods (Park and Lee, 2013), biogas reactor (Rastogi et al., 2009) stone (Kussmaul et al., 1998), sediments (Bowman et al., 1997), farmyard-manure (Singh et al., 2010b), farming soil, marine sediments (Semaru et al., 2008; Park and Lee, 2013), groundwater, tundra and polar lakes (Bowman et al., 1997). The methanotrophs are found in livestock manure (Rastogi et al., 2009), anaerobic environments (McDonald et al., 2008), continental margins (Tavormina et al., 2010, 2013) and extreme environments (Trotsenko and Khmelenina, 2002; McDonald et al., 2008; Tiwari et al., 2015) possibly hot springs and alkaline soda lakes (Semaru et al., 2008) and can survive in quite cold (4 °C) (Bowman et al., 1997) as well as hot temperature (> 81 °C) (Dunfield et al., 2014).

In recent years, due to the human activities the natural forest ecosystems are continuously being converted into farming lands for agricultural purposes. This transformation of forest into farm lands creates a deep impact on methanotrophic activity and community composition as a consequence an intense effect on global CH_4 budget (Dorr et al., 2010). Thus, the land use practices can alter the strength of soil CH_4 sink activity of methanotrophs (Mosier et al., 1991; Dorr et al., 2010). It has been suggested that deforestation and other land farming practices can result into alteration in the organic carbon content, soil moisture and N-nutrient status, which may adversely affect the number of viable methanotrophic community (Singh et al., 2007;

Singh et al., 2010a). Therefore, all the forest management related activities such as afforestation, reforestation and reduced deforestation can go a long way in achieving the balance in global CH_4 budget due to methanotrophic soil community.

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 CH_4 sink activity of methanotrophs too (Mosier et al. 1991; Dorr et al. 2010). Two main explanatory factors have been proposed to regulate the aerobic methanotrophs CH_4 consumption performance in soils under different land use: methanotrophic community composition and methanotrophs abundance (Malghani et al., 2016). To our knowledge, little or no investigations have previously examined the abundance of methanotrophs in dry tropical upland soils across different land uses, which acts as a strong sink of atmospheric CH_4 (Singh, 2011). The CH_4 consumption in aerobic soil by methanotrophs is typically assessed by the detection of the *pmoA* gene, which encodes the β-subunit of pMMO enzyme (Semrau 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 is 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), an important labile portion of the soil organic matter has been considered also as a source of plant nutrients in natural and agro-ecosystems (Singh et al., 2009; Silva Maganhotto De Souza and Fay, 2012). Although soil physico-chemical status, seasonality, soil temperature, and other environmental factors, are important major drivers in regulating the functioning of natural ecosystems, SMB may be one of the key ecological factors which can govern the productivity of tropical nutrient poor soils (Singh et al., 2010). The 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. As microbial adaptation and re-establishment in the form of SMB may play a major role in ecosystem responses to land use disturbances (Singh and Gupta, 2018), the long-term consequences of earlier reports on SMB levels are critical for predicting alterations in ecological and environmental functioning. The understanding of SMB levels can be used as a suitable indicator for quantifying and monitoring changes in SMB–C, –N and –P and beneficial microbial community structure as well as soil nutrient dynamics in response to anthropogenic mediated land use disturbances. 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.

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. Therefore, we were interested to study the influence of soil conditions and soil nutrient quality (organic matter and C/N ratio) on various microbial mediated activities across different land use changes and soil depths in dry tropical region of Vindhyan region. Furthermore, abundance, diversity of CH_4 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.**

CHAPTER 2

Review of Literature

Chapter 2

REVIEW OF LITERATURE

Land use changes (LUC) impacting soil physico-chemical properties

The present world human population of about 7.2 billion is expected to cross 9.6 billion by the end of year 2050. In order to provide food to all by that times, the annual production of cereals needs a jump of about 50%, i.e., from 2.1 billion tons to ~3 billion tons per year (Gabriel et al., 2014; Singh et al., 2016b). Growing demands for food, feed, fuel, fibre and raw materials create local and distant pressures for creation of new farming lands derived from natural forest covers (Lambin and Meyfroidt, 2011). This onerous target puts enormous pressure on agriculture sector to achieve the food security (Singh et al., 2016b). But such a quantum leap in food production can be achieved either by bringing more and more land under cultivation or by enhancing the productivity of cultivable land available. The first option remains a distant dream in the light of limited land and growing population. The option of increasing soil fertility and agricultural productivity with the help of better eco-friendly management tools, promises a successful food security (Upadhyaya et al., 2016). This boost up of human population across the Globe is making a pressure to fulfil the food demands and consequently, leading to the clearing of natural forests and gradual conversion into agriculture lands. These enormous lands use changes across the Globe creating disturbances in soil physico-chemical properties, and the major reasons for soil degradation. Changing climate patterns, economic globalization, population growth, increasing exploitation of natural resources and rapid urbanization are putting pressure on natural terrestrial ecosystems as never before, and virtually all of them are under stress. The cascade of outcomes resulting from these demands is complicated by urbanization and globalization, which separate the production of goods from their consumption over vast distances (Barles, 2010; Kissinger and Rees, 2010). The intensified demand for food and fuel has led to the conversion of forests and grasslands into intensively managed lands by deforestation and using agricultural practices such as tillage, fertilization and pesticide application (Francioli et al., 2014). These practices aim to maximize crop production in a short-term, impairing the ability of soils to provide other essential ecosystem services;

consequently, they reduces soil fertility and sustainability in the long-term (Liiri et al., 2012), often coupled with a decline in soil microbial biodiversity. The current perspective suggests that shifts in plant community composition may affect soil organic carbon (SOC) dynamics (Jin et al., 2010), and studies have indicated that the varied effects of forest conversion on soil C are related to the soil type, crops and pasture and management practices (Nunes et al., 2012). Tesfahunegn (2016) showed that in Ethiopia rapid population growth and environmental factors lead to the conversion of natural forestland and grassland into cultivated farmland. Such LUC have contributed to soil degradation and soil fertility loss by deteriorating the soil physical and chemical properties (Karltun et al., 2013).

The LUC can have negative or positive effects on soil quality (Adugna and Abegaz, 2015). Land use changes have remarkable effects on the dynamics of soil nutrients (Biro et al., 2013). The LUC from forest cover to cultivated land may reduce the input or organic residues that lead to a decline in soil fertility (Muñoz- Rojas et al., 2015), increased rates of erosion (Biro et al., 2013; Adugna and Abegaz, 2015), the loss of soil organic matter and inorganic nutrients (Saha and Kukal, 2015), and an accelerated rate of soil degradation (Barua and Haque, 2013). Vegetation cover is, therefore, a key indicator of soil stability as plants play a role in controlling soil erosion (Kropfl et al., 2013; Adugna and Abegaz, 2015). Biro et al. (2013) observed that the expansion of cultivated areas can substantially affect soil nutrient contents by reducing the composition of plant species, net primary productivity, above- and below-ground distribution in plants, and nutrient cycling. Saha and Kukal (2015) found a higher bulk density and lower macro-porosity and water retention in cultivated soils than in soils of grassland and forests. This indicates a degradation of soil physico-chemical properties due to the conversion of natural ecosystems to agricultural lands.

Soil erosion has been noted at higher rate in cropland compared to grassland (Adugna and Abegaz, 2015) and forest. The lower content of sand and higher content of clay fractions in the cultivated land may be attributed to the process of ploughing, clearing and the levelling of farming fields, because the clay particles are very small in size, silt, and sand fractions could be removed from the cultivated land due to surface runoff. The content of SOM was the highest in forest lands and the lowest in cultivated land and the differences were statistically significant (Adugna and Abegaz, 2015). The more rapid decrease in SOM contents in cultivated land may be attributed to accelerated rates of erosion and reduced decomposition because these processes were more active in cultivated lands than forest and grazing lands (Abegaz et al., 2016). Land management such as poorly designed terracing and cut off drainage, which was implemented on cultivated land, facilitated the drainage of water and soil from this land and deposited water and soil on forestland (Adugna and Abegaz, 2015). The traditional farming practices, also facilitates the removal of top soil in the cultivated land showing that SOM have a strong response to land use change (Vagen and Winowiecki, 2013). Thus, the high SOM in forest land may acts as a reservoir for the plant essential nutrients nitrogen, phosphorus, sulphur, etc. (Zeng et al., 2009). The greater SOM may also increase the soil water holding capacity and CEC and enhances the soil aggregation and the structure of soils of forest land. Adugna and Abegaz (2015) also reported that the total N was highest in soils of forest land and lowest in farming lands. The C: N ratio was reported highest in soils of forest land, while lowest in grazing and farming lands (Adugna and Abegaz, 2015). Due to the close relationship between SOM and total N, the soil C: N ratio indicates the status of soil fertility. The C: N ratio is often influenced by climate, soil condition, vegetation type and agricultural management practices (Oedraogo et al., 2006; Zhang et al., 2009).

The content of available P was the highest in cultivated land and the lowest in grazing land. The land use changes have also influence the available P level in the soil. Adugna and Abegaz (2015) showed that compared to the available P contents of forestland, the available P of cultivated and grazing lands is increased. Secondary minerals, organic, and inorganic fertilizer are important pools of soil phosphorous (P) (Assefa and van Keulen, 2009). Thus, those soils in the forestland have higher available P than in the grazing land may have to two reasons. Firstly, even though a pool of available P could be removed by trees in forestland, there is a probability of P return through litter fall to the soil surface (Asmamaw and Mohammed, 2013). Secondly, microbes, which are abundant in the litter layers of the forest, may quickly add a high proportion of P under forest cover. On the other hand, a higher available P in cultivated land than in grazing land may be due to (a) cattle dung applied to cultivated fields may increase the level of P concentration in this land use (b) the frequent application of inorganic P fertilizer to the cultivated fields may provide a considerable amount of inorganic P to the soil of cultivated fields (c) a higher P release as a result of greater weathering processes on cultivated land than on grazing land may provide a higher amount of available P to the soil of cultivated land.

Furthermore, Adugna and Abegaz (2015) reported that pH from forest soils was different from the cultivated and grazing land. Compared to the pH of soils of forestland, the pH of soils of cultivated and grazing lands was lower. Thus, soils in the cultivated land were more acidic than those of the forestland and grazing lands. This is because of intensive farming over a number of years with nitrogen fertilizers on cultivated land. Cultivated land is characterized by the acidifying effects of acid-forming nitrogen fertilizer, poor nutrient cycling, and the mining of basic cations through harvested crops, soil erosion, and acid rain. Soil acidity affects the process of other nutrient transformations, solubility, or plant availability of many plant essential nutrients (Barua and Haque, 2013). Compared to the CEC of the soils of forestland, the CEC of the soils of cultivated and grazing lands were decreased (Adugna and Abegaz, 2015).

It has been reported that SOM, TN, CEC, exchangeable cations, and pH are positively and significantly associated with each soil property except with available P, silt, and clay. SOM is significantly and strongly associated with pH. This finding was in agreement with other studies carried out in different parts of the country (Lelisa et al., 2010; Amare et al., 2013; Asmamaw and Mohammed, 2013). Thus, the conversion of forestland into cultivated lands likely leads to the degradation of SOM. Since SOM is the major influence for N in the soil, soil-available P, and CEC, it provides micronutrients through an effective soil food web. However, SOM in the soils of cultivated land can be increased through compost, cover crops, manures, minimum tillage, and crop rotation (Mikha et al., 2015; Martins et al., 2009). These can improve the concentration of physical, chemical, and biological soil parameters in the cultivated land. Adugna and Abegaz (2015) reported that there was no significant correlation between available P and any of the other chemical properties, most probably due to the limited range of pH in the soil sampled. They demonstrated that the finding did not agree with the fact that phosphorus availability is related to soil pH. CEC is significantly and strongly associated with exchange able Ca^{2+}, pH, and clay.

The impact of anthropogenic activities (land use) on soil characteristics such as the water content, soil texture, temperature, pH and soil nutrient levels with consequences for microbial communities is well documented (Noe et al., 2012). Soil is a highly complex biological system that is influenced by correlated physical-chemical and environmental parameters, and it presents a varied habitat for a diverse range of soil microorganisms (Pietramellara et al., 2002; Campos et al., 2012; Francioli et al., 2014). Soil microbes are closely linked to soil properties, and their diversity is critical for the maintenance of soil health because they are involved in principal soil functions (Parkinson and Coleman, 1991) and soil monolith and structure (Tisdall, 1991).

Soil is the largest organic carbon (C) reservoir in the terrestrial biosphere. It contains more than 1.5 trillion tonnes of C, roughly three times the C contained in all the vegetation worldwide and twice the amount of C stored in the atmosphere as CO_2 (Xu et al., 2017). Consequently, relatively minor changes in soil organic carbon (SOC) storage will result in substantial alterations in atmospheric CO_2 concentrations. The conversion of natural vegetation to agricultural ecosystems is regarded as one of the large sources of soil C losses (Xu et al., 2017). Several studies have shown that soil C status is impacted by land management (Chang et al., 2014), land degradation (Wen et al., 2012) and grazing intensity (Xu et al., 2017). Poor soil management practices deteriorate enormous amounts of land, which frequently becomes a major threat to rural sustenance in many developing and developed countries. Intensive land use can lead to negative impacts on soil quality (Siqueira et al., 2014). Conversely, impact of land use changes on soil can occur so unnoticed that land managers hardly contemplate initiating ameliorative measures. Land use changes have marked effects on soil quality, ecological processes and soil functions due to the modification of soil physical, chemical and biological properties (Bending et al., 2002; Zhao et al., 2013) the conversion of forest into grassland and of forest or grassland into agriculture can significantly affect the dynamics of soil organic matter (SOM), soil C and N cycles, and soil biological properties, thus altering soil quality and function (Chen et al., 2000; Zeng et al., 2009). Decreased content of organic matter, variation of soil microbial biomass and soil pollutions have been reported for park, roadside tree and other types of land use (Jim, 1998; Zhu qnd Carreiro, 1999; Wang et al., 2011). Cerli et al. (2009) reported that land use change from a primary floodplain forest to poplar plantation has modified many chemical and physical soil properties. The quantity and quality of organic input at the two sites influenced the C content and distribution along the soil profile. In the natural forest, the deposition of a litter layer and the natural incorporation of plant residues into the top mineral soil resulted in a C profile sharply decreasing with depth, thus contrasting the homogeneous depth distribution under the poplar plantation, which seems to be caused by cultivation practices. In the poplar plantation, the transfer of C into the deeper horizons by ploughing and the input of fine soil particles during flooding events resulted in unexpected similar C stocks at the two sites. In the natural forest, organic matter was partly free, thus bio-available, while another fraction was stabilized within aggregates and by formation of strong organo-mineral complexes, particularly in the deeper horizons, on the other hand, in the poplar stand the most organic matter was bound to mineral components, while little amounts were in the free and occluded light fractions (Cerli et al., 2009). From these results, it appears that the main effects of a 40-years change from pristine forest to poplar plantation are related to the C distribution both along the soil profile and among density fractions. Although C storage was apparently little affected, the soil biological activity, fertility, and structure declined under the poplar plantation. Adequate levels of organic matter are essential to maintain or improve chemical fertility, soil porosity, infiltration capacity, moisture retention, and resistance to water and wind erosion. On a global scale, organic matter represents the largest terrestrial repository of C (~1500 Pg C) and, thus, it is a key component of the C cycle (Cerli et al., 2009). Changes in land use and management can have profound effects on quantity and dynamics of soil organic matter and, in turn, on the soil ecosystem functions. On global scale, inaccurate managements have a large impact on the atmospheric CO_2 concentration (IPCC, 2001; Allmaras et al., 2000). It is clear that conversion of natural forests or grasslands into agricultural fields leads to the reduction of organic matter (Ellert and Gregorich, 1996). Similarly, cultivation generally decreases the total organic matter, but there are contrasting results in the literature on the impact of fast-growing plantation forests and their management (Cerli et al., 2009). Various patterns of change in soil C in fact have been associated with short-rotation tree plantations, including transient losses (Hansen, 1993), subsequent gains (Hansen, 1993; Makeschin, 1994) and no change (Ulzen-Appiah et al., 2000), even if often detection of short or even medium term land-use and/or management induced changes in total organic matter is difficult, due to high natural soil variability (Smith, 2004). Cerli et al. (2009) reported that periodical flooding events and erosion by the Ticino River and anthropogenic disturbances (land use change and cultivation) the natural forest and poplar plantation, resulted in modification of vegetation, fauna activity and site conditions. Land use is one of the main drivers of many processes of environmental change, as it influences basic resources within the landscape, including the soil resources (Gonzalez et al., 2014). Rejman et al. (2014) addressed the role of land use change in soil losses and relief modification in Loess areas of Poland. Shangguan et al. (2014) showed that at least two dozens of soils have already gone extinct due to inadequate land use. Liang et al. (2014) showed that short term impact of the studied tillage treatments varied in the different fractions analysed. McGranahan et al. (2014) analysed the reliability of carbon sequestration estimations associated with the effect of unexplained variability and due to interactions of vegetation, land use management, and soil properties with belowground ecosystem function.

The soil nitrogen (N) is a key element in primary productivity and soil fertility of ecosystems (Gonzalez-Pedraza and Dezzeo, 2014). N dynamics in tropical dry forest have been related to the rapid rate of mineralization, high available mineral concentrations, and the low efficiency of plant nutrient use (Matson et al., 1987; Johnson and Wedin, 1997). Great effort has been dedicated to understand the dynamics of this element after clear-cutting and burning of these forests (Vitousek and Matson, 1985). The N mineralization involves a series of actions mainly mediated by soil microorganisms (Campbell et al., 1981). Therefore, this is a sensitive process to disturbance in most forest ecosystems (Vitousek and Matson, 1985). In a tropical forest, N mineralization depends on the amount and type of organic matter and microbial activity, as well as on soil physicochemical properties and soil moisture content (Anaya et al., 2007. When the tropical dry forest is cut down and burned to be converted into pasture, the soil temperature increases, causing rapid ammonification and nitrification. Consequently, great losses of N by volatilization, erosion, and leaching occur. Additionally, vegetation absorption and microorganism immobilization are insufficient to prevent these losses. Later, during the early stages of re-vegetation under pasture, the mineralization and nitrification rates are lower than those in the original forest (Neill et al., 1997). The tropical dry forest has been considered one of the world's most threatened ecosystems (Kennard, 2002). However, there is little information about land use changes effects for this ecosystem. Most of the studies on this topic have been conducted in rainforest areas. Tropical dry forests are characterized by the marked seasonality of rainfall that influences its primary productivity (Murphy and Lugo, 1995). In Venezuela, this ecosystem represents the most important life zone of land and includes large areas covered by dense seasonal dry forests developed on relatively fertile soils (Gonzalez-Pedraza and Dezzeo, 2014). These forests have been subjected to a high pressure of use associated with population growth and the expansion of the agricultural frontier. After tree cutting, one of the main uses has been the establishing of pastures. However, there is no detailed information about changes occurred in the soil nitrogen once the forests have been cleared and converted into pasture (Gonzalez-Pedraza and Dezzeo, 2014).

The land use change may significantly affect the ammonium and nitrate content in soils. As mentioned in the results, pasture lands showed ammonium and nitrate losses compared to the forest. Johnson and Wedin (1997), Neill et al. (1997) and Ellingson et al. (2000) showed the similar results, who found that in pasture lands, especially young pastures, the ammonification and nitrification are lower than in original forest. During early stages of the pastures establishing, the large losses of nitrate have been associated with volatilization, erosion, and leaching due to a decrease in the absorption rate by vegetation and immobilization of soil microorganisms (Vitousek and Melillo, 1997; Vitousek and Matson, 1997). N availability also has been positively correlated with the C and N levels in savannas, pastures, and agricultural crops' soils (Salinas-Garcia et al., 1997). Johnson and Wedin (1997) demonstrated that woody species in tropical forests, perennial grasses, particularly those adapted to fire, generally have a high C/N ratio in senescent aerial and underground tissues, allowing them to immobilize N during decomposition. Hassink (1994) and Garcia-Oliva et al. (2006) also found higher C/N ratio in pastures than in forest soils.

Land use changes, soil methane consumption and methanotrophs

Methane (CH_4), a potent greenhouse gas, contributes about one third to the Global green house gas emissions in the atmosphere after CO_2 and water vapour (Barcena et al., 2014; Singh et al., 2016). The CH_4 is 26-30 times more efficient in absorbing and re-emitting infrared radiation than CO_2. The concentration of CH_4 in the atmosphere is increasing due to discrepancy in CH_4 emission and removal. Although its global emissions, estimated at around 550 Tg CH_4 yr^{-1} (Kirschke et al., 2013), are only 4% of the global CO_2 anthropogenic emissions in units of carbon mass flux, atmospheric CH_4 has contributed 20% of the additional radiative forcing accumulated in the lower atmosphere since 1750 (Ciais et al., 2013; Saunois et al., 2016). This is because of the larger warming potential of CH_4 compared to CO_2, about 28 times on a 100-year horizon as re-evaluated by the Intergovernmental Panel on Climate Change (IPCC) 5th Assessment Report (AR5) (Myhre et al., 2013; Saunois et al., 2016). The atmospheric concentration of CH_4 has doubled during past 200 years and its concentration is rising by an average rate of 1% yr^{-1} over the past few years. The atmospheric level of CH_4 has gone up to 1,840 parts per billion (ppb) from 700 ppb since 19th century; i.e., the current atmospheric amount of CH_4 is more than 2.5 times higher than pre-industrial concentrations (Lelieveld et al., 1998; Smith et al., 2000; IPCC, 2001). The lifetime of this gas in the atmosphere is about 12 and half years (Tiwari et al., 2015). Therefore, its continuous increasing concentration in the atmosphere due to imbalances between genesis and oxidation is a great cause of concern.

The CH_4 plays an important role in the earth's atmospheric chemistry and radiative balance, being the second most important greenhouse gas after carbon dioxide. It is released into the atmosphere by a large number of sources, both natural and anthropogenic, with the latter being twice as large as the former (IPCC, 2001). It has recently been established that significant amounts of geologic CH_4, produced within the earth's crust, are currently released naturally into the atmosphere (Etiope et al., 2008). Volcanic/geothermal systems emit endogenous gases, including CH_4, by widespread degassing from soils. In atmosphere, the methane is emitted from a range of natural and anthropogenic (human intervention) sources as a result of the anaerobic decomposition of organic matter, land use changes and fossil fuel related emissions and is oxidised generally in tropospheric zone, upland soils and surface layers of waterlogged areas. The main sources of methane emission and consumption have been shown in **Figure 2.1.**

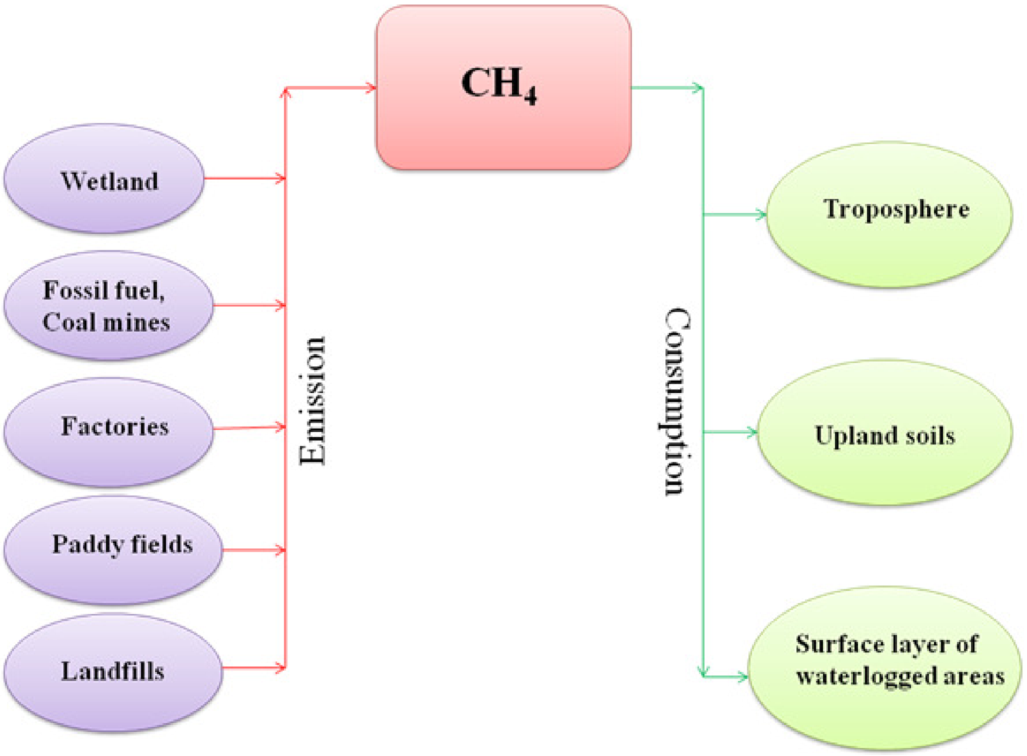


Figure 2.1. Methane emission and consumption in the environment

Methane emissions

Methane is emitted by a variety of sources in the atmosphere (Singh and Singh, 2012; Kirschke et al., 2013). These can be sorted by emitting process (thermogenic, biogenic or pyrogenic) or by anthropogenic vs. natural origin (Saunois et al., 2016). Biogenic methane is the final product of the decomposition of organic matter by Archaea in anaerobic environments, such as water-saturated soils, swamps, rice paddies, marine sediments, landfills, waste-water facilities, or inside animal intestines (Kirschke et al., 2013; Saunois et al., 2016). Thermogenic methane is formed on geological timescales by the breakdown of buried organic matter due to heat and pressure deep in the Earth's crust (Saunois et al., 2016). Thermogenic methane reaches the atmosphere through marine and land geologic gas seeps and during the exploitation and distribution of fossil fuels (coal mining, natural gas production, gas transmission and distribution, oil production and refinery). Finally, pyrogenic methane is produced by the incomplete combustion of biomass (Saunois et al., 2016). Peat fires, biomass burning in deforested or degraded areas, and biofuel usage are the largest sources of pyrogenic methane. Methane hydrates, ice like cages of methane trapped in continental shelves and below sub-sea and land permafrost, can be of biogenic or thermogenic origin. Each of the three process categories has both anthropogenic and natural components (Saunois et al., 2016). Moreover, it seems that the overall contribution of CH_4 to the global emissions has been underestimated, since some studies demonstrated that CH_4 can be readily formed *in-situ* by terrestrial plants (Keppler et al., 2006). This newly identified source may have important implications in the global CH_4 budget, and may call for a reconsideration of the role of natural CH_4 sources in the ongoing climate change. These new findings are likely to increase the importance of CH_4 sinks in the mitigation of its atmospheric concentrations. It is well accepted that anthropogenic activities (land use changes and use of chemicals in agriculture) are contributing to the global declining soil CH_4 sink potential (Zheng et al., 2010). Land use type is one of the major causes of soil characteristics variations and consequently the CH_4 sink activity. It may be argued that a higher rate of CH_4 consumption in forest soils, compared to other ecosystems could be attributed to the greater viable population size of methanotrophs. During the last few decades, the atmospheric concentration of CH_4 has increased dramatically because of the imbalance between the overall sources and sinks (Singh, 2011). Anthropogenic nitrogen enrichment, grazing, deforestation, and alterations in water availability and temperature have received particular attention in terms of their effects on soil CH_4 oxidation strength (Smith et al., 2000; Dai et al., 2013). The recent reports indicate that the problems of excess atmospheric load of this potent GHG can be mitigated either by reducing CH_4 emissions or enhancing its consumption (Singh, 2011). It may be argued that ~10% uplifting in the soil CH_4 consumption could stabilize the current problem of atmospheric CH_4 build up (Singh, 2011). Consequently, it is imperative to adopt a more viable and eco-friendly approaches that could significantly contribute to augmentation of soil CH_4 sink potential.

Human activity is mostly responsible for the major part of global CH_4 emissions, (which may comprise up to the 63% of the CH_4 emission per year); the remaining emissions are natural. Agricultural livestock, paddy cultivation, burning of fossil fuels, waste disposal practices, landfills, coal mining, natural gas distribution, and biomass burning are human activities that generate CH_4. Whereas natural sources comprise wetlands, oceans, rivers, lakes, estuaries, gas hydrates, vegetation including terrestrial plants, wildfires, arthropods, wild animals and permafrost, etc. (**Figure 2.2**). The human generated CH_4 production has increased steadily since the dawn of the industrial revolution.

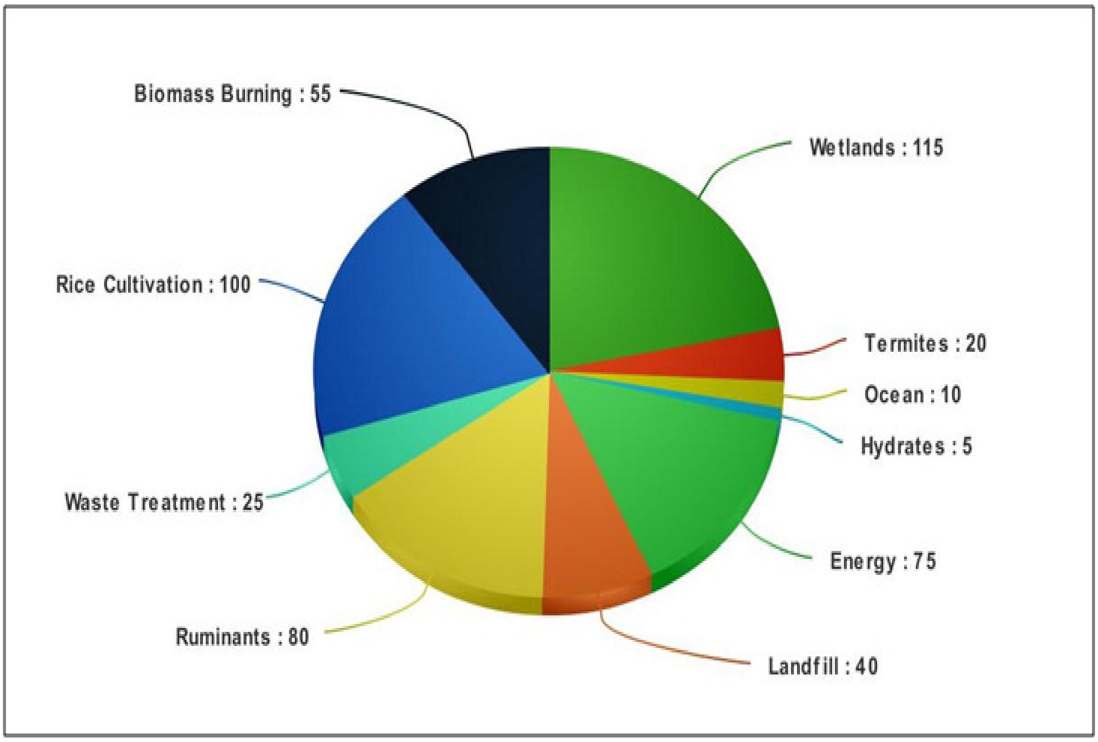


Figure 2.2. Chief methane emitters at global level (Mt/Yr) (Source: IPCC, 2001)

Biological CH_4 fermentation/ emission

Biologically, CH_4 is emitted by obligate anaerobic *Archaea* (methanogens). They play a vital role in anoxic environments of flooded soils like water-logged, swampy places and paddy fields (Dubey et al., 2014) for the generation of CH_4 and CO_2 (Conrad et al., 2006).

$$\underset{\text{(e.g. Cellulose)}}{C_6H_{12}O_6} \xrightarrow{\text{methanogens}} 3CO_2 + 3CH_4$$

The CH_4 is produced as the terminal step of the anoxic (absence of oxygen) decomposition of organic matter generating CH_4 and CO_2 (**Figure 2.3**). The anaerobic decomposition of organic matter and generation of methane in flooded places occur mainly through (a) hydrolysis of polymers by hydrolytic organisms, (b) action of fermentative bacteria to form acid from simple organic compounds, (c) formation of acetate from metabolites of fermentations by homo-acetogenic or cross-feeding bacteria, and (d) CH_4 formation from H_2/CO_2, acetate, simple methylated compounds or alcohols and CO_2. Methanogens use acetate (contributes about 80% to CH_4 production) as a carbon substrate, but another substrate like H_2/CO_2 and formats also accelerate 10–30 % CH_4 production (Conrad et al. 1995).

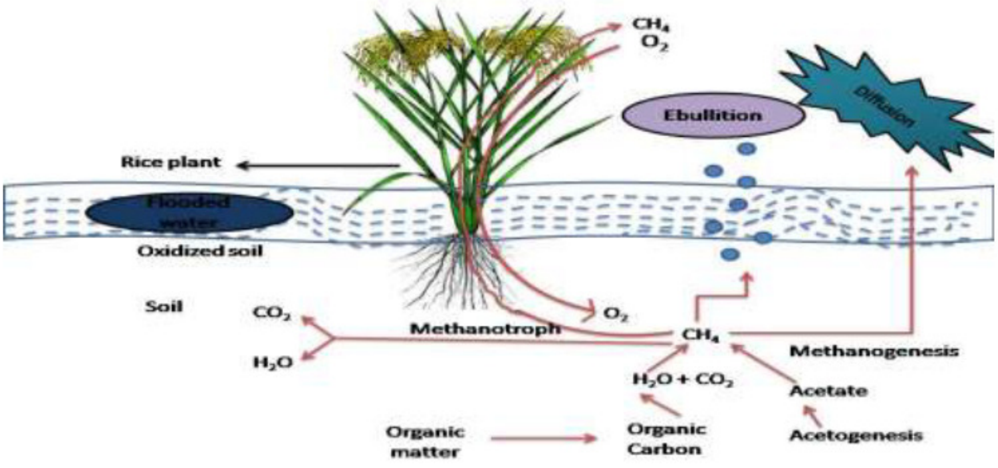


Figure 2.3. Production, consumption and transfer of CH4 to the atmosphere in paddy fields. Adapted from Singh et al. (2017)

Methanogens (methane-producing bacteria) are spherical (coccoid) or rod-shaped (Bacilli) *Archaebacteria* (considered ancient life forms that evolved separately from bacteria). They are anaerobes and are very sensitive to the presence of even very low level oxygen, i.e., cannot survive under aerobic environment. Over 50 species of methanogens have been isolated from water-logged places, sediments and many such places. Methanogens also thrive in the stomach of ruminants and produce CH_4 from CO_2 and hydrogen released by other microbes residing in their stomach. The main methanogenic strains are *Methanobacterium bryantii*, *Methanobacterium formicum,* *Methanobrevibacter arboriphilicus, Methanobrevibacter gottschalkii, Methanobrevibacter ruminantium, Methanobrevibacter smithii, Methanococcus chunghsingensis, Methanococcus burtonii, Methanococcus aeolicus, Methanococcus deltae, Methanococcus jannaschii, Methanococcus maripaludis, Methanococcus vannielii, Methanoflorens stordalenmirensis*, *Methanofollis liminatans, Methanogenium cariaci, Methanogenium frigidum, Methanogenium organophilum, Methanogenium wolfei, Methanomicrobium mobile,* etc.

Methane consumptions

Methane is removed from the atmosphere (i.e. converted to less harmful products) by a range of chemical and biological processes, which occur in different regions of the atmosphere (**Figure 2.4**). These include tropospheric oxidation, stratospheric oxidation and uptake by soils. In global perspective, most of the atmospheric CH_4 is oxidized through chemical reactions with hydroxyl radicals (OH-) in the tropospheric region (Schlesinger, 1997; Intergovernmental Panel on Climate Change, 2001; Hutsch, 2001). About 90 % of the total chemical oxidation occurs in the troposphere 'the detergent of the atmosphere' through reaction with free hydroxyl radical (OH-) (Schlesinger, 1997; Intergovernmental Panel on Climate Change, 2001; Hutsh, 2001).

Aerobic upland soils are the second largest biological sink for CH_4 due to methanotrophic bacteria. Methanotrophs (methane-oxidising bacteria, MOB), the only biological sink of CH_4, play an important role by consuming significant amount of CH_4 into the atmosphere (Singh, 2011). The amount of CH_4 oxidised by soil methanotrophic bacteria is estimated to be between 10 and 40 Tg yr^{-1} comprises approximately 6-10% of the total CH_4 oxidation of the atmosphere (Intergovernmental Panel on Climate Change, 2001). About 10-30% of the CH_4 produced by methanogens in rice field is consumed by aerobic methanotrophs associated with the roots of paddy crop (King, 1997; Schlesinger, 1997; Intergovernmental Panel on Climate Change, 2001; Mohanty *et al.,* 2007).

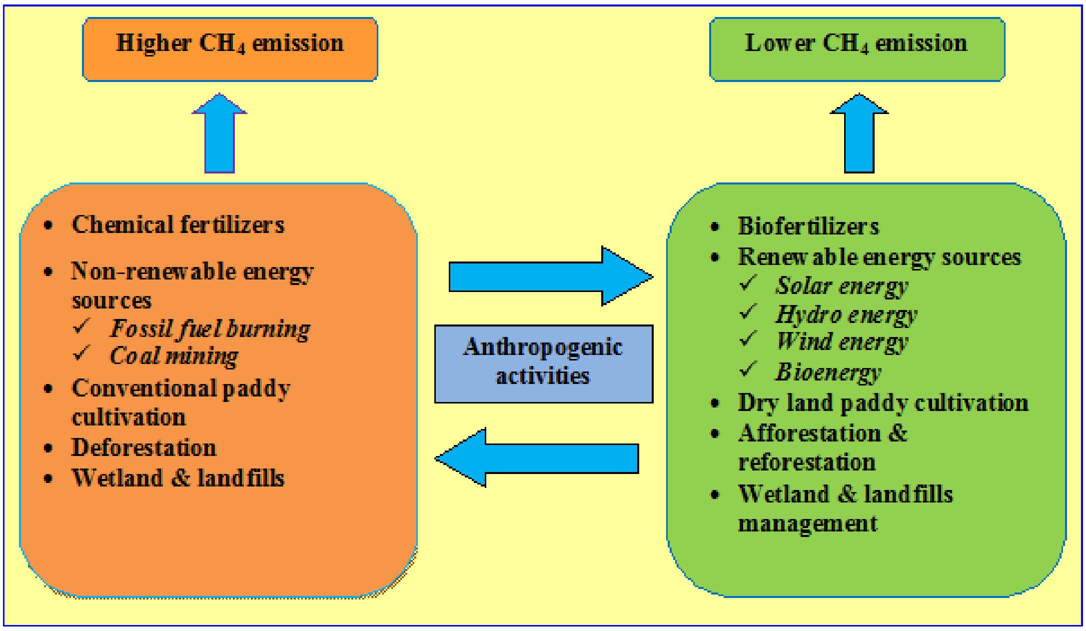


Figure 2.4. Production, consumption and transfer of CH_4 to the atmosphere in paddy fields.

Reforestation or afforestation and methane consumption

The CH_4 oxidation by forest soils has been studied extensively because these soils represent a major part of the soil sink in the global CH_4 budget (Adamsen and King, 1993; Kravchenko et al., 2002). It is well documented that forest soils are the more active sites for methanotrophy (CH_4 consumption), followed by grasslands and cultivated soils (Bender and Conrad, 1995; Le Mer and Roger, 2001). It has been noted that CH_4 uptake rates in indigenous forests are about 2–3 times higher than in adjacent pine and spruce plantations, indicating that forest type is an important factor in the rate of CH_4 sink activity (Le Mer and Roger, 2001). Native woodland in Australia has been shown to be a major sink for CH_4; taking up CH_4 significantly faster than managed soils (Livesley et al., 2009). In vitro investigations on the depth distribution of CH_4 oxidation found that the maximum rates of CH_4 oxidation occurred in the top layer of the forest soil, whereas in Tundra heath, this activity was more evenly spread over the organic horizon (Sjogersten et al., 2007).The soil CH_4 flux from grassland was positive in contrast to the negative CH_4 fluxes in different forest stands (McNamara et al., 2008). Thus, the varying CH_4 uptake rate in different soils demonstrates the role of vegetation cover in this process, which is quite suppressed in disturbed soils. It should be noted that there may be many more reasons for reduction in the CH_4 uptake process in such disturbed soils, and these need to be investigated. There is compelling evidence that forest degradation has a significant bearing on soil CH_4 fluxes (Verchot et al., 2000) and reforestation could be the potential strategy to mitigate the excess atmospheric CH_4 emissions by way of enhanced soil CH_4 consumption. Based on data from the previous studies, forest-related methane mitigation activities such as afforestation, reforestation, improved forest management and reduced deforestation can considerably contribute to reductions in excess atmospheric CH_4 load (McNamara et al., 2008). Conversion of a natural subtropical forest to farmland led to a loss of atmospheric CH_4 consumption, possibly because of the alterations in methanotrophic community composition (Dorr et al., 2010). Afforestation and reforestation of pastures exhibited a shift in the community composition of methanotrophs in soils taken from three different rainforest sites in New Zealand (Singh et al., 2007). Similarly, the conversion of forest to farmland also lowers the CH_4 sink capability of soil (Tate et al., 2007); however, the same may be recovered after afforestation (Verchot et al., 2000). In Western Australia, conversion of native woodlands to improved pasture led to a six fold decrease in CH_4 consumption. Significantly, this loss was partially reversed through subsequent re-afforestation to pine or eucalypt plantations (Livesley et al., 2007). Countries in tropical zones have also adopted the afforestation or reforestation of degraded land as a tool of forest of management, but they lack sufficient data in relation to methanotrophic bacterial diversity (Dalal et al., 2008). Very recently it has been observed a significant reduction in the number of methanotrophic bacteria in severely disturbed forest sites as compared to the undisturbed segment of the same forest. Based on these findings, it may be suggested that reforested ecosystems could provide a favourable soil environment and support the build-up of methanotrophs populations. The restoration of the ecological niche of the bacteria would perhaps allow the methanotrophic population to perform optimally. The disturbed soil with many stressful environmental conditions such as low soil moisture, nutrients, higher salinity and unfavourable pH suppresses the growth and population dynamics of methanotrophs and consequently the rate of CH_4 oxidation (Singh et al., 2010; Singh, 2011). It is possible that remaining spore-forming methanotrophs, lying dormant in the soil due to unfavourable ecological conditions, might help in restoring the population of methanotrophic bacteria once the ecological conditions become favourable. As the recovery of CH_4 sink capability is faster in tropical reforested sites than in temperate regions (Knief et al., 2005), there is a need to formulate an integrated approach to study the response of dry tropics and tropical rainforests ecosystems, with a special emphasis on soil methanotrophs dynamics. It is generally recommended that the best strategy to control further degradation of forests within the existing patches of remnant forest is to re-vegetate degraded areas with a mixed population of plant species. However, it seems essential to evaluate various strategies for the conservation of natural ecosystems, which might include the conversion of waste land into grassland or avoiding the compaction of soil to prevent further loss of CH_4 sink capacity (Vanitchung et al., 2004). The reforestation or restoration of degraded forests can be an effective and affirmative tool of environmental management, which simultaneously addresses the global concern for rising CH_4 concentrations in the atmosphere due to enhanced methanotrophic bacteria activity.

Consistent and significant increases in CH_4 sinks in forest soils indicate that afforestation can be effectively used to mitigate net CH_4 fluxes (Nazaries et al., 2013). Converting grasslands to forests could affect ecosystem–atmosphere exchanges of other greenhouse gases, such as nitrous oxide and CH_4, affects that are rarely considered (Menyailo et al., 2008). Menyailo et al. (2008) reported that afforestation on well-aerated grassland in Siberia reduces soil CH_4 uptake by a factor of 3 after 35 years of tree growth. The decline in CH_4 oxidation was observed both in the field and in laboratory incubation studies under controlled environmental conditions, suggesting that not only physical but also biological factors are responsible for the observed effect. Afforestation is an emerging policy recommended by the Kyoto protocol to increase the terrestrial uptake of atmospheric CO_2 (Schulze et al., 2002). It is assumed that forest ecosystems assimilate more CO_2 from the atmosphere and store more carbon in woody biomass than non forest ecosystems, but the full environmental consequences of afforestation (e.g. fluxes of other greenhouse gases) are rarely considered. An increase in CH_4 consumption after afforestation on drained peat soils in northern Europe was reported in several studies (Fowler et al., 1995; Maljanen et al., 2001; Ball et al., 2002), but the effect of converting upland grasslands into forests is less well understood. Grassland and peat differ considerably, in moisture, aeration regimes and site preparation before afforestation. They play opposite roles in the global CH_4 cycle: peat lands are a globally important CH_4 source, whereas upland grasslands are a sink. For these reasons, conversion of grasslands to forests may cause fundamentally different changes in CH_4 flux compared with conversion of peat lands. CH_4 consumption by soil is controlled by soil diffusion when the microbial activity is high or by microbial activities when the diffusion is large (Ridgwell et al., 1999). While many studies have considered the effects of land use change on CH4 uptake (Mosier et al., 1991, 1997; Sanhueza et al., 1994; Kruse and Iversen, 1995; Hutsch, 1998; Ball et al., 1999; Koga et al., 2004), less is known about the mechanisms of such effects (e.g. whether the effect is mediated by changed soil diffusion or by soil biology).

Physiology, biochemistry and molecular biology of methanotrophs

The ability of methanotrophs to oxidise CH_4 is due to unique enzyme called methane monooxygenase (MMO). The CH_4 oxidation reaction use reducing power to break the O-O bound in oxygen (O_2). One oxygen atom is incorporated into methanol (CH_3OH), while the other is converted to water (H_2O). The reducing power required for the oxidation of CH_4 to methanol and for bacterial growth is derived from further oxidation of methanol, via formaldehyde (HCHO) and formate (HCOOH) to carbon dioxide (CO_2) (**Figure 2.5**). The type I follows RuMP (Ribulose-bis-Phosphate) and Type II methanotrophs follow Serine pathway to assimilate the methane for their metabolic activities. Approximately 50% of the formaldehyde produced is assimilated into cell carbon and the remainder is oxidised to CO_2 and lost from the cell (Anthony, 1982). The MMO enzyme has been the subject of extensive biochemical and molecular research. There are two distinct forms of this enzyme, the membrane-bound particulate methane monooxygenase (pMMO) and the cytoplasmic soluble methane monooxygenase (sMMO) (Hanson and Hanson, 1996). The pMMO is integrated into the inner membrane of the bacterial cell wall and is a copper containing protein (Zahn and Dispirito, 1996). The other sMMO is present within the cellular fluid (cytoplasm) and is therefore called the soluble methane monooxygenase. The pMMO enzyme complex shares many similarities with the ammonia monooxygenase (AMO) enzyme complex found in ammonia-oxidising bacteria (Klotz and Norton, 1998). These similarities include a high degree of amino acid sequence identity, similar protein complex structures, and broadly similar substrate and inhibition profiles, while each play a crucial role in cell metabolism (Gilbert et al., 2000). Methanotrophs and ammonia-oxidising bacteria can oxidize both CH_4 and ammonia (NH_3); however, they can obtain energy only from the oxidation of CH_4 and ammonium (NH_4^+), respectively (Bedard and Knowles, 1989).

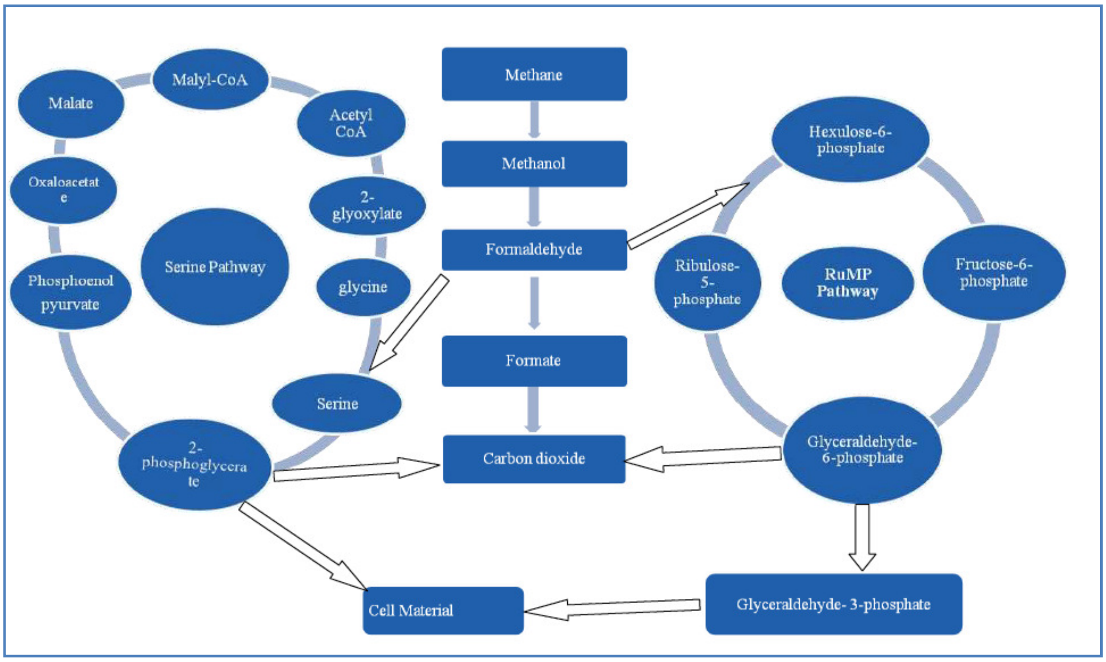


Figure 2.5. Methane oxidation pathway of Type I and Type II methanotrophs. Adapted from Singh et al. (2017)

There are several abiotic and biotic factors which affect the activities of CH_4 oxidising bacteria and also creating deep impact on the global CH_4 budget. The ecological distribution, diversity, and CH_4 oxidation activity of methanotrophs are affected by soil characteristics, pH, temperature, moisture, tillage, no-tillage and land use changes (Cole et al., 1997; Kessavalou et al., 1998; Knief et al., 2005; Horz et al., 2005; Mohanty et al., 2007; Ussiri et al., 2009; Singh et al., 2010; Zheng et al., 2012; Zheng et al., 2013). Chemical fertilisers are being used intensively at global level to fulfill the food demands because of continued population growth (Snyder et al., 2009). However, the use of fertilisers in agricultural systems often negatively affects the CH_4 sink potential of soils, which leads to increasing CH_4 concentrations in the atmosphere (Seghers et al., 2005). The increase in atmospheric CH_4 concentration is not clear whether it is due to increase in emission or reduced in consumption of CH_4. So, there is urgent need to identify the main factors for the increase concentration of CH_4 in the atmosphere. A new group of methanotrophs possessing MMOs gene has been cultured and sequenced belonging to phylum *Verrucomicrobia* (Islam et al., 2008) one of the primary lineages of the domain bacteria (Sangwan et al., 2004). These methanotrophs occupy their habitats generally in extreme environments such as extremely thermo-acidophilic (optimum growth pH was below 2 and optimum temperature 55 °C). During 2007-2008, the first extremophilic aerobic methanotrophs were reported from geothermal regions in New Zealand, Italy and Russia (Dunfield et al., 2007; Pol et al., 2007; Islam et al., 2008). Thompson et al. (2011) reported that the members of the *Verrucomicrobia* phylum remain absent in the Atlantic forest soil because the distribution of this phylum in soils is changeable and extremely sensitive to variation in the environment.

Methanotrophs in upland soils

The CH_4 sink mediated by soil methanotrophs can contribute up to 15% to the total global CH_4 destruction (Singh et al., 2010; Singh, 2011). De Bont et al. (1978) counted ten times more methane-oxidising bacteria in the rhizosphere than in the bulk anaerobic soil and one-third more than in the oxidised soil-water interface. They found significant increase in CH_4 emission by the rice cultivar IR36 when CH_4 oxidation was suppressed with acetylene at the soil water interface. However, acetylene had only a small effect on emission rates when applied to the rhizosphere. De Bont et al. (1978) concluded that the use of oxygen by reduced substances and microbes other than methanotrophs at the region of the root-soil interface exceeds the supply of oxygen by the root. Consequently, the aerobic zone surrounding the root of IR36 is too thin to oxidise the diffusing CH_4. Nevertheless, variability in root-oxidising power of rice cultivars is high, and the impact of roots on methane oxidation merits further study. In paddy fields, CH_4 oxidation greatly limits release of CH_4 to the atmosphere. Methanotrophs associated with the rhizosphere of rice plants oxidise significant of amount of CH_4 where concentration of molecular O_2 is abundant. Among different known land cover and land use the maximum CH_4 sink activity has been found in the soil of dry and paddy fields. Methanotrophs abundantly found in the oxidised flood water-soil interface and in the rice rhizospheric regions. It is reported that up to 60% of the CH_4 produced during a rice growing season may be oxidised before it reaches the atmosphere (Sass et al., 1991). Rice plants supply atmospheric oxygen to the roots for respiration via a special vascular system, the aerenchyma tissues. The aerenchyma has its own openings at the leaf sheath (Nouchi et al., 1991), and the oxygen supply to the roots is independent of transpiration and stomatal gas exchange. Because of the abundance of methane-oxidising bacteria present in the rhizosphere, the rhizospheric regions can contribute in CH_4 oxidation at a higher rate. It is clear that CH_4 consumption by methanotrophs in the rhizospheric regions of arable paddy crop and upland soils may greatly limit the diffusion of CH_4 to the atmosphere.

Land use changes and methanotrophs

Due to excessive need of food to satisfy huge population of human beings in present era of world the natural forests are continuously being converted into farming lands. This destruction of forests into farm lands creates a deep impact on methanotrophic community structure with intense effect on global methane budget (Dorr et al., 2010). Changes in land use practices can alter the strength of the soil CH_4 sink, altering the global CH_4 budget (Mosier et al., 1991). Zheng et al. (2010) showed that methanotrophic community structure was strongly affected by land use changes. They assessed the community structure in three plots under different land utilization patterns including disturbed, farming, and restoration on molecular basis. They found higher abundance of type I than those of type II methanotrophs. The application of treatments in farming land was significantly differed in terms of methanotrophic population from degraded and restored land. The lowest methanotrophic diversity indices were observed in the farming plot suggesting that the community structure was strongly affected by farming activities. The methanotrophic population abundance and diversity under different land uses is not very clear. Therefore, serious and extensive investigations related to impact of different land-use pattern on CH_4 consumption and methanotrophic numbers is urgently required.

Tillage may expose protected organic matter that may serve as a substrate for microbial growth (Rovira and Greacen, 1957). Tillage and the use of soils for arable cropping reduce the CH_4 oxidation capacity by 6 to 8 times compared with natural undisturbed soils (Hutsch, 1998). As the methanotrophic bacteria are highly sensitive to soil disturbance, reduced tillage may be beneficial for methane oxidation activity (Hutsch, 1998). Hutsch (1998) demonstrated that CH_4 oxidation rates were 4.5 to 11 times higher in the direct-drilled fields than in the continuously ploughed fields. He further showed that the oxidation rates in the forest soil were 11 times the highest rate measured at the field site, showing a different land use effect (Hutsch, 1998). However, CH_4 oxidising activity may also be reduced in tillage plots (Datta et al., 2013) and using conservation tillage systems, CH_4 emissions can be reduced in agricultural soils (Kessavalou et al., 1998; Ussiri et al., 2009). Tillage practices affect concentration and soil nutrient concentrations and availability to methanotrophs (Etana et al., 1999; Balesdent et al., 2000). Such agricultural practice also affects soil properties and hence, influences methane budget in the atmosphere (Ussiri et al., 2009). No-tillage farming has been promoted as an agricultural practice that creates a score-off situation by reducing soil erosion and enhancing agricultural sustainability with mitigating green house gas emissions such as CH_4 (Cole et al., 1997; Schlesinger, 1999). It may be suggested that plots under conventional tillage and no-tillage and found that no-tillage system acts as more beneficial for CH_4 sink than tillage system.

Land use type is one of the major causes of soil characteristics variations and consequently the CH_4 sink activity. It may be argued that a higher rate of CH_4 consumption in forest soils, compared to other ecosystems could be attributed to the greater viable population size of methanotrophs. However, the experimental evidences for such arguments are still to be investigated. During the last few decades, the atmospheric concentration of CH_4 has increased dramatically because of the imbalance between the overall sources and sinks (Singh, 2011). Anthropogenic nitrogen enrichment, grazing, deforestation, and alterations in water availability and temperature have received particular attention in terms of their effects on soil CH_4 oxidation strength (Smith et al., 2000; Dai et al., 2013). The recent reports indicate that the problems of excess atmospheric load of this potent GHG can be mitigated either by reducing CH_4 emissions or enhancing its consumption (Singh, 2011). It may be argued that ~10% uplifting in the soil CH_4 consumption could stabilize the current problem of atmospheric CH_4 build up (Singh, 2011). Consequently, it is imperative to adopt a more viable and eco-friendly approaches that could significantly contribute to augmentation of soil CH_4 sink potential.

In environments the high CH_4 content (fresh water wetlands, rice paddies, landfills, etc.) release to the atmosphere is controlled by methanotrophs via “low-capacity oxidation”. According to current concepts, the phylogenetically diverse uncultivable “high- affinity methanotrophs” are dominants in undisturbed soils and believed to be responsible for significant amount of atmospheric CH_4 destruction. Land use changes (conversion of natural forest and grassland ecosystems to agricultural lands) are the major consequences to the reduced soil CH_4 oxidation rate, and are likely to persist more extensive due to anthropogenic activities. Even when restored back to its original form, previously cultivated land may have continuous lower CH_4 sink activity than undisturbed lands. Variations in CH_4 uptake strength in soils under different land use practices could be due to the variation in methanotrophic community types. Based on the above arguments, it may be hypothesized that restoration of soil CH_4 sink strength in restored degraded land could be correlated to the variation in restored methanotrophic bacterial community composition. However, only a few studies addressed to methanotrophic activity and diversity in restored lands (Knief et al., 2005; Dorr et al., 2010; Levinet al., 2011; Zhou et al., 2013). The methanotrophs distribution and their CH_4 sink potential under prevailing influential environmental factors in resorted soils are still enigmatic. In addition, investigations on the influence of plant species on restored soil methanotrophs distribution are still very limited (Degelmann et al., 2010; Dorr et al., 2010), and the variation of methanotrophic communities in rehabilitated land with different plantation ways (natural and managed) remains unresolved (Dai et al., 2015). The previous investigations have typically assessed either CH_4 flux or the microbial methanotrophic community at similar ecosystems, but hardly ever has the role mediated by the efficient methanotrophic population size/community been studied concomitantly across a land-use gradient.

Due to the key role of methanotrophs in the biogeochemical carbon cycle and in global climate change, the influence of land use on methanotrophs diversity has attracted ample consideration (Robertson et al., 2000). A number of studies have been performed to evaluate the influence of different land use changes on the CH_4 uptake capacity with contrasting results (Prieme and Christensen, 1999; Suwanwaree and Robertson, 2005; Singh et al., 2008). For instance, Menyailo et al. (2008) confirmed that land use patterns suppressed the soil CH_4 uptake without affecting the diversity of methanotrophs. Similarly, tree species affected atmospheric CH_4 oxidation in grassland soil exclusive of altering methanotrophic community (Menyailo et al., 2010). The lower population size of methanotrophs in degraded lands oil could be due to the disturbances in soil conditions and micro-ecological niches of the bacteria. It may be argued that variations in CH_4 oxidation and methanotrophs diversity and abundance may largely result from, and soils analyzed across a wide variety of habitats, however, the exact explanations about these arguments have not been fully addressed.

Methanotrophs abundance across different land uses

Similarly, the conversion of natural forest to agricultural land also lowered the CH_4 consumption by soils; nevertheless, it could be restored through re-vegetation (Livesley et al., 2009; Zhang et al., 2014). A recent field study from natural ecosystem suggests that soil restoration, even if performed within a rather limited time, may have the positive effect on CH_4 consumption by terrestrial soils (Kizilova et al., 2013). It is suggested that the bio-fertilizer can be applied to minimize CH_4 emission from flooded paddy soils and also holds promise as the efficient device for controlling the potent greenhouse gas CH_4. Many studies claimed that long-term N fertilization in paddy soils alters the methanotrophic composition, resulting in inhibited CH_4 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). Several management practices, including the organic amendments and residue managements have been proposed to restore the microbial diversity and methanotrophs numbers in deteriorated agricultural lands (Singh et al., 2011; Singh and Pandey, 2013; Carlson et al., 2015). It has been proposed that direct introduction of beneficial bio-filmed bio-fertilizers may significantly on tribute to the revival of microbial diversity in degraded agro-ecosystems (Singh et al., 2011). Bio-fertilizer applications, particularly the nitrogen- fixing bio-agents, such as cyanobacteria, free-living diazotrophs, and *Azolla* may augment methanotrophs diversity and CH_4 oxidation while reducing the amount of N fertilizer applied (Singh and Strong, 2016; Singh et al., 2016). Cyanobacteria are the exceptional model systems that can offer the biotechnologist with novel genes and stress tolerance capability having diverse uses in environmental sustainability and also; hold promise as the effective tool for harnessing CH_4 (Prasanna et al., 2002). It may be argued that applications of above described microbial biofertilizers in place of their chemical counter parts may mitigate the onset of global CH_4 emission by conserving the viable methanotrophs diversity in disturbed agriculture soils (Pingak et al., 2014). The application of these bio-agents in paddy fields may be considered as the innovative tool for promoting the methanotrophs community composition. It is suggested that the application of bio-fertilizers as the nitrogen fertilizer replacement would be cost-effective, eco-friendly, and the safer means for degraded land restoration, and also to conserve the methanotrophic diversity and CH_4 consumption in the long term.

Land use changes and soil microbial biomass

It is now well accepted that soil microbial biomass (SMB) plays an important role in nutrient cycling in agro-ecosystems, and is limited by several factors, such as soil water availability (Rangel-Vasconcelos et al., 2015). The SMB is an important parameter of nutrient cycling in ecosystems, because it is the fraction of SOM that is most rapidly decomposed. The dynamics of SMB are determined by biotic and abiotic factors that vary in space and over time in both natural and man-modified ecosystems (Jenkinson and Ladd, 1981; Wardle and Hungria, 1994; Rangel-Vasconcelos et al., 2015). Variation in SMB over time is closely associated with changes in water availability in the soil (Patel et al., 2010), and its study contributes to the understanding of release and mineralization patterns (Wardle, 1998; Rangel-Vasconcelos et al., 2015) of nutrients that will consequently be available for plants (Singh et al., 1989; Luizão et al., 1992). The patterns in temporal variation of SMB in temperate climate are already well understood; they are closely associated with seasonal changes of temperature and hydrological regime (Wardle, 1998). In tropical soils, where temperatures do not vary greatly, water regime plays a major role in the dynamics of soil microbiota (Lodge et al., 1994). SMB, an important driver of ecosystem functioning, may be considered as the sensitive key biological indicator of perturbations owing to soil disturbances (Zornoza et al., 2009). SMB may be considered as important index for the soil health and environmental sustainability. Soil microbial biomass plays a crucial role in nutrient cycling (Amador, 2012; Yang et al., 2016). Soil microbial biomass was found to be a sensitive indicator of the dynamics of soil C and N cycles (Liu et al., 2012; Giese et al., 2013). The soil microbial biomass contributes 2-3% of the total organic carbon in soil and is a relatively labile fraction of soil organic matter (Yang et al., 2016). SMB pool can be revived by afforestation of deforested land can be achieved by mixed plantations in association with suitable bio-inoculants, enhancing the ecosystem productivity (**Figure 2.6**)

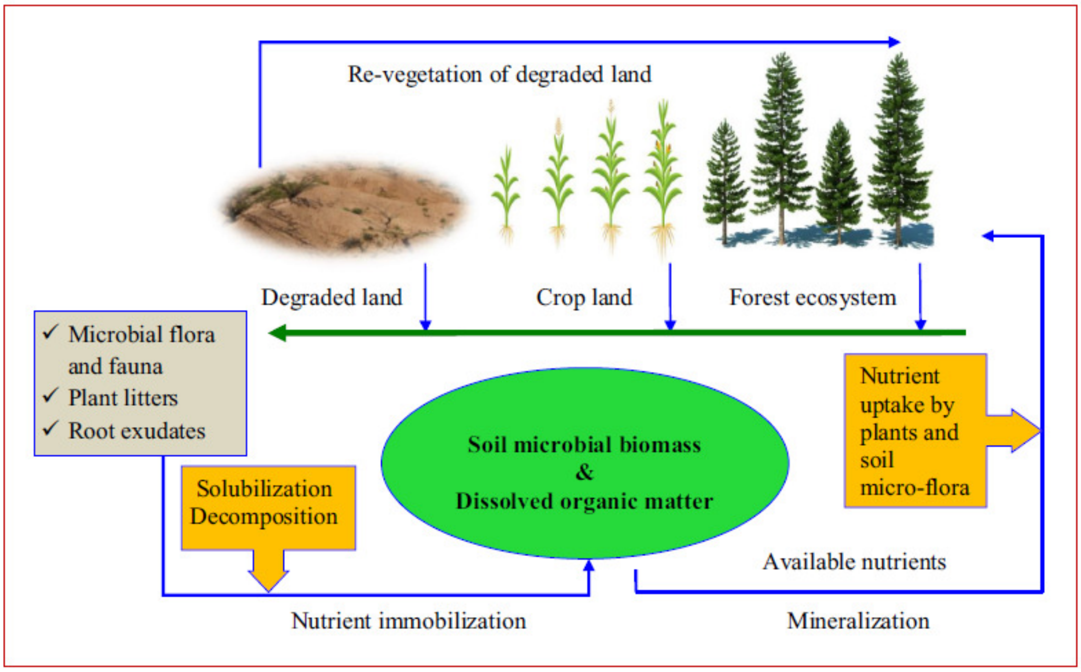


Figure 2.6. Pictorial presentation indicating contribution of SMB as source of plant available nutrients under different land use types (Taken from Singh and Gupta, 2018)

The microbial diversity and biomass have been described and reviewed at local and regional scales, a unifying driver, or set of environmental drivers affecting SMB pattern need to be investigated (Singh and Gupta, 2018). The SMB is considered widely as the index of soil fertility and ecosystem productivity. The escalating soil stresses due to land degradation and climatic variability are directly correlated with loss of microbial diversity and abundance or biomass dynamics (Singh and Gupta, 2018). The key ecological factors which stabilize the SMB and minimize its turnover, are supposed to play an important role in the soil nutrient dynamics and productivity of the ecosystems (Singh and Gupta, 2018). Soil microbial diversity and abundance/biomass play key roles in the ecosystem sustainability by maintaining essential functions of soil health, through carbon and nutrient turnover (Singh and Gupta, 2018). Even after disturbances, an ecosystem with a higher microbial diversity and biomass may have a higher capacity to sustain the ecological stability. Aboveground (plant litter quantity and quality) and belowground (soil microbial flora and fauna diversity) play significant roles in controlling ecosystem functioning (Wardle et al., 2004). The N deprived soils in Arctic and Alpine Tundra and Temperate forest ecosystems, nutrients release after soil micro-flora (SMB) death and decay has been pointed out as an important source of plant nutrient (van der Heijden et al., 2008). Moreover, many microbial communities have restricted bio-geographic distributions (e.g. N_2-fixing rhizobacteria in tropical forests and mycorrhizae in boreal temperate forests) it is suggested that disparity in the size of SMB can impact to variability of the functioning of various ecosystem types. As well as disturbances in climate conditions and anthropogenic interventions are the significant drivers to regulate the existence and survival of indigenous microbial diversity, and consequently, the essential soil functioning of the ecosystem (Singh and Gupta, 2018). The macroclimate, topography and soil characteristics are main influencing factors in controlling SMB dynamics across different ecosystems and locations (Wardle, 1992). The temporal dynamics of SMB may be the key factor in shaping the extent of release of immobilized labile nutrients due to microbial cell death, decay and decomposition, and the availability of released nutrients are key factor to plant growth and ecosystem functioning (Wardle, 1998). The factors that provide viability to the soil microbial community therefore, are assumed to enhance soil nutrient conservation in the form of higher SMB size (Singh and Gupta, 2018). Climate variability, land use types and the vegetation cover might be the key factors for SMB variability across different ecosystem types along the large geographical area (Singh and Gupta, 2018). Variations in the quantity and quality of substrate (organic C and N) inputs caused by varying plant residue types (litter and fine roots) and the associated nutrient specificity can be crucial drivers to influence the SMB across the ecosystem types. Accordingly, the higher SMB in soil of some ecosystems having vegetation cover with giant plant species might be due to the greater availability of organic substances. Wardle (1998) reported that persistent and fewer disturbed ecosystems had higher SMB values than the most disturbed one. This indicates that temporal SMB variability is exclusively governed by the dominant vegetation and land use type. Therefore, it might be suggested that the forest ecosystem having insignificant disturbances may have higher SMB pool as compared to other land use types such as savanna and arable land. In addition, Zhang et al. (2016) demonstrated that shifts in plant species composition during land use change can influence the microbial community composition dynamics and SMB basically by changing soil organic nutrient content. Though nutrient status, seasonality, temperature, soil and other factors are play an important role in controlling of dry tropical forest ecosystem, SMB pool may be one of the crucial factors affecting output in the tropical dry deciduous forests, as experienced in the Vindhyan plateau (Singh et al., 2010). For Indian point of view, the previous studies concerning SMB across the tropical dry deciduous forest ecosystems have been conducted in one or the other sites on a temporal scale, and without deciphering differences in SMB status and its role in distribution and variations of the dominant vegetation composition (Singh and Gupta, 2018). Variations in soil properties due to land uses and anthropogenic activities would correspond to differences in the SMB size and turnover rates and consequently, to loss of vegetation cover and ecosystem productivity.

In nutrient poor ecosystems, SMB acts as the major reservoir of available nutrients, and plays very crucial roles in the survival of plants (Singh et al., 2016a, Vimal et al., 2017; Singh et al., 2018). In the dry tropical deciduous forest ecosystem, nutrient withdrawal from the deteriorating leaves and its immobilization in the SMB pool has been considered as the nutrient assimilating adjustments in response to nutrient scarcity, and thus SMB constitutes the key available nutrient source for the survival of plant communities (Singh et al., 2010; Singh, 2015, Singh and Gupta, 2018). The conversion of forest into cropland and savanna due to plant harvesting may result a significant alterations in the size of SMB (Singh and Gupta, 2018). Thakur et al. (2015), reported that plant diversity is a key controller in SMB pool, therefore, any change in the soil physico-chemical properties due to forest clearing may have significant impact on SMB stability and its turnover rates (Singh and Gupta, 2018). Since, the deforestation is a common practice for the generation of agricultural managed land use systems; however, afforestation has resulted establishment of SMB pool in soil due to the regain of soil nutrient status via litter residues decomposition (Singh et al., 2010). The enhance level of agricultural land expansion accelerates deforestation and consequently disturbances in SMB trends (Singh and Gupta, 2018).

The SMB pool can provide one of the most satisfactory estimates of the productivity of any restored degraded land (do Couto et al., 2016). A lower ratio of

SMB to the total soil organic nutrients seems to indicate good strength of degraded land rehabilitation (Singh and Gupta, 2018). Consequently, the ratio of SMB to the total organic nutrients may be considered as a reliable parameter for the success of degraded land rehabilitation. The re-established SMB status and its dynamics under prevailing environmental drivers may provide valuable information about the restoration progress and productivity potential of derived lands for agriculture purposes (Singh and Gupta, 2018). The soil microbial properties between degraded and restored lands have been compared (Bini et al., 2013; Singh, 2016). The study of SMB patterns and its relationship to climate and soil drivers can enhance our knowledge in rehabilitation of land uses like vast degraded lands (e.g. spoiled coalmine areas). It is proposed that restoration practices with mixed plant species having high biomass producing potential, can contribute to speedy recovery of lost microbial diversity and SMB pools (Singh, 2013; Araujo et al., 2013; Singh, 2014). The fast recovery of microbial communities and SMB size due to mixed plantations cover (Lange et al., 2015) compared to the monoculture plantations can help in the returning of the huge bulk of diverse types of plant derived resources (leaf litter, fine root biomass, root exudates or rhizodeposits) and favourable soil conditions.

The values for SMB-C and SMB-N agreed with results of earlier studies conducted in the same region (Rangel-Vasconcelos et al., 2005; Sotta et al., 2008; Lopes et al., 2011; Melo et al., 2012). Bittencourt et al. (2006) found a higher SMB-N content in Amazonian soils under secondary vegetation in the rainy season. Other studies in tropical forests indicated that seasonal variation in soil moisture is associated with seasonal variation in soil microbial C and N (Singh et al., 1989; Luizão et al., 1992; Srivastava, 1992). The high concentrations of SMBC and SMBN in the first soil sampling, during the dry season, may have been the result of nutrient release from dead SMB and/or the decomposition of leaf litter, in addition to rainfall in the days before sampling. Prolonged dry periods followed by rainfall cause osmotic stress in microbial cells and promote cell lysis, resulting in the release of a pulse of nutrients (Lodge et al., 1994; Wardle, 1998; Yang et al., 2008) that become available to the soil micro-biota and plants (Singh et al., 1989; Srivastava, 1992). Bini et al. (2013) demonstrated that soils under forests had more organic C compared to other ecosystem. Ma et al. (2015) reported that land use types had significant role in SMB level and lowest in paddy fields as compared to other uses.

Tropical and subtropical ecosystems are very sensitive to land use changes, because much higher temperature lead to decomposition rates of organic substrates that are much higher compared to temperate ecosystems (Mganga and Kuzyakov, 2014). Therefore, there is considerable concern that land-use change from natural ecosystems to agricultural land lead to a depletion of soil C and subsequent increases in CO_2 levels in the atmosphere (Murty et al., 2002). Conversion of natural ecosystems to intensive agricultural used ecosystems (among others: maize and coffee) is common in East Africa (Mganga and Kuzyakov, 2014). However, the Chagga home gardens in Tanzania well exemplify a traditional sustainable land use system with many ecosystem services (Soini, 2005).

Natural ecosystems are continually being converted to agricultural land. Therefore, there is an urgent need to improve the management of organic inputs and soil organic matter (SOM) dynamics in tropical land-use systems (Lal, 2004). Input of organic substances such as crop residues and litter, play a critical role in the tropical and sub-tropical ecosystems because they provide both a short-term supply of nutrients and substrate for synthesis of SOM in the long-term (Fernandes et al., 1997). Mganga and Kuzyakov (2014) reported that MBC in the natural ecosystems was between 3 and 6-fold higher than in intensively used agricultural soils. Ecosystems located at higher elevation favour microorganisms because of higher amounts of precipitation, which result in higher plant biomass. Higher amounts of MBC content in undisturbed tropical forest soils than savannah and grassland soils have been reported in India (Singh et al., 2010). Ecosystems with higher amounts of easily available OM compounds (e.g. rhizodeposits released by grass roots) tend to have higher MBC contents (Mganga and Kuzyakov, 2014) because organic substrates are the preferred energy source for microorganisms (Blagodatskaya et al., 2009). The dense root system characteristic of grassland and savannah ecosystems contributed to a higher MBC content (Singh and Gupta, 2018) compared to agro-ecosystems at located at the same elevation (Mganga and Kuzyakov, 2014). Low amount of MBC content in agricultural soils of Mt. Kilimanjaro ecosystems and higher MBC content in the upper layers seems to be significant role of land use changes in MBC status (Mganga and Kuzyakov, 2014). In addition, higher MBC content in the natural and semi-natural ecosystems compared to agricultural lands shows that intensive cultivation depletes soil of its nutrients leading to soil degradation because soil

(Mganga and Kuzyakov, 2014). Savannah soils incorporated the highest amount of glucose into microbial biomass strongly suggests that savannah ecosystems are more efficient in utilizing available organic substrates as compared to agriculture land (Mganga and Kuzyakov, 2014). Yang et al. (2016) showed that MB-C & -N significantly decreased in grazing grassland compared to the untouched grassland. Thus, it is clear that the ecosystem having insignificant disturbances may have higher level of SMB content as compared to the ecosystems naturally or anthropogenically intervened.

CHAPTER 3

Study Area, Experimental Sites and Soil Sampling

Chapter 3

STUDY AREA, EXPERIMENTAL SITES AND SOIL SAMPLING

Study Area

Land use changes (LUC), one of the environmental disturbances, greatly contributing to soil degradation, loss of ecosystem productivity and microbial community compositions (Leeuwen et al., 2017). Land-use conversion has been considered as an intensive anthropogenic activity contributes to the loss of soil organic matter and plant available nutrients (Kumar and Ghoshal, 2017). Nowadays, to satisfy the human developments and food requirements, better land use management practices may be crucial for the improvement of soil and environmental quality management.

The viability and future of present study area (northern India) is associated with the eco-friendly and efficient soil natural resource management and environment restoration. In this investigation, we focused on the four kinds of land use/covers, including (i) natural forest, (ii) mixed forest, (iii) savanna, and (iv) agriculture land.

Study Sites

The selected land use types/covers for this study are located in Mirzapur and Sonbhadra districts (Vindhyan region) of Uttar Pradesh, India (**Figure 3.1**). The coordinates of the selected sites ranges between 24° 17' 52" – 25° 10' N lat. and 82° 45' – 83º 6' 36" E long. The Vindhyan region, considered as one of the densely forested sites in the region witnessing the industrial growth since last several decades. The climatic condition in the region are dry tropical monsoonal, and vary in terms of topography and dominant vegetation cover with the weather divisible into winter (November-February), summer (April- mid June) and rainy (late June-October) per year (Singh and Kashyap, 2007; Singh et al., 2010). The mean monthly temperature ranges between 18.5 to 43.7 ºC. The annual precipitation of the selected ecosystems ranges between 684.5 (Mirzapur) to 706.6 mm (Sonebhadra) receiving nearly 87% of the total annual rainfall (mm) due to the south-west monsoon (Singh et al., 2010). It has been observed that nearly 9 months of the year are dry and remaining 3 months are humid (Singh et al., 2010). The rainfall is irregular, random and mostly falls to three months of rainy season. Moreover, the probability of raining in winter season is quite vague; however the weather from March to Mid-June is entirely dry with sporadic rains. This leads to moisture stress for maximum duration of the year.

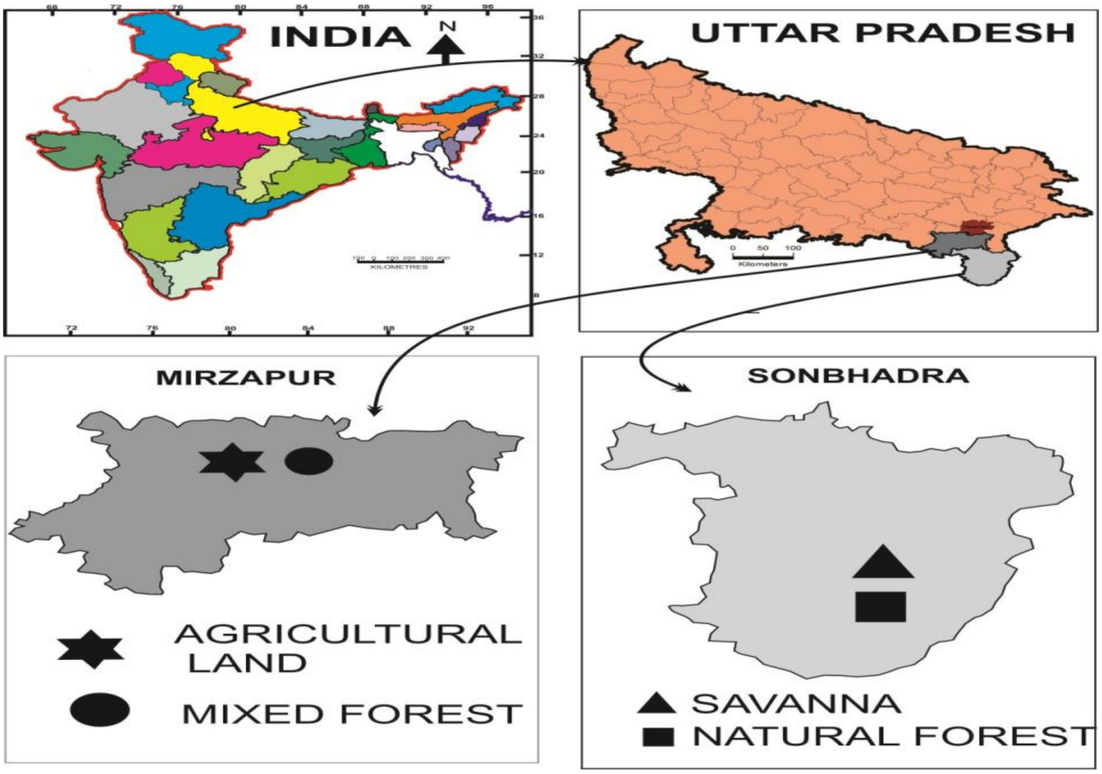


Figure 3.1. Location of different land use types across Mirzapur and Sonbhadra districts of Uttar Pradesh, India.

The flora and fauna of dry tropical region of Vindhyan plateau has been extremely distorted and/or destroyed by human interventions for agriculture and building purposes (Singh et al., 2010). Moreover, the climatic conditions have also been severely disturbed for a long time. Four types of vegetation cover have been reported in dry tropical region of Vindhyan region: i) forest, ii) mixed deciduous forest, iii) savanna, and iv) grassland (Singh et al., 2009; Singh et al., 2010).

Dominant vegetation

The dominant vegetation covers at natural forest ecosystem mainly *Shorea robusta*, *Madhuca longifolia*, *Haldina cordifolia*, *Terminalia tomentosa*, *Anogeissus* *latifolia*, *Boswellia serrata*, *Acacia catechu*, *Hardwickia binnata*, *Diospyros melanoxylon*, *Senegalia catechu*, *Buchanania lanzan*, etc., and mixed forest covers the *Boswellia serrata*, *Terminalia tomentosa*, *Ziziphus oenoplia*, *Acaciabnilotica*, *Emblica fficinalis*, *Dendrocalamus*, etc. However, the savanna site had the vegetation cover *Shorea robusta*, *Butea monosperma*, *Panicum* sp., *Andropogon* sp., *Heteropogon contortus*, etc. The agriculture land (Rajiv Gandhi South Campus of Banaras Hindu University, Barkachha, Mirzapur) was covered earlier by pastureland, thorny semi-arid bushes and scattered plantation. The localities in the area are uneducated and belong to lower income status, as a result in late 80s the vegetation was removed in agriculture land to drive livelihood. Therefore, the transformation of vegetation cover and savannah for agriculture purposes best exemplifies the land use change practice in the Vindhyan region. The crop rotation, tillage practices, use of pesticides and fertilizers at agriculture site may also affect the native soil microbial biomass.

Meteorological data

The average monthly rainfall and temperature of the study area during the study period (January–December 2015) are given in **Figure 3.2.** The study area is characterized by vide variations in land use covers due physiographic and intense anthropogenic disturbances (**Table 3.1**). 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. For present study, 4 different land uses selected are demonstrated in

Table 3.1 Dominant vegetation covers of the different land use types in dry tropical region at Vindhyan plateau. Source: Singh *et al*. (2010).

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	*Boswellia serrata, Acacia nilotica,* *Areca catechu*, *Phyllantus emblica*, *Dendrocalamus,* etc.	419	25° 0’ 6” N	82° 37’ 18” E	100
Sonbhadra district	Savanna (199 ha)	*Butea monosperma*, *Panicum* sp., A*ndropogon* sp., etc.	310	24° 25’ 55” N	83° 5’ 30” E	299
	Natural forest (2555 ha)	*Hardwickia binnata*, *Shorea robusta, Acacia catechu*, *Terminalia tomentosa*, etc.	2112	24° 25’ 57” N	83° 6’ 27” E	355

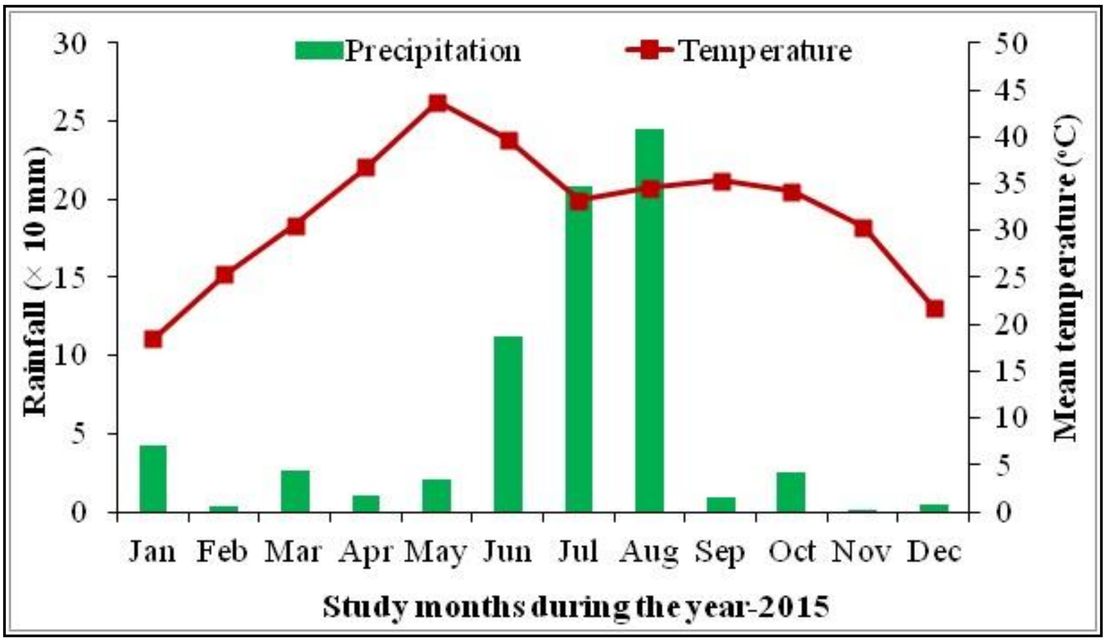


Figure 3.2. Mean monthly temperature and precipitation of the study area of Mirzapur and Sonebhadra districts of Uttar Pradesh during January– December, 2015. The data were obtained through the courtesy of Department of Meteorology, Ministry of Earth Sciences, Government of India.

Soil Sampling

Fore present study while selecting the different land use/covers, care was taken to ensure the homogeneity in elevation, slope type and characteristic. The experimental design was completely randomized block design, involving 4 land uses/covers (**Figure 3.3**).

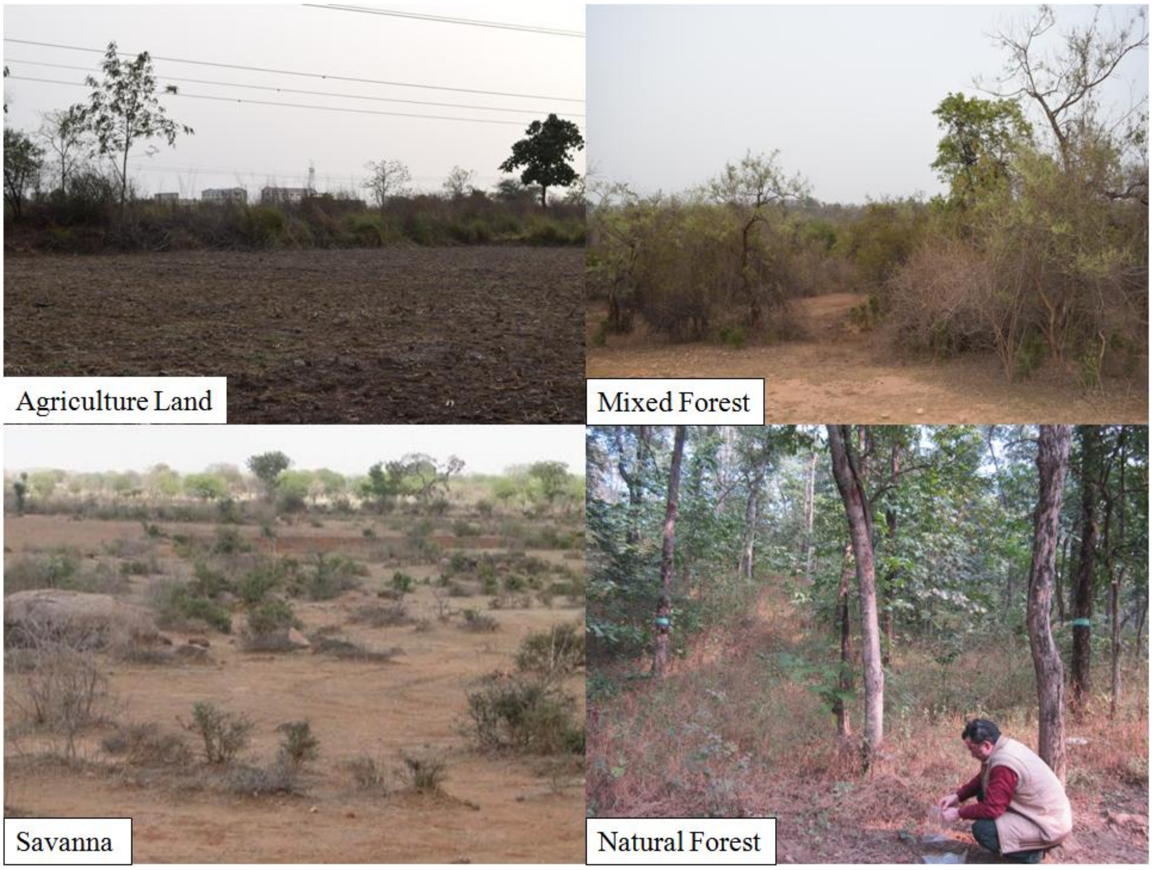


Figure 3.3. The 4 land uses/covers selected for collection of soil samples

For each land use/cover types, about 50×100 m area was demarcated for soil collections in the year 2016. Form each selected land use type, three soil samples (2.5 × 45 cm soil cores) were dug (July 02, 2017) at three different soil depths (0-10, 10-20 and 20-30 cm) of land use/cover, resulting in 9 soil samples (3 replicates × 3 soil depths) for each land use type and a grand total of 36 soil samples for all four selected land use types (**Figure 3.4**). Soils were air-dried and passed through 2-mm sieve (aggregates were broken to pass through a 2 mm sieve). Part of the soil samples were transferred to insulated containers and transported to the laboratory as soon as possible and was stored at 4 °C till further analysis.

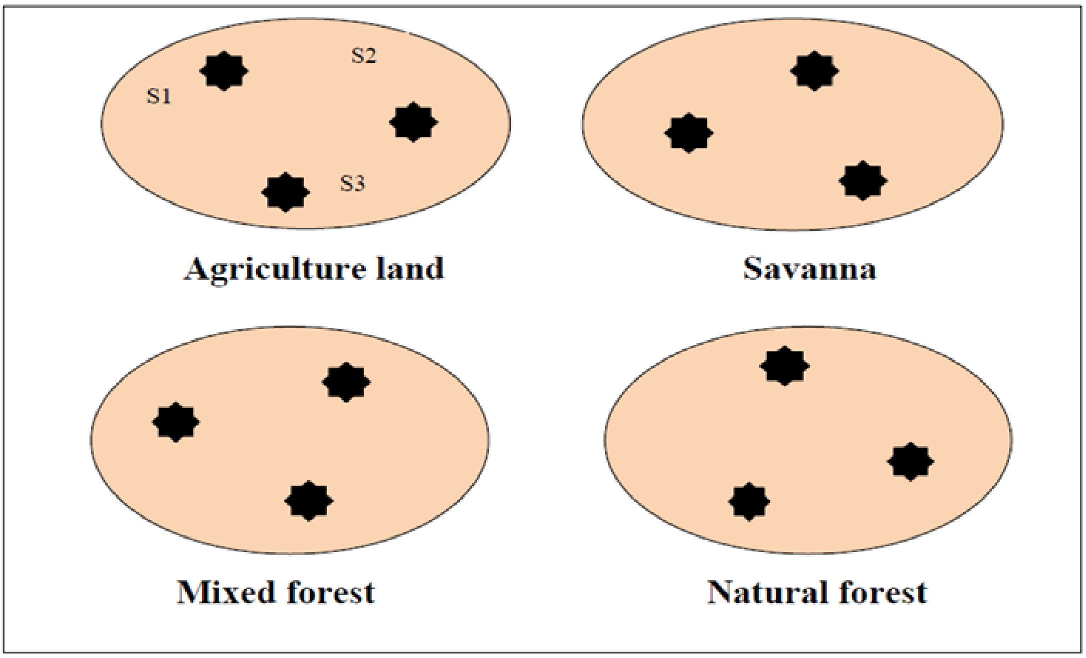


Figure 3.4. The 4 land uses/covers used for collection of soil samples.

Figure 3.5 Soil sampling at different selected land use types. The soil samples were collected with the help of soil cores (2.5 cm diameter and 45 cm length) on May 26, 2015.

Land use types = 4
Soil depths = 3 (0-10, 10-20 and 20-30 cm)
For each land use type N=09 (3 soil depths × 3 replicates)
Total number of soil samples were N=36 (4 land use types × 3 soil depths × 3 replicates)

The collected soil samples (**Figure 3.5**) further were analysed to explore the physico-chemical characteristics, methanotrophs abundance and community composition and soil microbial biomass across different soil depths (0-10,10-20 and 20-30 cm) and land use types (agriculture land, mixed forest, savanna and natural forest).

Figure 3.6. Collected soil samples (in air tight polyethylene bags) from different selected land uses were brought to laboratory and stored at 4^{o}C till further analysis

CHAPTER 4

To Study the Impact of Land Use Changes On Physico-Chemical Properties of Soil

Chapter 4

Objective 1

To study the impact of land use changes on physico-chemical properties of soil

Introduction

The soil physico-chemical characteristics of any ecosystems may be directly correlated with the disturbances in soil conditions due to land use/cover changes. Few studies in this region, owing to land uses, have been carried out to show the disturbances in soil organic matter inputs/removal in a variety of forms; can significantly impact the soil physico-chemical properties (Singh and Gupta, 2018). The earlier study related to soil physico-chemical properties from dry tropical regions (India) were conducted by selecting only one agro- or forest ecosystem or the other selected sites on local level with very small area and it was not able to decipher differences in vegetation cover or topographical variations. Therefore, assessments of soil properties relevant to soil fertility/organic matter, such as organic-C, total-N and –P, inorganic-N nutrients, soil moisture contents, C/N ratio, etc. could better reflect changes in soil quality and productivity, which alter labile available soil nutrient and microbial community dynamics. The previous works limited to show that changes in land covers would influence abiotic and biotic factors which subsequently govern the soil physico-chemical status (Kumar and Ghoshal, 2017). 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 soil fertility/organic matter, such as organic-C, total-N and –P, inorganic-N nutrients, soil moisture contents, C/N ratio, etc. due to land use changes remains poorly understood. Therefore, a better understanding to the effects of current land use practices may provide further insight into restoration of soil fertility and environmental management. As microbial adaptation and re-establishment in the form of SMB may play a major role in ecosystem responses to land use disturbances (Singh and Gupta, 2018), the long-term consequences of earlier reports on soil fertility/organic matter, such as organic-C, total-N and –P, inorganic-N nutrients, soil moisture contents, C/N ratio, etc. levels are critical for predicting alterations in ecological and environmental functioning. The understanding of soil properties levels can be used as a suitable indicator for quantifying and monitoring changes in beneficial microbial community structure as well as soil nutrient dynamics in response to anthropogenic mediated land use disturbances. Very little is known about the effects of changes in land use/cover on soil fertility/organic matter, such as organic-C, total-N and –P, inorganic-N nutrients, soil moisture contents, C/N ratio, etc. 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 soil properties 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 fertility/organic matter, such as organic-C, total-N and –P, inorganic-N nutrients, soil moisture contents, C/N ratio, etc. Therefore, the consequences of changes in physico-chemical properties during land use changes should be studied to offer relevant information on soil microbial activity levels to changes in the soil conditions and interactions between soil microbial communities and soil physico-chemical properties. The viability and future of present study area (northern India) is associated with the eco-friendly and efficient soil natural resource management and environment restoration. In this investigation, we focused on the four kinds of land use/covers, including (i) natural forest, (ii) mixed forest, (iii) savanna, and (iv) agriculture land. This study tried to assess the impact of various types of land use/covers on soil physico-chemical (bulk density, soil moisture content, pH, organic-C, total-N and available nutrients) properties. The present study hypothesized: i) different land use type/cover may affect soil physico-chemical properties in top surface soil (0–10 cm), but would have less effects in sub-soil profiles (20–30 cm); and ii) soil physico-chemical properties in top surface soil are will be highest in natural forest, followed by mixed forest and then savanna and agriculture land sites.

Materials and methods

Soil sampling and analyses

The details of soil sampling are already described in **Chapter 3**. The collected soil samples stored at 4 °C were used for the analyses of soil physico-chemical properties. The soil texture was analyzed using the sieves of different mesh size (Indian Standards, 1965). Soil temperature was measured using Digital Soil Thermometer (Luster Leaf 1625). The air-dried soil was analyzed for electrical conductivity (EC; 1: 5 soil to water ratio) and pH (1: 2.5 soil to water ratio). Soil bulk density (BD) and water holding capacity (WHC) were analyzed according to Piper (1944). Soil moisture content (%) and soil organic-C was determined as per protocol respectively, by Buresh (1991) and Walkley (1947). Soil inorganic-N (NH_4-N and NO_3-N) and -P were determined according to Eno (1960). The soil total-N and total-P was analyzed as described by Jackson (1958).

Comparative IR spectra (FTIR analysis) of soil samples of different land uses

The soil samples were characterized by infrared spectroscopy (IR), using FTIR (Nicolet^{TM} 6700, Thermo Scientific, USA). Samples were kept in Desiccators and dried in an oven at 105 ^{o}C for 48 h until the measurements were made. The samples were grounded with KBr in the ratio of 100:1 (100 mg KBr and 1 mg soil sample) in an agate motor to make the pellets by using a hydraulic press (CAP-15T). The spectra were obtained at 4,000–400 cm^{-1} mid-IR range (Narain et al. 2012; Tripathi et al. 2014). Spectra were collected with a total of 32 scans (resolution 4 cm^{-1}) against a KBr background and manipulated using the OMNIC software supplied by the manufacturer of the spectrometer.

Statistics

The values given are means of three replicates ±SE. The one-way analysis of variance (ANOVA) was used to compare the soil physico-chemical properties across selected land use changes/covers and soil depths. Duncan's MRT test ($p < 0.05$) was also applied to assess the differences among land use types and soil depths. All data in the experiment were processed and calculated using the SPSS version 20 (IBM, Armonk, NY, USA).

Results

The soil colour was almost brownish-black of all the selected regions, except the savanna site having brownish colour. The pH of soil was variable across the land uses (**Chapter 3 Table 1**). In present study the land use changes had significant effects on soil physico-chemical properties particularly in top soil (0-10 cm depth) (**Table 4.1**). One-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) (**Table 4.1**). The soil temperature of savanna land (46.0 ^{o}C) was highest followed by agriculture land (44 ^{o}C), mixed forest (43 ^{o}C) and natural forest (42 ^{o}C). The surface soil layer of agriculture (6.5), mixed (7.3), savanna (8.9), and natural forest (7.6) land was slightly acidic to alkaline in nature. 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 ≈ 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 C/N ratio was in decreasing order from natural forest (25.25) to mixed forest (23.58), savanna (17.25) and agriculture land (11.55).

Table 4.1 Description of soil characteristics across different land use types at different soil depths of Vindhyan plateau. Values are means (N=12) of three replicates with standard error (SE). Based on one-way ANOVA, values are statistically significant due to the impact of land use types. (N=not significant; *$P < 0.05$; **$P < 0.001$).

Soil parameters	Soil depth (SD) (cm)	Land use covers (LUC)				Two-way ANOVA
		Agriculture land	Mixed forest	Savanna	Natural forest	
Temperature (ºC)	0-10	44.2 ±0.4Aa	43.5 ± 0.15Ab	46.4 ± 0.20Ac	42.3± 0.11Ad	LUC: df=3, F=287.6***
	10-20	37.5 ±0.6Ba	38.4 ± 0.76Ba	38.5 ± 0.76Ba	35.7 ± 0.47Bb	SD: df=2, F=31360.6***
	20-30	26.8 ±0.3Ca	25.6 ± 0.51Cb	25.8 ± 0.75Cb	25.4 ± 0.70Cb	LUC×SD: df=6, F=88.5***
pH	0-10	5.9 ±0.01Aa	7.3 ± 0.05Ab	8.9 ± 0.03Ac	7.6 ± 0.04Ad	LUC: df=3, F=510.9***
	10-20	5.7 ±0.04Ba	7.3 ± 0.02Ab	8.8 ± 0.02Ac	7.6 ± 0.02Ad	SD: df=2, F=0.3^{NS}
	20-30	5.7 ±0.02Ca	7.3 ± 0.03Ab	8.9 ± 0.02Ac	7.6 ± 0.01Ad	LUC×SD: df=6, F=0.3^{NS}
EC (µs/cm)	0-10	93.3 ±2.5Aa	84.5 ± 0.49Ab	190.8±0.49Ac	119.9±0.23Ad	LUC: df=3, F=110085.8***
	10-20	84.8 ±2.3Ba	77.3± 2.4Bb	154.1±1.3Bc	102.8±1.4Bd	SD: df=2, F=19732.8***
	20-30	70.5±1.6Ca	65.5± 1.2Cb	146.0±1.8Cc	89.8±1.4Cd	LUC×SD: df=6, F=1930.0***
BD (g cm^{-3})	0-10	1.5± 0.01Aa	1.4± 0.02Ab	1.5± 0.01Aa	1.3± 0.01Ac	LUC: df=3, F=2.96*
	10-20	1.6 ± 0.02Aa	1.5± 0.01Bb	1.5± 0.03Ab	1.4 ±0.02Bb	SD: df=2, F=3.0*
	20-30	1.7 ±0.01Ba	1.5 ± 0.01Bb	1.6± 0.04 Ba	1.4±0.01Bd	LUC×SD: df=6, F=0.1^{NS}
WHC (%)	0-10	15.2±0.8Aa	17.9± 0.2Ab	17.1± 0.3Ab	20.6±0.4Ac	LUC: df=3, F=1102.2***
	10-20	13.7±0.1Ba	16.4± 0.4Bb	14.9± 0.2Bc	18.4±0.5Bd	SD: df=2, F=1271.1***
	20-30	11.3±0.4Ca	14.9± 0.7Cb	13.3± 0.2Bc	15.9±0.7Cd	LUC×SD: df=6, F=13.1***

Soil parameters	**Soil depth (SD) (cm)**	**Land use covers (LUC)**				**Two-way ANOVA**
		Agriculture land	**Mixed forest**	**Savanna**	**Natural forest**	
Organic-C ($\mu g\ g^{-1}$ dry soil)	0-10	5266.6±152.7Aa	15800.0±264Ab	14533.3±702.3Ac	17900.0±602Ad	LUC: df=3, F=3417.4***
	10-20	4200.0±251.6Ba	10200.0±755Bb	11200.0±709.4Bc	9700.0±776Bd	SD: df=2, F=6051.1***
	20-30	3600.0±208.1Ca	6900.0±318Cb	5900.0±513.1Cc	6500.0±351Cd	LUC×SD: df=6, F=524.0***
Total-N ($\mu g\ g^{-1}$ dry soil)	0-10	453.3±40.4Aa	773.3±20.8Ab	633.6±40.4Ac	933.3±40.4Ad	LUC: df=3, F=21639.1***
	10-20	373.3±20.8Ba	583.3±25.1Bb	513.5±30.2Bc	720.0±30.0Bd	SD: df=2, F=27311.6***
	20-30	330.0±36.1Ca	446.6±23.1Cb	383.3±25.1Cc	520.0±26.4Cd	LUC×SD: df=6, F=1351.7***
Total-P ($\mu g\ g^{-1}$ dry soil)	0-10	456.6±11.5Aa	543.3±15.2Ab	346.6±40.4Ac	626.6±35.1Ad	LUC: df=3, F=30481.09***
	10-20	343.3±25.1Ba	396.6±15.2Bb	193.3±20.8Bc	383.3±11.5Bd	SD: df=2, F=92273.3***
	20-30	203.4±23.1Ca	266.6±15.5Cb	76.6±11.5Cc	256.6±25.1Cd	LUC×SD: df=6, F=1089.8***
C/N ratio	0-10	11.61	20.43	22.93	19.17	
	10-20	11.25	17.48	21.81	13.47	
	20-30	10.90	15.45	15.39	12.50	

Different capital letters designate significant differences at $p < 0.05$ at soil depths for each land use type; different lowercase letters indicate significant differences at $p < 0.05$ among different land use types at a same soil depth. NS = not significant; *P < 0.05; **P < 0.01; ***P < 0.001; df = degree of freedom; EC=electrical conductivity; BD= bulk density; WHC= water holding capacity; CEC= cation exchange capacity;

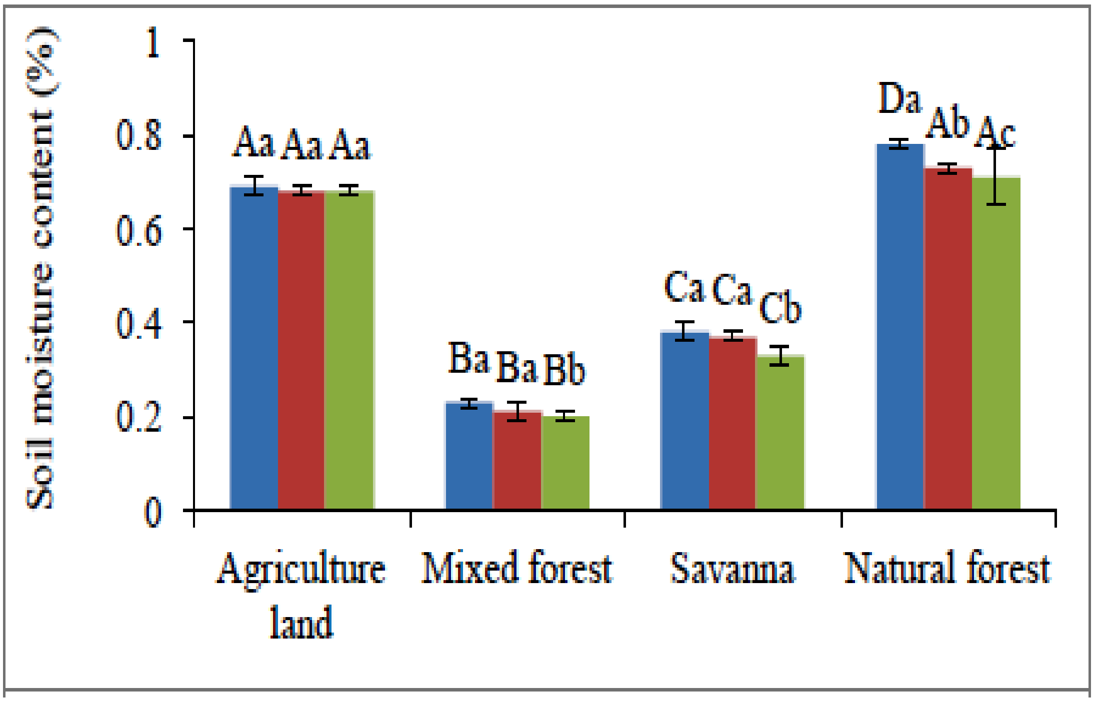


Figure 4.1. Variations in soil moisture content (%) across different land use types and soil depths. Values presented are means of three replicates ±SE. Different capital and small alphabets in uppercase values show statistical significance (Tukey at 0.05) among land use types and soil depths, respectively.

The soil of natural forest/mixed forest had significantly higher soil moisture content than all the other land uses. The soil moisture content (0.78%) and water holding capacity (29.73%) was highest for the natural forest soil followed by agriculture land, savanna and mixed forest (**Figure 4.1**). Across various land uses the soil ammonium-N (7.46 µg g^{-1} dry soil) (**Figure 4.2**) and nitrate-N (5.93 µg g^{-1} dry soil) (**Figure 4.3**) contents were greater at natural and mixed forest with top soil layer (0-10 cm depth) (**Table 4.3**).

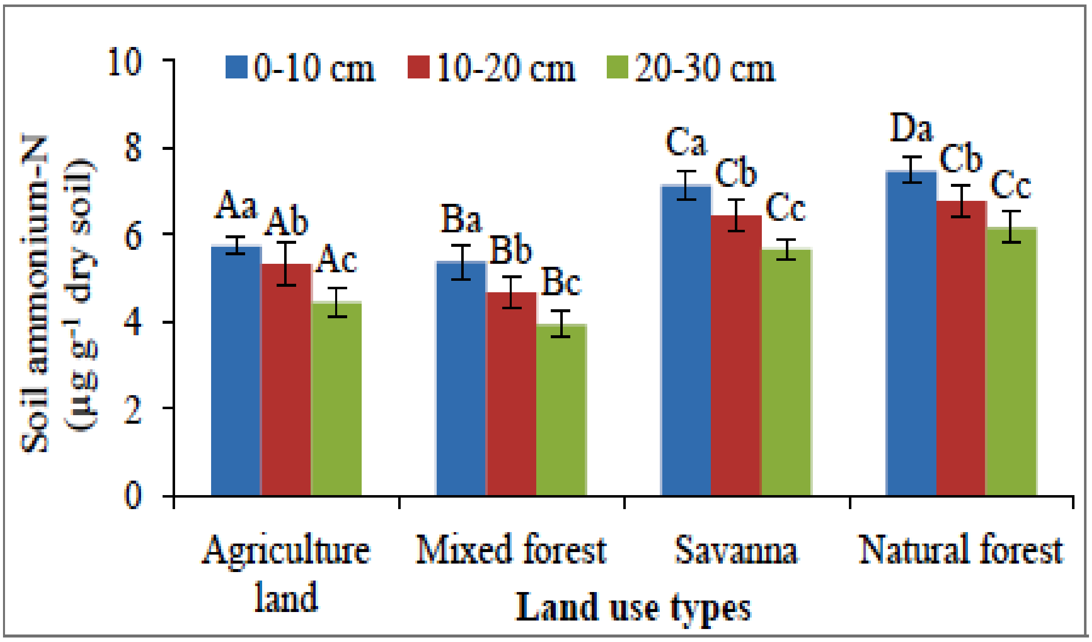


Figure 4.2. Variations in soil ammonium-N content across different land use types and soil depths. Values presented are means of three replicates ±SE. Different capital and small alphabets in uppercase values show statistical significance (Tukey at 0.05) among land use types and soil depths, respectively.

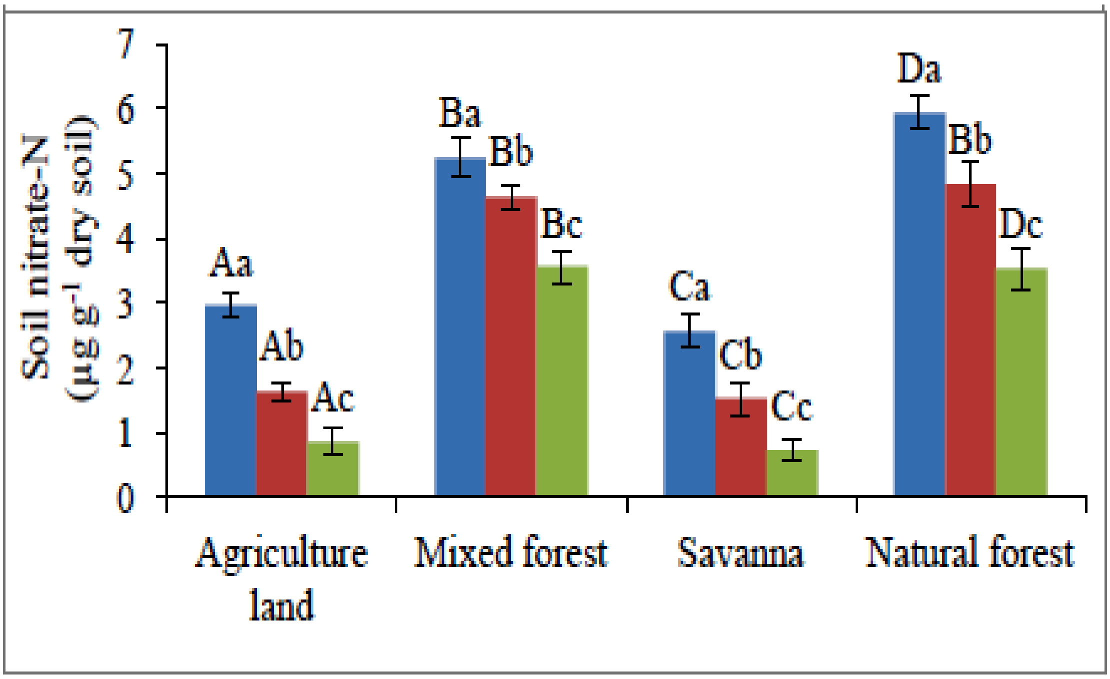


Figure 4.3. Variations in soil nitrate-N across different land use types and soil depths. Values presented are means of three replicates ±SE. Different capital and small alphabets in uppercase values show statistical significance (Tukey at 0.05) among land use types and soil depths, respectively.

The FTIR was used to analyze the characteristics and the functional chemical groups present in the soil samples collected from different land uses (**Figure 4.4**). Since, the soils have been sampled from different land uses distributed in Mirzapur and Sonbhadra districts of Uttar Pradesh of Vindhyan region. It is assumed that basic soil chemical constituents and characteristics could be more or less same at all the selected sites, because during course of soil weathering and paedogenesis the soil chemical constituents are derived from the similar parent rocks. However, during passage of time due to anthropogenic activities and natural calamities the soils sampled from different land uses may differ in terms of different groups at altered peaks in the FTIR graph. Compared to other land uses, the savanna soil sample provides substantial evidence to have higher level of silicate/lime which might be the reason for higher pH, unproductive and hard nature of the soil. These results are in conformity to the study of Ning et al. (2016) who also reported that higher level of soil silicate are responsible for elevated level of soil pH. Compared to other sites, the 1427.7 peak at savanna site (**Figure 4.4**) may be indicative of higher amount of calcium due to lime deposition. The soils of other land uses showed no variability in peaks that may create any discrepancy.

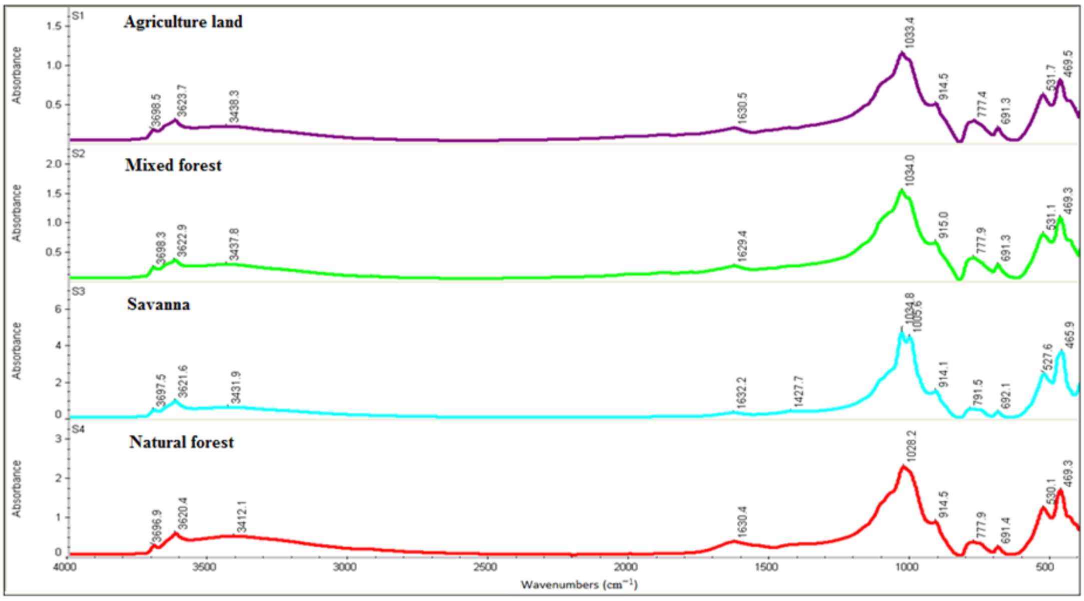


Figure 4.4. FTIR analysis of soil sample collected from different land use types

Discussion

The different land use sites selected for the present investigation may be divided into forest ecosystems (natural and mixed); savanna and agriculture land (**Table 4.1**). A wide variation in vegetation composition occurs within each of the selected land use types due to anthropogenic activities, physiographic differences, mining activities, deforestation, industrialization, etc. The selected land uses have a wide range of environmental characteristics (e.g. soil pH, temperature, moisture, etc.) and provide baseline data for most diverse soil disturbances of Vindhyan uplands. Since, the selected sites varied in terms of dominant vegetation cover, physiography and anthropogenic activity so a wide variation in soil properties might be expected across different land uses. The greater tree density at natural and mixed forest site compared to agriculture land and savanna site 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. (**Table 4.1**) might be expected. The nutrient rich soil especially the organic-C, acts as a major controlling key soil driver in terrestrial ecosystem which manage the vital interactions between the beneficial microbes, soil and plants (Maharana and Patel, 2013).

The conversion of natural forest into agriculture land disturbs most of the soil functioning due to disturbances between microbial-soil-plant interactions (Raiesi, 2017; Vimal et al., 2017) and ultimately decomposition process. The mean surface temperature of soil on the sampling sites (agro-ecosystem and mixed forest) located in Mirzapur district was 46 ºC, whereas the mean surface temperature of soil on the sampling sites (savanna and natural forest) located in Sonbhadra district was 47 ºC (**Table 4.1**). The temperature continuously declined when from surface to inner depth. The moisture content remains present in different soil layers which keeps it wet and cold as compared to the surface layer soil. So, intensive forest clearing due anthropogenic involvement and cattle grazing at agro-ecosystem and savanna sites may reduce the input of organic-C via plant residues and therefore, a disturb soil physico-chemical resulted. Luneberg et al. (2018) also reported that land use change with agricultural farming in dry lands, as in many other ecosystems, alters the quantity and quality of organic matter, destroying the soil carbon stocks.

The soil pH of the study sites seems to be quite different across land uses. The agriculture soil is moderately acidic (5.9) and forest soil perform neutral behaviour, however savanna being slightly alkaline (8.9) in nature (**Table 4.1**). The soil pH at all the sites slightly decreased when we move from surface to deeper regions. Singh et al. (2009) also showed that pH of forest soils was lower than savanna site may be because of the enhanced nitrification rates that may add higher H^+ ions from NH_4^+-N oxidation. In addition, Tripathi and Singh (2007) reported that conversion of forest ecosystem into savanna may slightly increase the soil pH level due to enhancement in salt concentration. The electrical conductivity (EC) of savanna was highest, followed by natural, agriculture and mixed forest soil. Similar to the pH, the electrical conductivity also decreased towards greater depth. A greater amount of accumulation of salts in the surface soil of savanna compared to natural forest soil may results a higher EC.

The result shows that BD of agro-ecosystem and savanna soils was higher than natural forest ecosystems (**Table 4.1**). The soil BD increased with the soil depth at all the sites. It may be due to changes in soil organic matter content, porosity and soil compaction (Chaudhari et al., 2013). Zhang et al. (1988) and Singh et al. (1989, 2009) have also reported that an increase in cultivation of land increases the soil BD. In contrast, water holding capacity was recorded higher in forest soil compared to cropland and savanna ecosystem. The higher total-C contents at natural forest site due to return of greater amount of litter resides may result in higher values of WHC.

High organic content in the mixed forest and savanna in other study is also reported by Bui and Handerson (2003) and Fazhu et al. (2015). As nitrogen is concerned, it was maximum (low C/N ratio) in agriculture land. Addition of inorganic fertilizers may increase the soil N content which ultimately leads to a decreased C/N ratio in agriculture land. Singh et al. (2009) reported that soil total-N and -P concentrations and C/N ratios were also higher at the two forest sites compared to savanna and cropland. Across the different study sites soil total-N and -P ranged from 143 to 1342 and 43 to 378 $\mu g\ g^{-1}$ dry soil, respectively. The C/N ratio of the natural and mixed forests ranged from 16.6 to 20.4, while that of agriculture land and savanna ranged from 15.0 to 15.3 (**Table 2**). The well constrained C/N ratio along soil profile experiences the principles that the formation of spoil organic matter necessitates a certain amount of N in a relatively fixed ratio with C (Sterner and Elser, 2002). Interestingly, although insignificant, soil C/N ratio in savanna and agriculture land tended to decrease with soil depth, possibly reflecting a greater degree of breakdown and older age of the humus stored in the deeper soil layer. As decomposition proceeds, easily decomposed material disappears and nitrogen is immobilized in microbial biomass and decay products, leaving behind more recalcitrant material with slower decomposition rates and lower C/N ratio (Post et al., 1985). The organic matter in subsoil layers is older and more humified than that in topsoil layers, and thus it is frequently observed a decreasing soil C/N ratio with soil depth (Callesen et al., 2007). The higher C/N ratio in natural forest soil than the agriculture land and savanna may be attributed to the addition of higher amount of organic matter due to greater density of plant species cover. But at disturbed sites the lower C/N ratio could be due to because the above ground biomass at agriculture land and savanna is usually periodically removed by harvest, fires and intense cattle grazing. Furthermore, a higher C/N ratio at natural forest site could be due to higher plant litter accumulation at upper soil layer can transform the organic matter into mineral soil due to the degradation of labile organic matter and the formation of humus.

The soil moisture content of the natural forest soil was highest (0.78%) compared to the soil of other sites (**Figure 4.1**). Singh et al. (2009) reported that the reason for reduction in moisture content level in the farming land as compared to natural forest ecosystem may be due to the least quantity of organic matter as well as aeration promoted drying. Further, the soil moisture content vary temporally and spatially due to different parameters such as soil texture, vegetation cover, litter density, root distribution and density (Griffiths et al., 2009; Xu et al., 2013; He et al., 2014; Ma et al., 2014).

Due to anthropogenic activities and natural calamities the soils sampled from different land uses may differ in terms of different groups at altered peaks in the FTIR graph (**Figure 4.4**). The savanna soil sample provides substantial evidence to have higher level of silicate/lime which might be the reason for higher pH, unproductive and hard nature of the soil. The 1427.7 peak at savanna site may be indicative of higher amount of calcium due to lime deposition and subsequently elevated level of pH (Ning et al., 2016).

Conclusions

The results show that soil physico-chemical properties are good indicators of the soil quality following changes in land use/cover. The land cover changes resulted in disturbances of soil properties in agriculture and savanna soil compared to soils under natural and mixed forest. It is evident that over time recycling of inorganic and organic nutrients via litter returns significantly increases the soil organic matter contents status of undisturbed natural forest. Present study also indicate that inorganic nutrients 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). 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 physico-chemical properties across different land uses and soil depths. Thus, land use changes can serve as potential key ecological indicators of soil quality changes resulting from land use management practices to top and sub-soils of dry tropical region of Vindhyan uplands.

CHAPTER 5

To Isolate and Identify the Methanotrophic Bacterial Abundance Under Different Land Use Changes

Chapter 5

Objective 2

TO ISOLATE AND IDENTIFY THE METHANOTROPHIC BACTERIAL ABUNDANCE UNDER DIFFERENT LAND USE CHANGES

Introduction

The land use changes (LUC), an anthropogenic intervention may be considered as one of the major key drivers that may affect soil characteristics, microbial diversity and functioning of terrestrial ecosystems (Tian et al., 2017; Luneberg et al., 2018). The LUC is also responsible for microbial biodiversity disturbances (Haines-Young and Potschin, 2009) and loss of soil microbial biomass of tropical forest soils (Lima et al., 2014; Qi et al., 2018). The conversion of forest cover, savanna vegetation and grasslands into cultivated land may lead to negative alterations of soil functioning (Raiesi, 2017), microbial community structure (Li et al., 2015) and the ecosystem functioning (Singh and Gupta, 2016). The soil methanotrophs diversity and their abundance may contribute significantly in the methane (CH_4) consumption (Singh and Gupta, 2016). Thus, the land uses may cause long lasting impacts on the plant species composition, soil nutrient status, soil microbial properties including soil methanotrophs abundance. However, the abundance of methanotrophs in altered soil characteristics due to land uses is almost unknown in the dry tropical soils of Vindhyan plateau. Therefore, it is important to find out in details about the impacts of land use changes on soil properties and methanotrophs abundance in dry tropical region of India.

Due to anthropogenic activities, natural forest ecosystems are continuously being converted into farming lands for agricultural purposes. It has been suggested that deforestation and other land farming practices can result into alteration in the soil organic carbon content, soil moisture, C/N ratio and N-nutrient status (Singh et al., 2007). The soil C/N ratio level may be an important physico-chemical parameter for understanding the functioning nature the soil of an ecosystem. High C/N ratio in the soil clearly reflects the high content of carbon compound and N-poor soil ecosystem.

The change in land use might affect the soil nutrients dynamics and microbial interaction in soils (Fazhu et al., 2015; Singh and Gupta, 2018). Since the soil C/N ratio is a valuable index for understanding the turnover of nutrients and microbial growth efficiency in an ecosystem (Agrawal and Ghoshal, 2016). Consequently, in this study impact of soil C/N ratio and its correlation with soil methanotrophs abundance will be also assessed. It is hypothesized that the methanotroph abundance and their performance under contrasting land uses can be positively correlated with the soil nutrient status and physico-chemical properties. For example, the influences of land management practices, such as tillage and fertilizer use in agriculture lands, afforestation or the type of dominant vegetation cover, on the methanotrophs performance of soil may be linked to altered soil characteristics. In addition, the soil methane uptake potential of different soils to inorganic fertilizers amendment have also been related to the methanotroph abundance, with some taxa having higher tolerance to soil ammonium-N and C/N ratio compared to others (Jang et al., 2011). However, the mechanisms underlying these relationships (methanotrophs abundance and soil N status) remain unknown. Given the soil disturbances due to land use patterns and variation in soil physico-chemical status, we hypothesize that soil conditions in terms of moisture, organic carbon, C/N ratio, etc. could limit methanotrophs abundance. Overall, the forest management related activities such as deforestation, tillage, grazing, afforestation, reforestation, etc. can influence the aerobic soil methanotrophs abundance in upland soils. Since, the population of soil methanotrophs and their activity in altered soil characteristics due to different land uses are relatively unknown therefore; there are strong reasons to investigate the land use changes on methanotrophs abundance in upland soils of dry tropical region.

Soil methanotrophs, a functional group of bacteria with proficiency of utilizing CH_4 as their sole carbon and energy source (He et al., 2012), represents the sole biological sink for potent greenhouse gas- CH_4. Aerobic methanotrophs are the chief dominant CH_4 consuming bacteria in upland soils, but they have been also isolated from a wide variety of other terrestrial ecosystems (Dedysh et al., 2001; Knief et al., 2005, Zheng et al., 2012). The conversion of natural ecosystem 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). The conversion of forest into farm lands may create deep impacts on CH_4 consumption and global CH_4 budget (Dorr et al., 2010). Thus, the land use practices can alter the strength of soil CH_4 sink activity of methanotrophs (Mosier et al., 1991; Dorr et al., 2010). Two main explanatory factors have been proposed to regulate the aerobic methanotrophs CH_4 consumption performance in soils under different land use: methanotrophic community composition and methanotrophs abundance (Malghani et al., 2016). To our knowledge, little investigations have previously examined the abundance of methanotrophs in dry tropical upland soils which acts as a strong sink of atmospheric CH_4 (Singh, 2011). The objective of this study was to determine the impact of converting natural dry tropical forest to savanna and agriculture land on soil methanotrophs bacterial abundance. Since, land use changes also alter the soil properties and microbial abundance by affecting the transport of soil nutrients in deeper soil horizon either through alteration of belowground input of organic matter or surface mixing by soil micro-flora. Study of soil properties and methanotrophic abundance in deeper soil horizons is required to draw the complete understanding of impact of land use types on various soil drivers and viable methanotrophic bacterial population through the entire soil profile. Since, the soils of the selected area are nutrient poor and dry tropical in nature therefore, we interested to study the influence of soil moisture status and soil nutrient quality (organic matter and C/N ratio) across different land use changes and soil depths in dry tropical region of Vindhyan region.

The oxidation of atmospheric CH_4 by methanotrophs is performed due to broad spectrum CH_4 monooxygenase (MMOs) enzyme (Singh et al., 2018). The particulate methane monooxygenase (pMMO), a species of MMOs is found in all aerobic methanotrophs except *Methylocella* and *Methyloferula* (Kizilova et al., 2014). The CH_4 consumption in aerobic soil is typically assessed by the detection of the *pmoA* gene, which encodes the β-subunit of pMMO enzyme (Semrau et al., 2010). However, no studies have been conducted to assess the impact of land uses on abundance of soil *pmoA* gene copies of methanotrophs in dry tropical regions of Vindhyan uplands, which acts as strong sink of atmospheric CH_4 (Singh, 2011). Therefore, in this study, we used *pmoA* genes to quantify the soil methanotrophs abundance across different land uses in uplands of dry tropical region. Additionally, we correlated the soil properties of each land use system with the changes in the structure of soil methanotrophic bacterial *pmoA* genes abundance. We hypothesize that alterations in soil physico-chemical properties due to land uses may influence the soil methanotrophs abundance and *pmoA* gene quantity. In addition, we also predict a higher bacterial numbers and *pmoA* gene copies in natural forest soil than in agricultural systems. We also expected differences in the number of the methanotrophs and *pmoA* genes at different soil depths of four land use types. Our study may provide a better understanding on how land use practices with various soil drivers (soil moisture, nutrient status, etc.) would govern the viable and active soil methanotrophs abundance and *pmoA* gene copies in agriculture and natural forest ecosystems of dry tropical region of Vindhyan plateau.

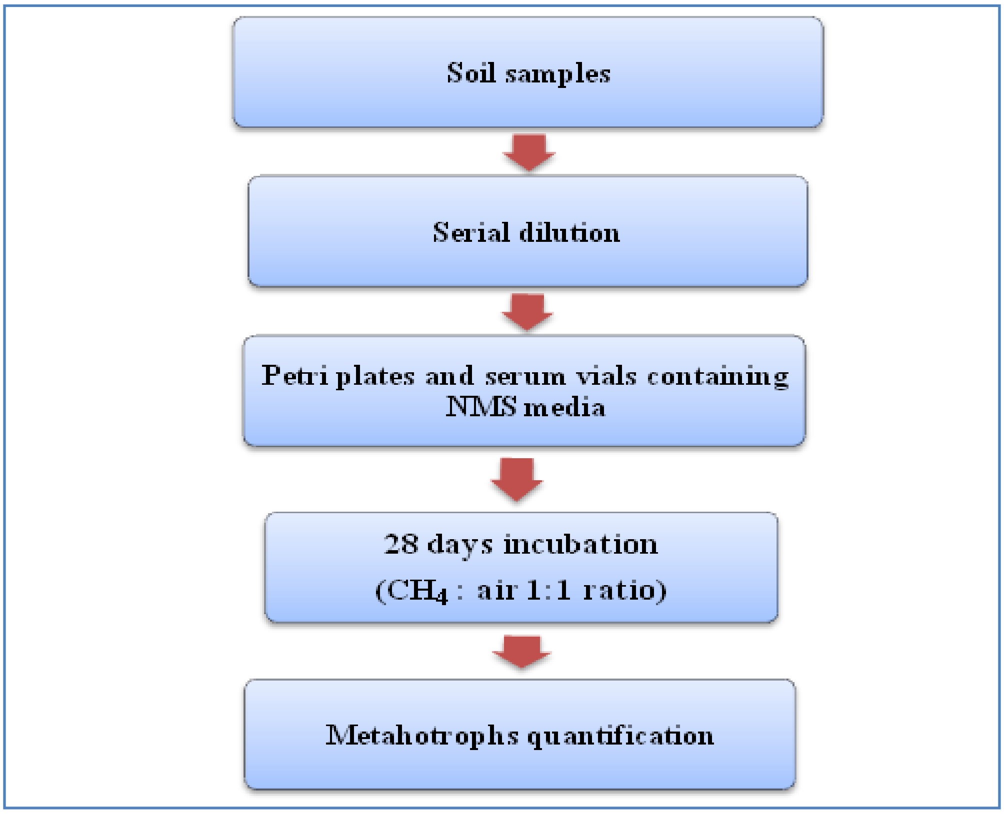
Materials and methods

Soil sampling and analyses

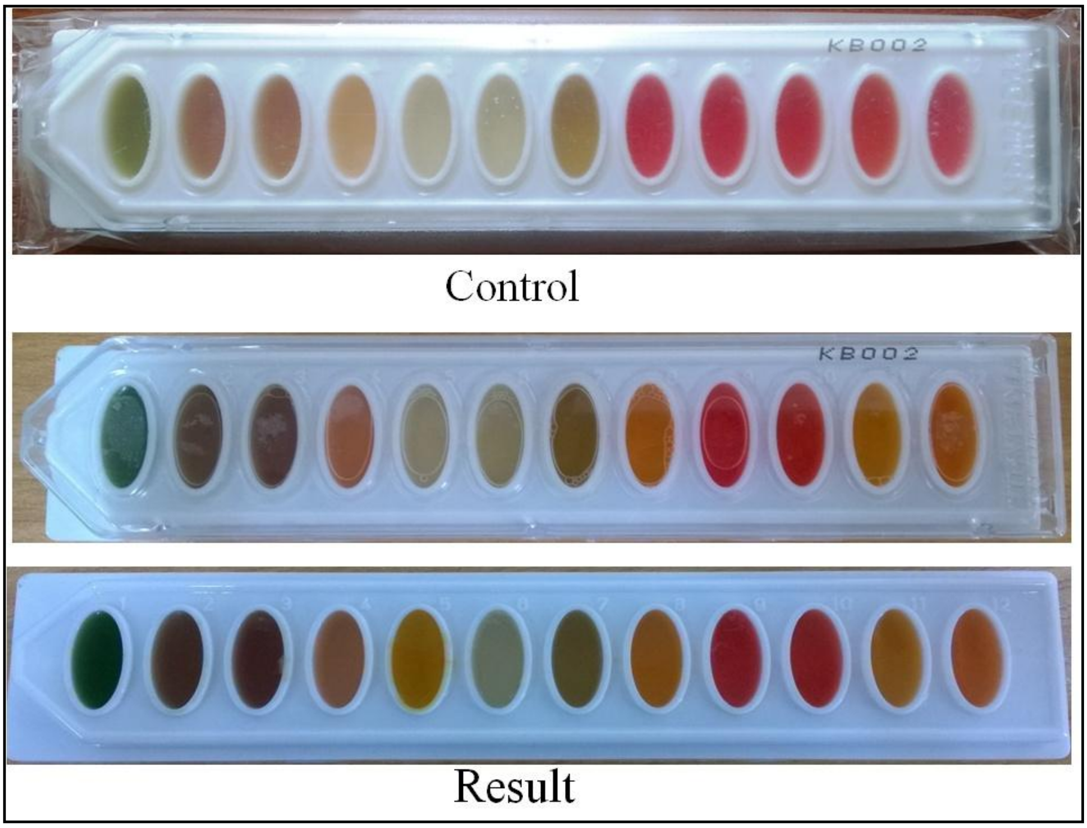
The details of soil sampling are already described in **Chapter 3**. The collected soil samples stored at 4 °C were used for the analyses of soil methanotrophs abundance and community composition.

Methanotrophs abundance quantification by MPN methods

The culturable methanotrophic community structure was identified using modified most probable number (MPN) (Saitoh et al., 2002) method. A modified nitrate mineral salt (NMS) medium (Whittenbury et al. 1970) was used to isolate the methanotrophs. The NMS media contains: $MgSO_4.7H_2O$, 1 gm; KNO_3, 1 gm; $Na_2HPO_4.12H_2O$, 0.71 gm; ferric ammonium EDTA, 5 gm, chelated iron solution 2.0 mL which contains ferric (III) ammonium citrate 0.01 gm or ferric chloride 0.05 gm, EDTA sodium salt 0.2 gm, conc. HCl 0.3 mL, distilled deionised water 100 mL and Trace element solution 1 mL. The composition of trace element solution is DiSodium EDTA, 50 mg; $FeSO_4.7H_2O$; 20 mg; H_3BO_3, 3 mg; $CoCl_2.6H_2O$, 2 mg; $CuSO_4.5H_2O$; 3 mg; $ZnSO_4.7H_2O$; $MnCl_2.4H_2O$, 3 mg; $Na_2MoO_4.2H_2O$, 3 mg; $NiCl_2.6H_2O$, 2 mg. The pH of the medium was maintained at 6.8. The media was autoclaved at 121 ^{o}C for 15 minutes for sterilization. The serially diluted soil samples of different land use sites were spread on Petri plates containing NMS media for the enumeration of methanotrophs abundance. The CH_4 gas was injected in 1:1 ratio with air in the Petri plates as carbon source for the growth of methanotrophs. After the growth on NMS medium, the methanotrophic bacterial isolates from different soil samples were considered for biochemical characterization according to Chandra and Singh (2014).



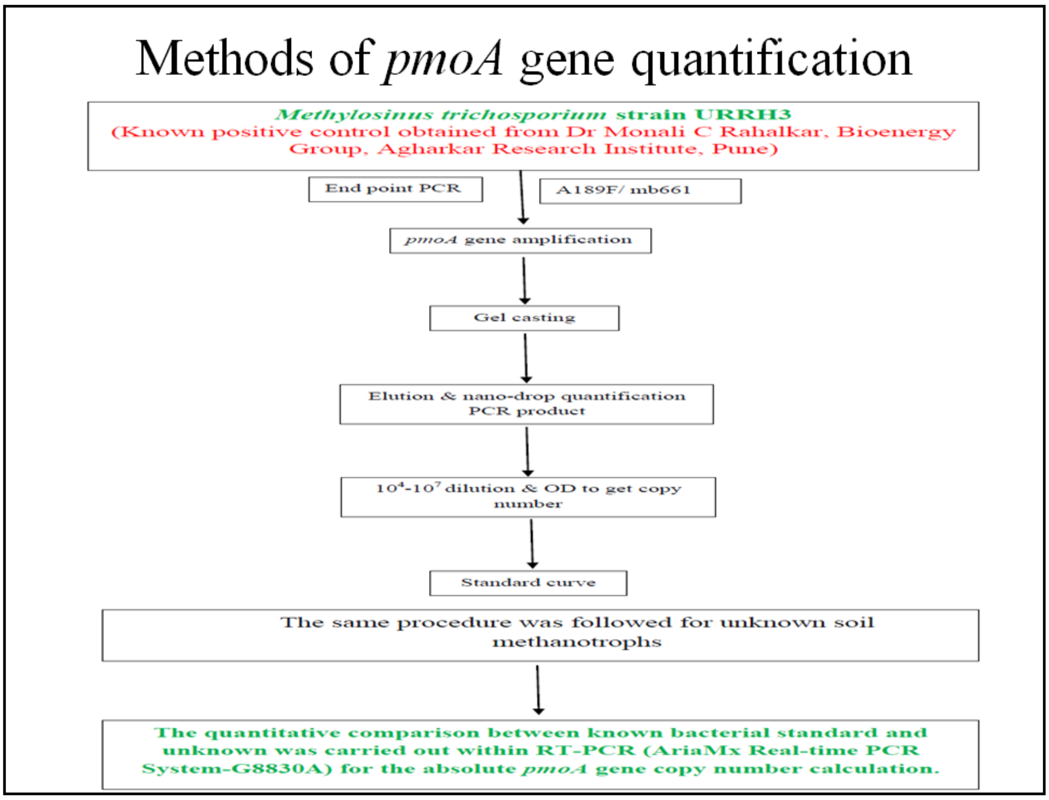
Quantification of methanotrophs by MPN methods



Biochemical test of soil methanotrophs

Soil pmoA gene quantification of methanotrophs

The *pmoA* gene was used to enumerate methanotrophic bacteria present in the soil samples across different land uses. We applied the method of Kizilova et al. (2014) for the quantification of *pmoA* gene copies of upland soils. The DNA was extracted from 0.25 g of soil in triplicate with Power Soil DNA Kit (MO BIO, United States) including a short-term heating to 80 ºC and bead beating step. Extracted DNA preparations were used for quantitative PCR (qPCR). The Real-Time PCR (RT-PCR) (AriaMx Real-time PCR System-G8830A) was performed with the primers A189F (5'GGTACTGGGACTTCTGG3') and mb661R (5'CCGGMGCAACGTCYTTACC 3') to amplify the β-subunit of the *pmoA* gene encoding pMMOs. The PCR reactions were performed with 29 cycles and annealing temperature was 56 ºC. A pure culture of *Methylosinus trichosporium* strain URRH3 was used as positive control. The PCR product of the pure culture was serially diluted from 10^4 to 10^7 copies μL^{-1} and amplified by quantitative PCR to construct standard curves. The distilled water was used for the negative control having no nucleic acid content. The reaction mixture contained ROX (carboxy-X-rhodamine) passive reference dye for the normalization and correction of well-to-well optical variations and reporter dye SYBR Green I to obtain fluorescence signal. The DNA concentrations of the PCR products were measured spectrophotometrically (UV-Visible Spectrophotometer, EVOLUTION 201, Thermo Scientific, USA). On completion of each PCR cycle, the melting curves for the amplicons were measured by increasing the temperature 0.5 ºC (from 50–56 to 95 ºC) while monitoring the fluorescence. The *pmoA* genes copies in soil samples were calculated using standard calibration curve and PCR products.



Methods of *pmoA* gene quantification

Methylosinus trichosporium strain URRH3
(Known positive control obtained from Dr Monali C Rahalkar, Bioenergy Group, Agharkar Research Institute, Pune)

End point PCR

A189F/ mb661

pmoA gene amplification

Gel casting

Elution & nano-drop quantification PCR product

10^4-10^7 dilution & OD to get copy number

Standard curve

The same procedure was followed for unknown soil methanotrophs

The quantitative comparison between known bacterial standard and unknown was carried out within RT-PCR (AriaMx Real-time PCR System-G8830A) for the absolute *pmoA* gene copy number calculation.

Molecular analysis of methanotrophic community across different land uses

DNA extraction from soil, PCR amplification and sequencing

The DNA was extracted, using the QIAGEN Kit (Zymo Research, Thermo Fisher) following the manufacturer's protocol from soil samples. The extracted DNA was subjected to DNA quality check by using Nano Drop quantification and GEL electrophoresis before PCR amplification. The DNA samples exhibiting Nano Drop readings of 260/280 at value of 1.8 to 2 were assumed as appropriate for further studies.

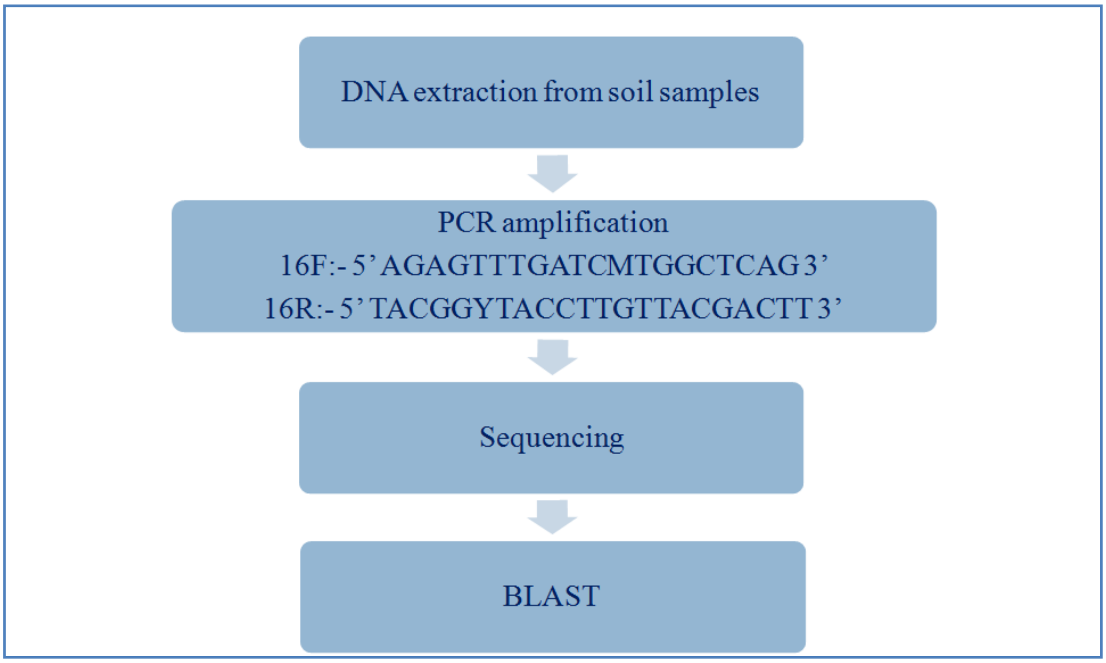
The PCR amplification of 16S Gene was done using TAQ Master Mix (High-Fidelity DNA Polymerase, 0.5mM dNTPs, 3.2mM $MgCl_2$, PCR Enzyme Buffer) and primers (16F:- 5' AGAGTTTGATCMTGGCTCAG 3' & 16R:- 5' TACGGYTACCTTGTTACGACTT 3'). Then 40 ng of DNA was used to amplify along with 10 pM of each primer (25 cycles of the following condition, initial denaturation @ 95 ºC for 15 sec, annealing @ 60 ºC for 15 sec, elongation @ 72 ºC for 2 minutes, final Extension at 72 ºC for 10 minutes and hold at 4 ºC. The obtained products were electrophoresed on Agarose gel (1% w/v) and then applied for sequencing.

Overview of sequencing protocol

- Nanopore sequencing was performed by using 1μg of DNA template.
- End repair/dA tailing Ligation of Barcode Adapter
- Barcoding PCR
- End repair/dA tailing
- Blunt end Adapter Ligation
- Purification using AM Pure XP bead binding
- Priming and loading the SpotON flow cell

Bioinformatics protocol

EPI2ME 16S analysis workflow used to perform genus-level identification from single reads; with access to base called files for detailed investigations at the species and sub-species level. The phylogeny analysis of query sequence with the closely related sequence of blast result was performed followed by multiple sequence alignment. The workflow is designed to BLAST base sequence against the NCBI 16S bacterial database, which contains 16S sequences from different organisms. Each read is classified based on % coverage and identity. The 16S workflow was useful in exploring the composition of methanotrophic community.



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 ±SE.

Results

Soil methanotrophs abundance and pmoA genes copies

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 (**Figure 5.1**).

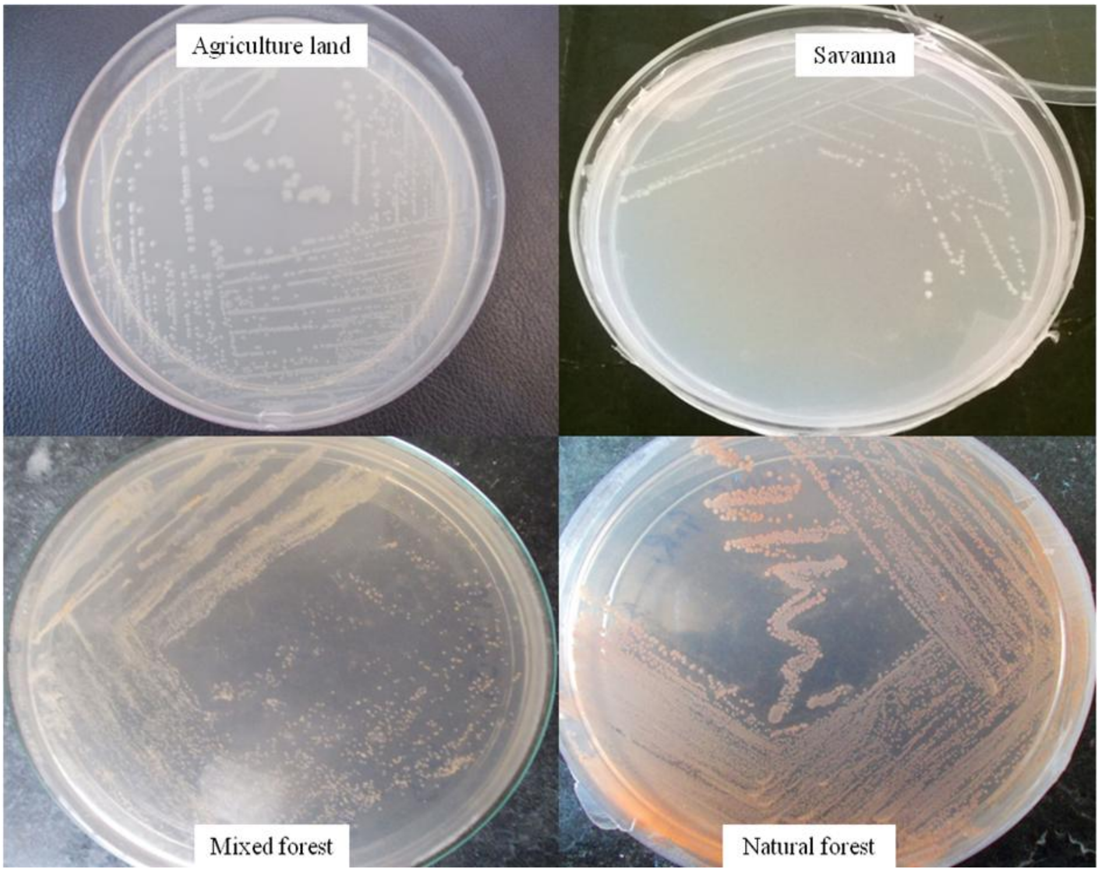


Figure 5.1. The colonies of aerobic soil methanotrophs across different land use types growing on NMS medium after 3 week of incubation at 30 ^{0}C.

During cultivation and incubation of methanotrophic bacterial growth (**Figure 5.2**) and abundance (**Figure 5.3**) 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 (**Figure 5.3**). ANOVA indicated significant difference in viable methanotrophs abundance due to land uses (P=<0.001), soil depths (P=<0.001) and land uses × soil depths interaction (P=<0.001).

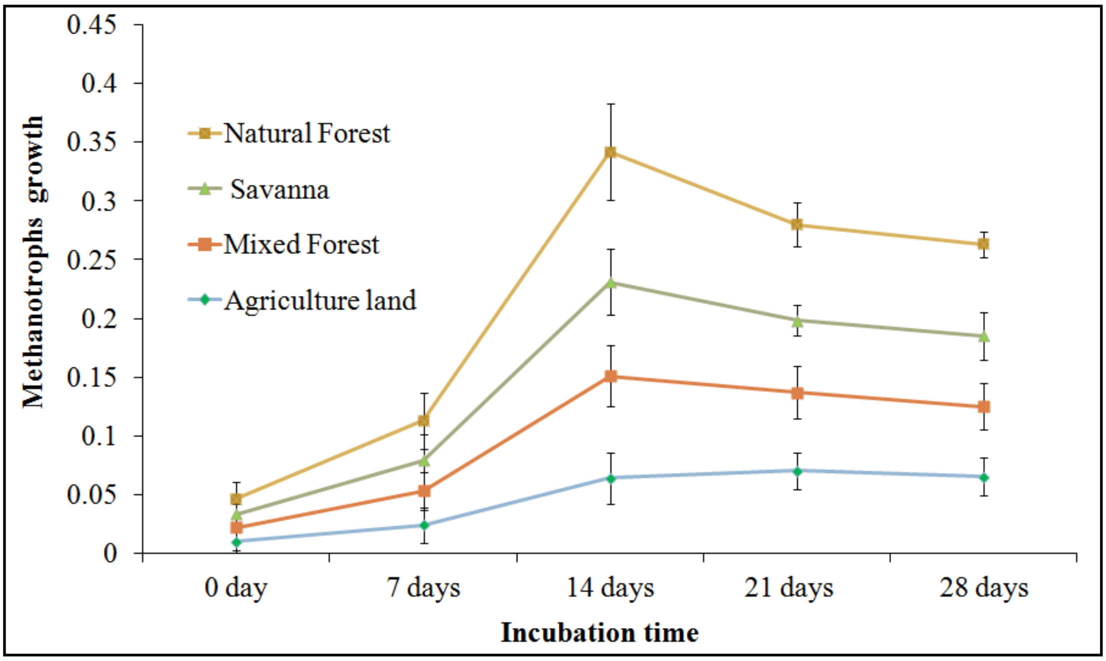


Figure 5.2 Variations in soil methanotrophs growth across different land use types during different incubation days. Values presented are means of three replicates ±SE. Different capital and small alphabets in uppercase values show statistical significance (Tukey at 0.05) among land use types and soil depths, respectively.

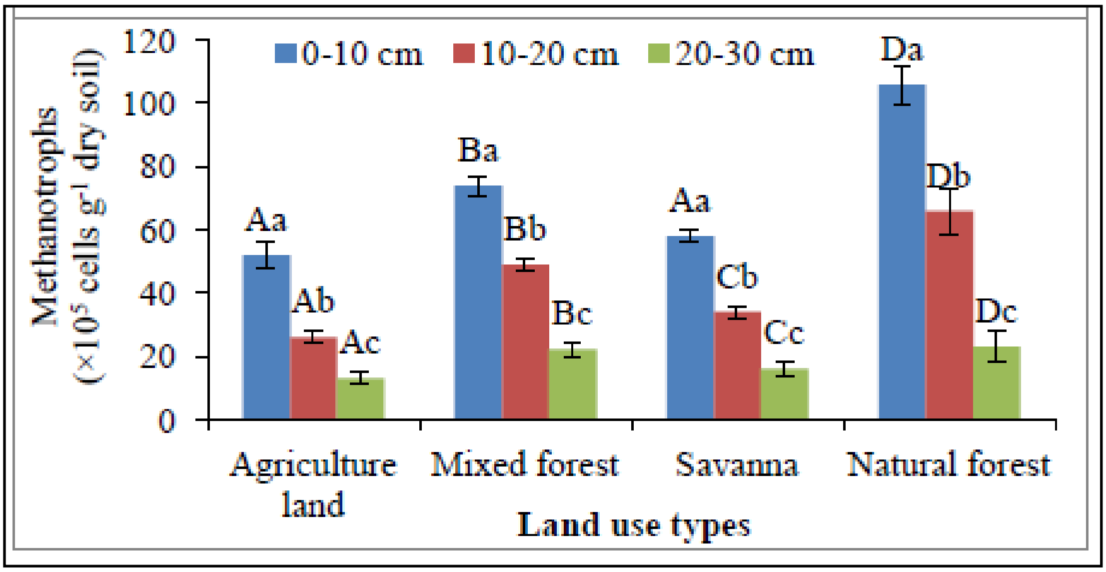


Figure 5.3 Variations in soil methanotrophs abundance across different land use types and soil depths. Values presented are means of three replicates ±SE. Different capital and small alphabets in uppercase values show statistical significance (Tukey at 0.05) among land use types and soil depths, respectively.

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 (**Figure 5.4**) 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 × depths interaction (F = 5.916; P = <0.001) (**Table 5.1**).

Table 5.1 Two-way ANOVA to assess the impact of various land use types and soil depths on *pmoA* genes copies (g^{-1} dry soil) in upland soils of Vindhyan plateau. Pooled data across different land uses and soil depths used for the analyses was N=36 (4 land use types × 3 soil depths × 3 replicates).

Variables	N	R^2	F	P
Land use types	4	27.749	83.246	< 0.001
Soil depths	3	46.024	92.048	< 0.001
Land use types × soil depths	12	0.994	5.916	< 0.001

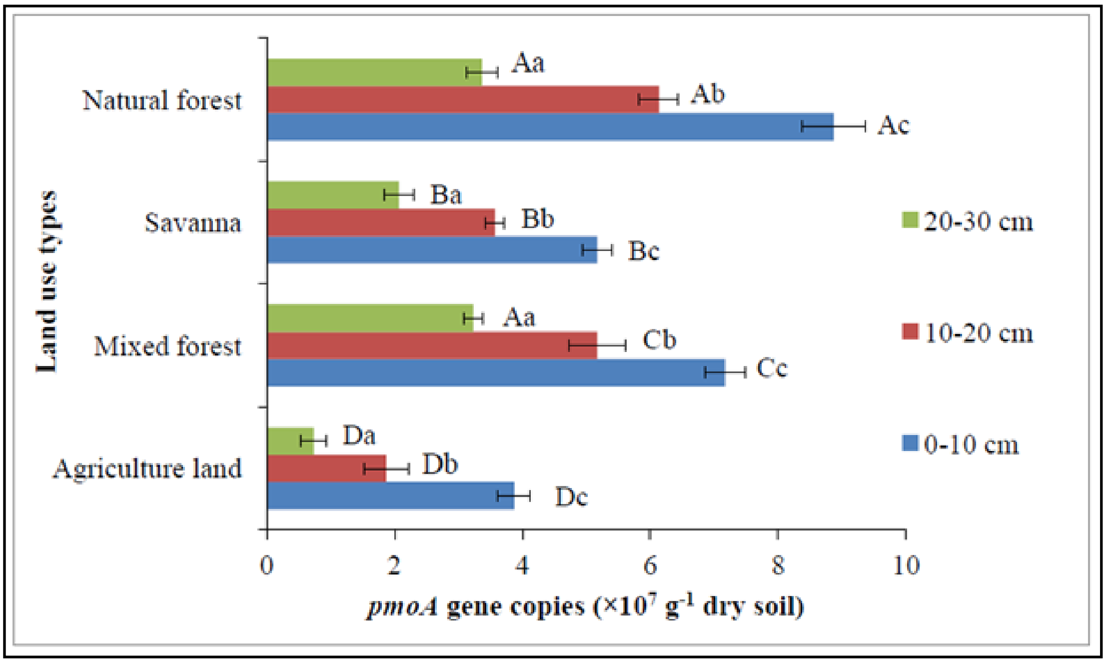


Figure 5.4 Variations in *pmoA* gene copies across different land use types and soil depths at Vindhyan upland soils. Values presented are means of three replicates ±SE. Different capital and small alphabets in uppercase values show statistical significance (Tukey at 0.05) among land use types and soil depths, respectively.

Methanotrophic community composition across different land uses

Across different land uses the molecular community composition analyses of methanotrophs revealed that agricultural land soil contained 83043 reads having 70774 classified and 12269 non-classified reads of bacterial population, whereas the natural forest had 89043 reads with 69714 classified and 19323 non-classified reads of bacterial population (**Appendix**). The metagenomics of soil samples showed that the undisturbed soil of natural forest soil sample contain higher number of methanotrophic community compared to agricultural land soil (**Table 5.3** and **5.4**).

Table 5.3 Methanotrophic community composition in agricultural land

Family	Genus	Species
Methylobacteriacece	*Methylobacterium*	*Methylobacterium nodulans* ORS 2060
		Methylobacterium aquaticum
		Methylobacterium phyllosphaerae
		Methylobacterium radiotolerans JCM 2831
		Methylobacterium extorquens
		Methylobacterium populi
Methylophilaceae	*Methylotenera*	*Methylotenera mobilis* JLW8
	Methylophilus	*Methylophilus* sp. TWE2
	Methylobacillus	*Methylobacillus flagellatus* KT
Methylocystaceae	*Methylocystis*	*Methylocystis bryophila*
		Methylocystis sp. SC2
	Methylosinus	*Methylosinus trichosporium* OB3b
Methylacidiphilaceae	*Methylacidiphilum*	*Methylacidiphilum infernorum* V4
		Methylacidiphilum fumariolicum SolV
Methylophilaceae	*Candidatus Methylopumilus*	*Candidatus Methylopumilus turicensis*
		Candidatus Methylopumilus planktonicus
	Methylovorus	*Methylovorus*
Methylococcaceae	*Methylococcus*	*Methylococcus capsulatus* str. Bath
		Methylomicrobium alcaliphilum 20Z
Methylococcaceae	*Methylomonas*	*Methylomonas denitrificans*
		Methylomonas methanica MC09
Sterolibacteriaceae	*Methyloversatilis*	*Methyloversatilis* sp. RAC08
Beijerinckiaceae	*Methylocella*	*Methylocella silvestris* BL2
Piscirickettsiaceae	*Methylophaga*	*Methylophaga frappieri*
		Methylophaga nitratireducenticrescens

Table 5.4 Methanotrophic community composition in natural forest

Family	Genus	Species
Methylobacteriacece	*Methylobacterium*	*Methylobacterium* sp. C1 *Methylobacterium* sp. 4-46 *Methylobacterium nodulans* ORS 2060 *Methylobacterium aquaticum* *Methylobacterium phyllosphaerae* *Methylobacterium* sp. PR1016A *Methylobacterium radiotolerans* JCM 2831 *Methylobacterium extorquens* *Methylobacterium populi*
Methylophilaceae	*Methylotenera*	*Methylotenera versatilis* 301 *Methylotenera mobilis* JLW8
	Methylophilus	*Methylophilus* sp. TWE2
	Methylobacillus	*Methylobacillus flagellatus* KT
Methylocystaceae	*Methylocystis*	*Methylocystis bryophila* *Methylocystis* sp. SC2
	Methylosinus	*Methylosinus trichosporium* OB3b
Methylacidiphilaceae	*Methylacidiphilum*	*Methylacidiphilum infernorum* V4 *Methylacidiphilum fumariolicum* SolV
Methylophilaceae	*Candidatus Methylopumilus*	*Candidatus Methylopumilus turicensis* *Candidatus Methylopumilus planktonicus*
	Methylovorus	*Methylovorus*
Methylococcaceae	*Methylococcus*	*Methylococcus capsulatus* str. Bath
	Methylomicrobium	*Methylomicrobium alcaliphilum* 20Z
Methylococcaceae	*Methylomonas*	*Methylomonas denitrificans* *Methylomonas methanica* MC09
Sterolibacteriaceae	*Methyloversatilis*	*Methyloversatilis* sp. RAC08
Rhizobiales (order)	*Methyloceanibacter*	*Methyloceanibacter caenitepidi*
Beijerinckiaceae	*Methylocella*	*Methylocella silvestris* BL2
Burkholderiales (order)	*Methylibium*	*Methylibium petroleiphilum* PM1
Piscirickettsiaceae	*Methylophaga*	*Methylophaga frappieri* *Methylophaga nitratireducenticrescens*

Discussion

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. The pink colour colonies and morphology (cocci shape: **Table 5.2**) of methanotrophs, isolated from mixed and natural forest soil suggest that these sites contains similar type of methanotrophic communities.

Table 5.2 Morphological and biochemical test of CH_4 consuming bacteria of soil samples collected from different land use types.

Biochemical test	Land use types			
	Agriculture land	Mixed forest	Savanna	Natural forest
Gram's staining	-	-	-	-
Colour	Cream	Pink	Cream	Pink
Shapes	Cocci	Cocci	Cocci & Rod	Cocci
Motility test	Motile	Motile	Motile	Motile
Catalase test	+	+	+	+
Oxidase test	+	+	+	+
Amylase production	-	-	-	-
Urease test	-	-	-	-
Lipase production	-	-	-	-
H_2S	-	-	-	-
Citrate utilization	-	-	-	-
Lysine	-	-	-	-
Ornithine	-	-	-	-
Phenyalanin deamination	-	-	-	-
Nitrate reduction	-	-	-	-
Glucose	-	-	-	-
Adonitol	-	-	-	-
Lactose	-	-	-	-
Arabinose	-	-	-	-
Sorbitol	-	-	-	-

While ago-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 (**Figure 5.5)** 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.

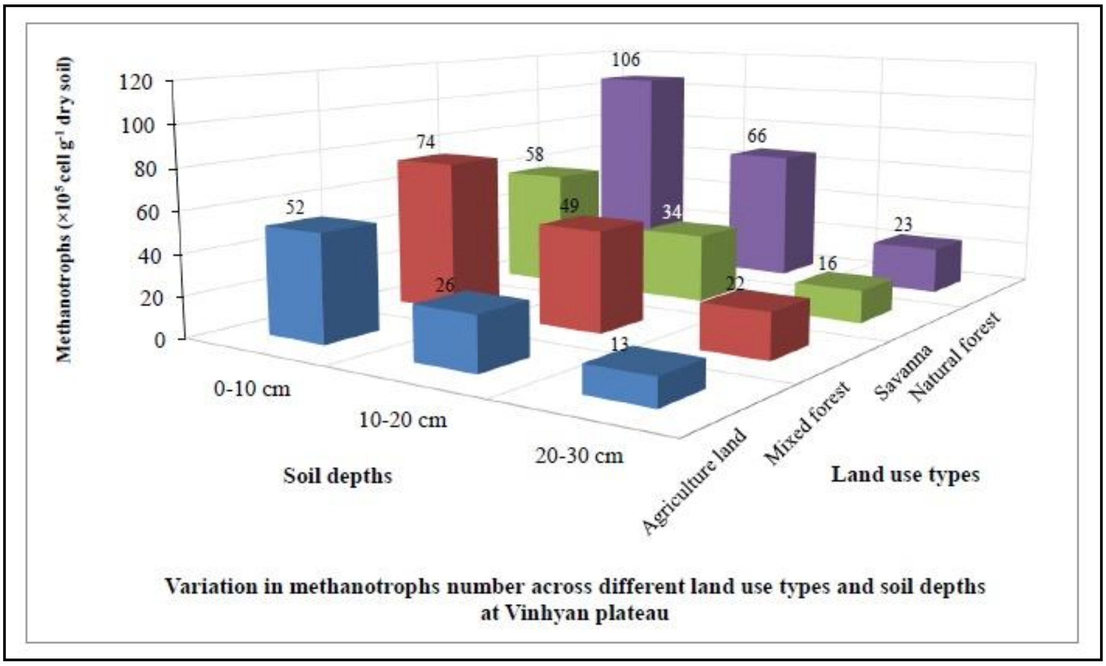


Figure 5.5 Variations in soil methanotrophs abundance across different land use types and soil depths.

The conversion of natural forest and grassland ecosystems into crop lands are the important indicator of variations in the abundance and/or type of methanotrophic communities in soils (Singh and Gupta, 2016). Zheng et al. (2010) showed that methanotrophic community structure was strongly affected by land use changes. Land use change intensely influences the soil physico-chemical characteristics, particularly pH and P that may be primarily responsible for the differences in methanotrophs population density (Menyailo et al., 2008). Levine et al. (2011) demonstrated that alteration of natural forest and native lands to agriculture land led to a seven fold reduction in methanotrophs community diversity. The deforestation and land use practices changed the soil properties adversely affecting the viable soil methanotrophic population (King and Nanba, 2008; Dorr et al., 2010). A harsh change in soil characteristics in a particular ecosystem owing to land use changes could be a potential threat to adversely affect the community composition of methanotrophs (Singh and Gupta, 2016). 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 (Megonigal and Guenther, 2008; Shoemaker et al., 2014). Therefore, methanotrophic numbers in this study (**Figure 5.3**) 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 CH_4 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 (**Figure 5.4**) 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 CH_4 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 NH_4^+-N inhibits the methanotrophs activity and growth in forest, grassland, arable and paddy soils (Cai and Yan, 1999) because of the molecular analogy of NH_4^+ with CH_4 molecules and consequently interfere the uptake of CH_4 by methanotrophs. The competitive inhibition of NH_4^+ to CH_4 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 NH_4^+-N content confirmed the inhibitory effect of NH_4^+-N treatments to the growth and multiplication of bacteria.

This study indicated wide variations in methanotrophic community compositions due to land use changes (**Table 5.3** and **5.4**), that may be attributed to variations in soil physico-chemical properties of soils. Similar reports regarding variations in methanotrophic species across different ecosystems types is reported by Knief et al. (2005), Singh et al. (2007), Zheng et al. (2010, 2012).

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. The favorable soil conditions (greater organic and inorganic nutrients, soil moisture and optimum pH) at mixed and natural forest sites exhibited higher number of CH_4 consuming bacteria and community compositions 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 and their diversity. 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 community composition. The lower quantity and diversity of methanotrophs 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. 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 diversity and their CH_4 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 CH_4 load.

CHAPTER 6

Soil Microbial Biomass Under Different Land Use Changes

Chapter 6

Objective 4

SOIL MICROBIAL BIOMASS UNDER DIFFERENT LAND USE CHANGES

Introduction

Land use management practices may influence the functional roles of soil microorganisms through modification of the quantity and quality of organic matter inputs (Steenwerth et al., 2002; Jin et al., 2010). Conversion of forest to agriculture and intensive farming practices contribute to the loss of soil organic matter (Lagomarsino et al., 2011), alter microbial activities and ultimately affect soil microbial biomass (SMB) (Schloter et al., 2003; Kumar and Ghoshal, 2017). The rapid growth rate of population caused by urbanization in northern India requires additional farmlands for the production of crops. One way is to expand the tillable area by clearing the forests and converting pastures into farming land. Consequently, deforestation and land use changes have caused serious environmental problems such as soil erosion/desertification, eutrophication, green house gases emissions and other environmental threats to northern India. Among the various ecological drivers, microbes and their biomass pools may be considered as major ecological impetus in controlling the diverse ecosystem functions globally (Singh and Gupta, 2018). Plant litter and soil microbial flora are key drivers that play fundamental roles in controlling ecosystem processes and is assumed that variations in the size of SMB can impact the functioning of various ecosystem types. Differences in the quantity and quality of substrate (organic C and N) inputs caused by varying plant residue types (litter and fine roots) and the associated nutrient specificity can be crucial drivers to influence the SMB across the ecosystem types (Singh and Gupta, 2018). It is also suggested that shifts in plant species composition during land use changes can influence the microbial community composition dynamics basically by changing the soil organic nutrient status (Zhang et al., 2016).

The 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 region, owing to land uses, have been carried out to show 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). The earlier study related to soil microbial biomass from dry tropical regions (India) were conducted by selecting only one agro- or forest ecosystem or the other selected sites on local level with very small area and it was not able to decipher differences in vegetation cover or topographical variations. Therefore, assessments of microbial properties relevant to soil fertility/organic matter, such as SMB–C, –N and –P could better reflect changes in soil quality and productivity, which alter labile available soil nutrient dynamics. The previous works limited to show that changes in land covers would influence abiotic and biotic factors which subsequently govern the soil physico-chemical status (Kumar and Ghoshal, 2017). 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. Therefore, a better understanding to the effects of current land use practices may provide further insight into soil microbial communities and SMB mediated restoration of soil fertility and environmental management. As microbial adaptation and re-establishment in the form of SMB may play a major role in ecosystem responses to land use disturbances (Singh and Gupta, 2018), the long-term consequences of earlier reports on SMB levels are critical for predicting alterations in ecological and environmental functioning. The understanding of SMB levels can be used as a suitable indicator for quantifying and monitoring changes in SMB–C, –N and –P and beneficial microbial community structure as well as soil nutrient dynamics in response to anthropogenic mediated land use disturbances. 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. Therefore, the consequences of changes in SMB–C, –N and –P levels and soil physico-chemical properties during land use changes should be studied to offer relevant information on SMB levels to changes in the soil conditions and interactions between soil microbial communities and soil physico-chemical properties. The viability and future of present study area (northern India) is associated with the eco-friendly and efficient soil natural resource management and environment restoration. In this investigation, we focused on the four kinds of land use/covers, including (i) natural forest, (ii) mixed forest, (iii) savanna, and (iv) agriculture land. This study also tried to assess the relationships among various types of land use/cover, SMB–C, –N and –P, and soil physico-chemical (bulk density, soil moisture content, pH, organic-C, total-N and available nutrients) properties. The present study hypothesized: i) different land use type/cover may affect SMB levels in top surface soil (0–10 cm), but would have less effects in sub-soil profiles (20–30 cm); and ii) SMB levels in top surface soil are will be highest in natural forest, followed by mixed forest and then savanna and farming lands.

Materials and methods

Soil sampling

The details of soil sampling are already described in **Chapter 3**. The collected soil samples stored at 4 °C were used for the analyses of soil microbial biomass (SMB)-C, -N and –P analyses.

Soil microbial biomass (SMB) analyses

SMB-C

The conditioned soil samples (50 gm) were saturated with purified liquid chloroform (Analytical reagent grade, Qualigens, India) for 10-20 hrs (Srivastava and Singh, 1988) and subsequently removed by evacuation. The soil was then extracted with 0.5 M K_2SO_4 (1: 4, soil: extractant) and shaked for 30 minutes. Similarly, the unfumigated soil sample was also extracted following the same protocol. MB-C in 0.5 M K_2SO_4 soil extract was measured by dichromate digestion (Vance et al., 1987) and estimated by the equation MB-C= 2.64 X Ec (Vance et al., 1987), where Ec is the difference between C extracted from fumigated and unfumigated treatments, both expressed as $\mu g\ g^{-1}$ oven dry soil basis (Jenkison and Ladd, 1981).

SMB-N

The conditioned soil samples (25 gm) were saturated with purified liquid chloroform for 18-20 hrs. The procedure of extraction was similar to as described for MB-C. The 0.5 M K_2SO_4 extracts of fumigated and unfumigated soil samples were analysed for MB-N employing the microKjeldahl digestion procedure (Brookes et al., 1985). The MB-N was calculated by the equation $MBN = X - Y/K_N$; where X= total N in K_2SO_4 extract of fumigated soil and Y = total N in K_2SO_4 extract of unfumigated soil; K_N = fraction of biomass-N extracted after $CHCl_3$ treatment. A K_N of 0.54 (Brookes et al., 1985) was taken by assuming that 54% of the MB-N was extracted by $CHCl_3$ treatments.

SMB-P

The conditioned soil sample (5 gm) was saturated with purified liquid chloroform for 18-20 hrs. Two portions of soils (fumigated and un-fumigated) were extracted with 0.5 M $NaHCO_3$ solution. MB-P was determined as inorganic-P (Pi) in 0.5 M $NaHCO_3$ extract of fumigated and un-fumigated soil samples by ammonium molybdate-stannous chloride method (Sparling et al., 1985). The MB-P was calculated from $CHCl_3$ released Pi dividing by a K_P value of 0.40 (Brookes et al., 1982) by assuming that 40% of P in biomass is released as Pi by $CHCl_3$ treatment (Brookes et al., 1982; Srivastava and Singh, 1988).

All data in the experiment were processed and calculated using the canonical correspondence analysis using SPSS version 20 (IBM, Armonk, NY, USA). The canonical correspondence analysis (CCA) between SMB-C, -N and -P and soil physico-chemical properties, creating ordination, was performed using PC-ORD software package (McCune and Mefford, 1999).

Soil samples collected from different selected sites were analyzed for the SMB-C, -N and -P. The SMB-C, -N and -P (μg g^{-1} dry soil) were determined by the chloroform ($CHCl_3$) fumigation-extraction procedures as described by Brookes et al. (1985) and Vance et al. (1987). Liquid chloroform after purification was used for soil fumigation (Srivastava and Singh, 1988). The 0.5 mol L^{-1} K_2SO_4 (1:4 Soil: extract) was used for extraction for about 30 minutes. Similar extraction procedures were also adopted for no-fumigated soil samples. The SMB-C and -N in 0.5 mol L^{-1} K_2SO_4 soil extract was determined by dichromate digestion (Vance et al., 1987). SMB-P was analysed as inorganic-P (Pi) in 0.5 mol L^{-1} $NaHCO_3$ extract of both fumigated and non-fumigated soil samples by ammonium molybdate-stannous chloride method as described by Sparling et al. (1985)

Statistics

The values given are means of three replicates ±SE. Two-way analysis of variance (ANOVA) was used to compare the soil physico-chemical properties and soil microbial biomass–C, –N and –P values across selected land use changes/covers and soil depths. Duncan's MRT test ($p < 0.05$) was also applied to assess the differences among land use types and soil depths. All data in the experiment were processed and calculated using the SPSS version 20 (IBM, Armonk, NY, USA). The canonical correspondence analysis (CCA) between SMB-C, -N and -P and soil physico-chemical properties, creating ordination, was performed using PC-ORD software package (McCune and Mefford, 1999).

Results

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

The level of SMB-C differed significantly among the land-use types and soil depths ranging from 297.65 to 768.25 μg 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 μg g^{-1}) in the natural forest soil, followed in decreasing order in mixed forest, savanna, and the minimum (297.65 μg 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 μg g^{-1} dry soil). The SMB- P ranged from 22.45 in the agriculture land to 48.9 μg 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 (**Figures 6.1 A, B and C**).

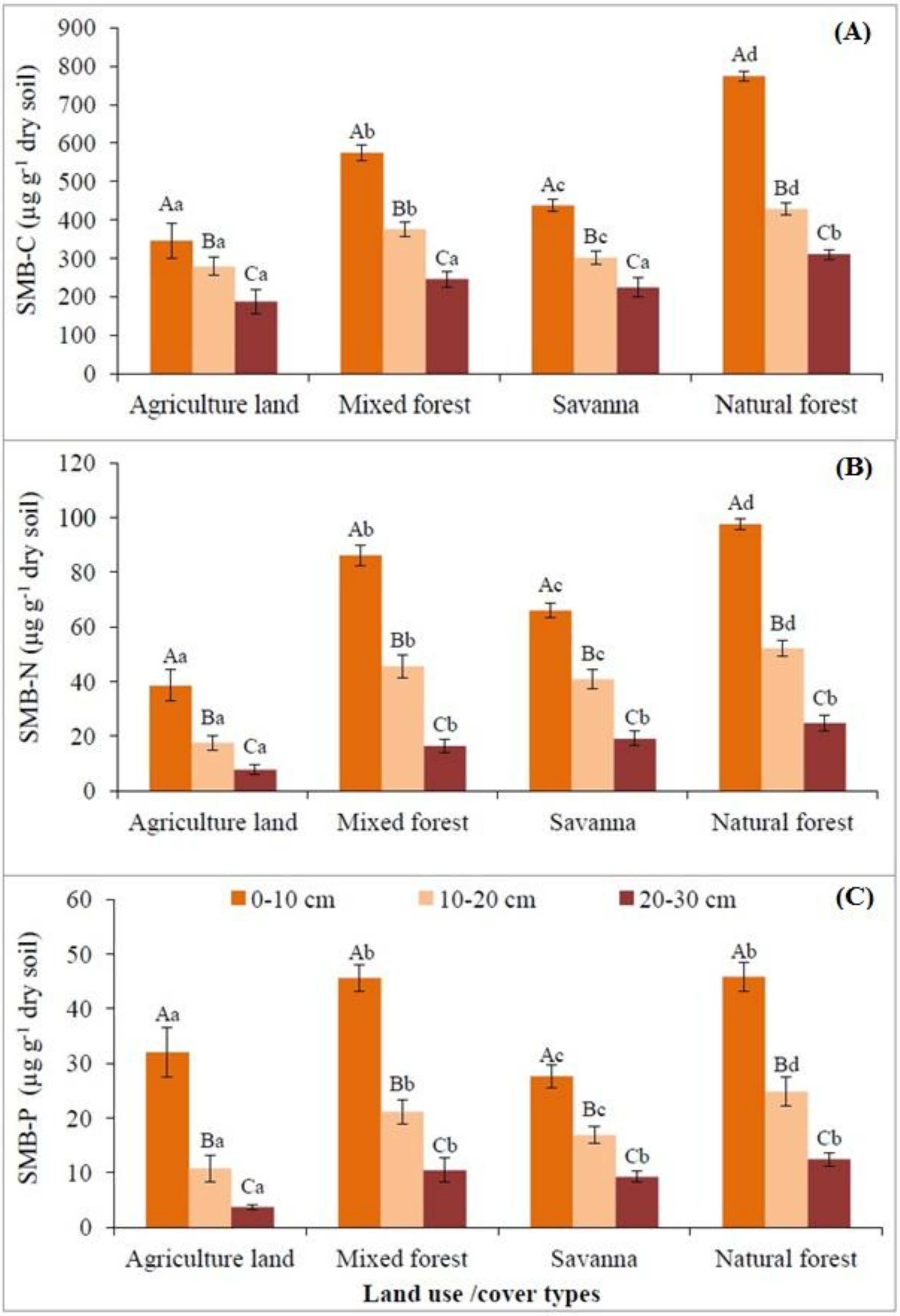


Figure 6.1 Dynamics of soil microbial biomass (SMB)–C (**A**), SMB–N (**B**) and SMB–P (**C**) at different soil depth (0–10, 10–20 and 20–30 cm) and land use/cover types. Values are means of three replicates ±SE. Different capital letters designate significant differences at $p < 0.05$ at soil depths for each land use type; different lowercase letters indicate significant differences at $p < 0.05$ among different land use types at a same soil depth. Results of two-way ANOVA on impact of land use types, soil depths and their interaction for SMB-C, -N and -P is given in **Table 6.1**.

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 × soil depths interaction ($P < 0.001$) (**Table 6.1**), indicating that effect of land use changes on different soil depths (0-10, 10-20 and 20-30 cm) were different.

Table 6.1 Results of two-way ANOVA on the impacts of land use changes (LUC), soil depth (SD) and their interactions for soil microbial biomass (SMB)-C, N and P. The data for this analysis were considered from **Figure 6.1 A, B and C**.

Soil parameters	**Land use covers (df=3)**	**Soil depths (df=2)**	**Land use covers × Soil depths (df=6)**
SMB-C	F=2518.7***	F=7564.1***	F=431.7***
SMB-N	F=559061.2***	F=2310004.3***	F=77069.0***
SMB-P	F=787210.8***	F=6477696.0***	F=123058.8***

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; df = degree of freedom

The canonical correspondence analysis (CCA) between SMB-C, -N and -P and soil physico-chemical properties across different land use types was also performed and details of Figures are given in **Chapter 7**. The detail CCA analysis summary between SMB-C, -N and -P and soil physico-chemical properties across different land use types is given in **Table 6.2**. The Eigen values of SMB-C and -N on ordination Axis 1 and 2 respectively, were 0.011 and 0.028. Results suggest, between SMB-C and soil physico-chemical characteristics, the ordination Axis 1 explained 28.5 % variability while Axis 2 reported 67.9% of total variation. In case of SMB-N, the ordination Axis 1 explained 27.2% variability while the Axis 2 explained 71.1% of total variation. The Eigen values of SMB-P on ordination Axis 1 and 2 respectively, were 0.022 and 0.016. The total variability for ordination Axis 1 in case of SMB-P was about 56.8% while the total variations noted for Axis 2 was 41.3%. The ordination plots 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.

Table 6.2 Details of axis summary statistics of canonical correspondence analysis (CCA) between the soil physico-chemical parameters and soil microbial biomass (SMB)-C, -N and -P quantity across various land use types of Vindhyan uplands. This detail of CCA analyses are based on **Figures 7.3, 7.4 and 7.5**.

Variables	Eigen-value		Variance in species data (% of variance explained)	Cumulative % explained
SMB-C	Axis 1	0.011	28.5	28.5
	Axis 2	0.028	67.9	96.4
SMB-N	Axis 1	0.011	27.2	27.2
	Axis 2	0.028	71.1	98.3
SMB-P	Axis 1	0.022	56.8	56.8
	Axis 2	0.016	41.3	98.1

Discussion

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 (**Figures 6.1 A, B and C**). 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 grater in to 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 resides. 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 (**Figures 6.1 A, B and C**). 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 (**Figures 6.1 A, B and C**). 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.

Across different land uses the CCA showed that SMB-C, -N and -P contents are very closely related with soil pH, soil moisture (SM), water holding capacity (WHC), soil temperature (Temp), total-N (TN), C/N ratio and organic-C (OC) (**Chapter 7**). The study of Xiangmin, et al. (2014) also showed a strong positive correlation between SMB and soil moisture contents across different land uses in the Changbai Mountains of Northeast China. Across different land uses it supposed that high microbial biomass may be attributed to more favourable environmental factors, such as dense vegetation covers, optimum soil moisture contents, nutrient richness (organic-C and total-N contents) and availability of greater amount of plant residues/litters (Chen et al., 2010). It expected that low soil moisture condition in agriculture and savanna ecosystems generally may have low organic matter levels, because a stress (low) soil moisture may negatively affect the rate of microbial decay and decomposition (Xiangmin et al., 2014) and therefore, significantly reduces the soil organic matter accumulations and SMB quantity at disturbed savanna and agriculture land uses. The results of CCA between SMB and important soil parameters of present study are in accordance with the study of Wallis et al. (2010). They performed CCA between physico-chemical variables and microbial community composition and demonstrated that especially soil organic-C content plays a significant role to govern the soil microbial community composition in KwaZulu-Natal midlands, South Africa. Several study also confirmed that soil pH, soil moisture, organic-C, total-N and -P contents were the most important factors regulating the microbial community structure and biomass under different land uses/ecosystems (Liu et al., 2014; Cao et al., 2017). Moreover, Cao et al. (2017) showed that pH was a significant factor that deciding the size and abundance of the soil microbial community/biomass. Mendes et al. (2015) confirmed that pH is strongly correlated with the bacterial community structure in south-eastern region of the Amazon. In addition, Cong et al. (2015) showed that soil temperature and soil moisture contents could have important effects on soil population and their biomass. The soil moisture status effectively increases substrate fluxes towards microbial cell surface, whereas low soil moisture availability can hinder microbial activity by decreasing the intracellular water potential and enzymatic activities (Stark and Firestone, 1995).

Conclusions

The results show that soil physico-chemical properties and SMB levels are good indicators of the soil quality following changes in land use/cover. The land cover changes resulted in disturbances of soil properties in agriculture and savanna soil compared to soils under natural and mixed forest. It is evident that over time recycling of inorganic and organic nutrients via litter returns significantly increases the soil organic matter contents and improves the soil microbial community composition and SMB status of undisturbed natural forest. Present study also 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.). Since, soil parameters are indices of active soil microbial community stability that ultimately controls the ecosystem services; decline in SMB quantity reveals poor disturbed soil that can neither sustain soil microbial biodiversity nor any overlying crop/vegetation. The CCA between soil properties (soil organic–C, total–N, moisture contents, etc.) and SMB–C –N and –P levels underline the fact that variations in SMB quantity are dependent on changes in soil nutrient conditions. 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.Thus, SMB levels can serve as potential key ecological indicators of soil quality changes resulting from land use management practices to top and sub-soils of dry tropical 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.

CHAPTER 7

Correlation Between Land Use Changes and Methanotrophic Bacterial Abundance and Soil Microbial Biomass

Chapter 7

Objective 5

CORRELATION BETWEEN LAND USE CHANGES AND METHANOTROPHIC BACTERIAL ABUNDANCE AND SOIL MICROBIAL BIOMASS

Introduction

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 and its correlation with other soil microbial properties in dry tropical soils of Vindhyan region.

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 CH_4 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 is soil physico-chemical properties due to land use changes would be correlated to the variation in soil methanotrophs abundance and their *pmoA* gene copies at Vindhyan upland soils.

Currently, it is 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. Variations in quantity of SMB–C, –N and –P due to land use changes and its correlation with soil physico-chemical properties remains poorly understood in dry tropical regions of Vindhyan uplands. Therefore, a better understanding to the impacts of variations in soil properties owing to 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

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. Therefore, we were interested to study the influence of soil conditions and soil nutrient quality (organic matter and C/N ratio) on various microbial mediated activities across different land use changes and soil depths in dry tropical region of Vindhyan region. Furthermore, abundance, diversity of CH_4 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 correlations among soil physico-chemical properties and abundance, diversity of CH_4 oxidizing bacteria, *pmoA* gene copies, and SMB levels at different soil depths, across different land use types.

Materials and methods

The relationships between soil physico-chemical properties and soil methanotrophs abundance and *pmoA* gene copies across different land use types and soil depths were examined by Pearson's correlation analysis. We also used canonical correspondence analyses (CCA) between SMB-C, -N and -P and soil physico-chemical properties, creating ordination, using PC-ORD software package (McCune and Mefford, 1999). All data in the experiment were processed and calculated using the SPSS version 20 (IBM, Armonk, NY, USA).

Results and Discussion

The data on soil methanotrophs abundance in this study showed that natural and mixed forest soil have a statistically higher number of viable soil methanotrophic populations as compared to the savanna and agriculture land. The results also exhibited that methanotrophic abundance at all the land uses was significantly higher in top (0-10 cm) soil layer than the deeper soil profiles.

The correlation analysis revealed significant ($P = <0.01$) positive interaction between methanotrophs population with WHC, total-C,-N and -P (**Table 7.1**), nitrate-N (**Figure 7.1B**) soil moisture content (**Figure 7.2A**) and C/N ratio (**Figure 7.2B**) and negative with BD (**Table 7.1**) and ammonium-N contents (**Figure 7.1A**) at different soil depths.

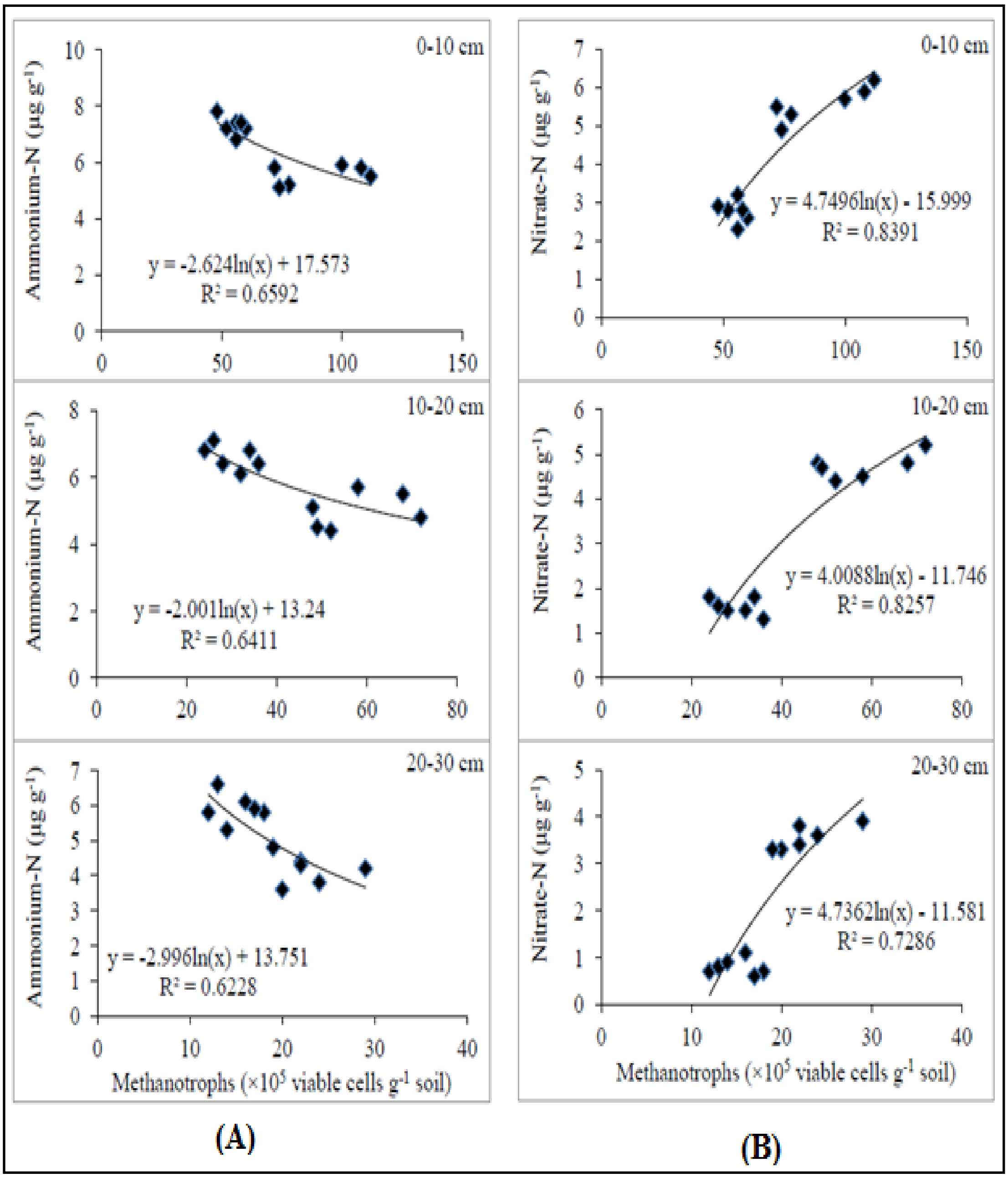


Figure 7.1. At different land uses the relationship of methanotrophs abundance to soil ammonium-N (A), nitrate-N (B) across soil depths. For the correlation analyses total soil sample used was N=12 (4 land uses × 3 soil depths).

Table 7.1 Pearson’s correlation matrix of soil physico-chemical characteristics and methanotrophs abundance under different land use types at three (0-10, 10-20 and 20-30 cm) soil depths. Total samples used during correlation analyses for each depth was N=12 (4 land use types × 3 replicates).

Parameters	Temperature (ºC)	pH	EC	BD	WHC	CEC	OC	TN	SCNR	TP
0-10 cm depth										
pH	0.655*									
Electrical conductivity (µs cm^{-1}) (EC)	0.731**	NS								
Bulk density (g cm^{-3}) (BD)	NS	-0.661*	NS							
Water holding capacity (%) (WHC)	NS	0.811**	NS	-0.804**						
Cation exchange capacity (meq/100g) (CEC)	NS	NS	0.612*	NS	NS					
Organic -C (%) (TC)	NS	0.720**	NS	-0.931**	0.912**	NS				
Total- N (%) (TN)	NS	0.698*	NS	-0.854**	0.898**	NS	0.969**			
Soil C/N ratio (SCNR)	NS	0.834**	NS	-0.946**	0.918**	NS	0.946**	0.882**		
Total -P (µg g^{-1} soil) (TP)	NS	NS	-0.632*	-0.841**	0.522	NS	0.757**	0.655*	0.684*	
Methanotrophs (× 10^{6} viable cells g^{-1} soil)	NS	0.533	NS	-0.914**	0.833**	NS	0.955**	0.938**	0.859**	0.803**
10-20 cm depth										
pH	NS									
EC	NS	NS								
BD	NS	-0.737**	NS							
WHC	NS	0.932**	NS	-0.876**						

Parameters	Temperature (ºC)	pH	EC	BD	WHC	CEC	OC	TN	SCNR	TP
CEC	NS	NS	0.690*	NS	NS					
OC	NS	0.707*	NS	-0.953**	0.810**	NS				
TN	NS	0.628*	NS	-0.863**	0.718**	NS	0.960**			
SCNR	NS	0.863**	NS	-0.958**	0.941**	NS	0.909**	0.813**		
TP	NS	NS	-0.836**	NS	NS	NS	NS	NS	NS	
Methanotrophs	NS	0.619*	NS	-0.949**	0.814**	NS	0.948**	0.895**	0.904**	NS
			20-30 cm depth							
pH	-0.841**									
EC	NS	NS								
BD	0.735**	-0.735**	NS							
WHC	-0.710**	0.873**	NS	-0.858**						
CEC	NS	NS	0.682*	NS	NS					
OC	-0.584*	0.609*	NS	-0.895**	0.741**	NS				
TN	NS	NS	NS	-0.740**	0.602*	NS	0.934**			
SCNR	-0.782**	0.839**	NS	-0.936**	0.872**	NS	0.850**	0.696*		
TP	NS	NS	-0.821**	-0.598*	NS	NS	NS	NS	NS	
Methanotrophs	NS	0.607*	NS	-0.829**	0.782**	NS	0.744**	0.599*	0.700*	NS

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed)

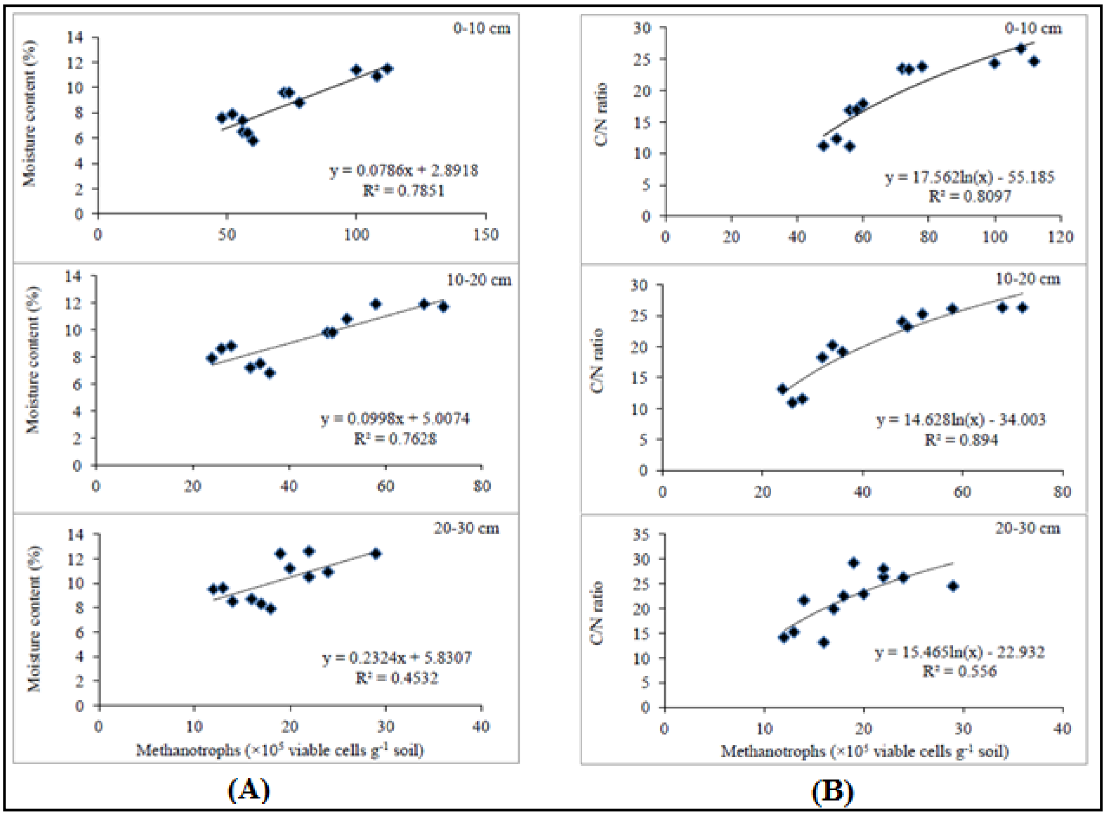


Figure 7.2. At different land uses the relationship of methanotrophs abundance to soil moisture contents (%) (A) and C/N ratio (B) across soil depths. For the correlation analyses total soil sample used was N=12 (4 land uses × 3 soil depths).

The result showed that compared to the savanna and agriculture soil the methanotrophic abundance 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 and soil moisture can contribute significantly to growth of methane-oxidizers in such well aerated soils (Megonigal 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 soil moisture and aeration because of high amount of organic contents. Due to these factors the number of methanotrophs shoed positive relationship soil moisture, organic-C, total-N, etc. (**Table 7.1** and **Figure 7.2A**). 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.

At all the land use sites the methanotrophs abundance increased with increases of C/N ratios. Across the land uses and soil depths the highest methanotrophs abundance is observed at natural forest with a C/N ratio 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 CH_4 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), or stimulatory effects (Mohanty et al., 2006) or no effects (Delgado and Mosier, 1996) on methanotrophic population. The addition of NH_4^+-N inhibits the methanotrophs activity and growth in forest, grassland, arable and paddy soils (Cai and Yan, 1999) because of the molecular analogy of NH_4^+ with CH_4 molecules and consequently interfere the uptake of CH_4 by methanotrophs. The competitive inhibition of NH_4^+ to CH_4 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 NH_4^+-N content and C/N ratios (**Figure 7.1A** and **Figure 7.2B**) confirmed the inhibitory effect of NH_4^+-N treatments to the growth and multiplication of bacteria. 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 CH_4 consuming activity of methanotrophs in cropland soils.

Correlation between soil physicochemical properties and SMB - C, -N and -P

The canonical correspondence analysis (CCA) performed between SMB-C, -N and -P and soil physico-chemical properties across different land use types are given in **Figures 7.3, 7.4 and 7.5**.

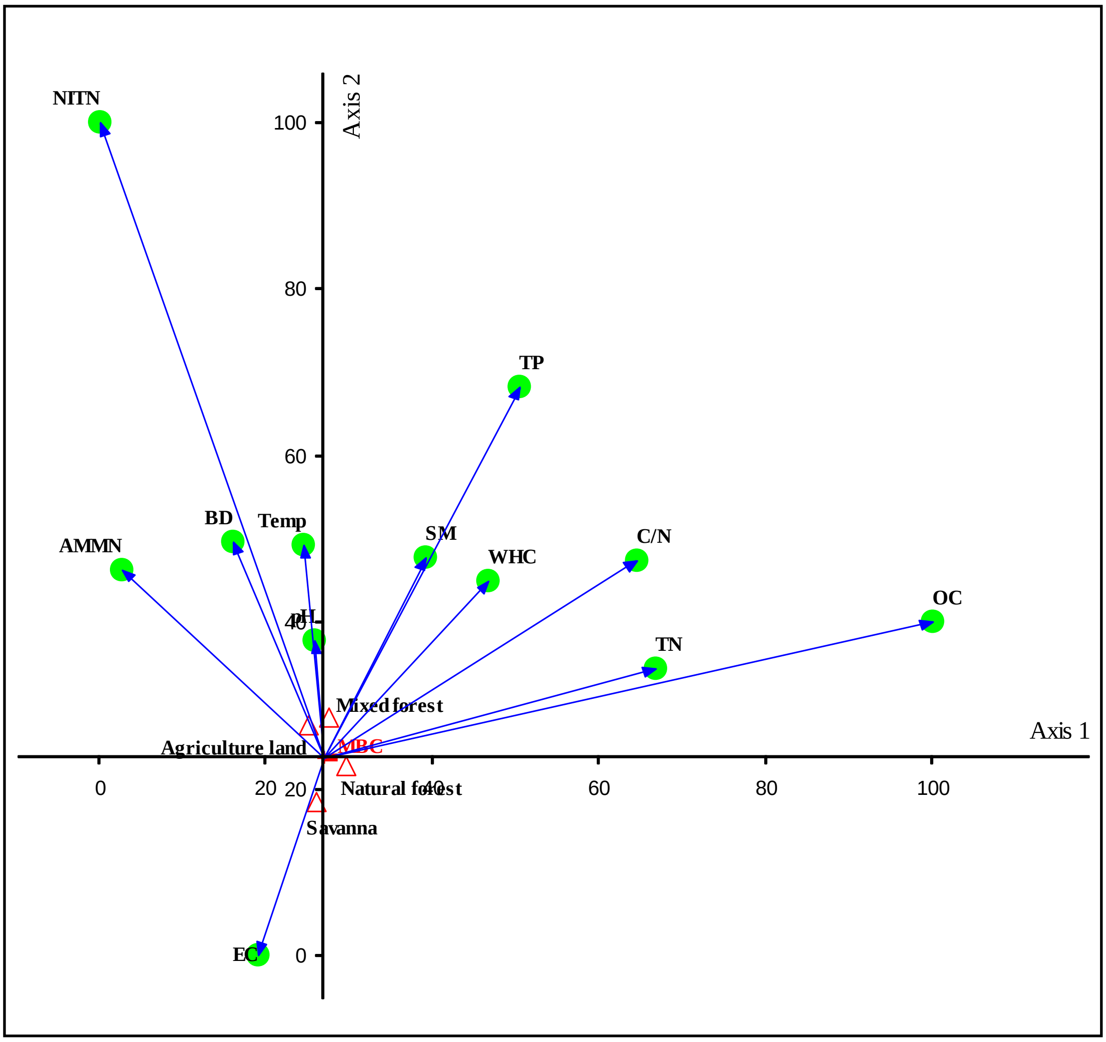


Figure 7.3. The canonical correspondence analysis (CCA) ordination showing the relationship between the SMB-C quantity and soil physico-chemical properties quantity across various land use types of Vindhyan uplands. AMMN=ammonium-N, NITN=nitrate-N, BD=bulk density, Temp=temperature, C/N= C/N ratio, OC=organic-C, SM=soil moisture, TN= total-N; TP=total-P, WHC=water holding capacity.

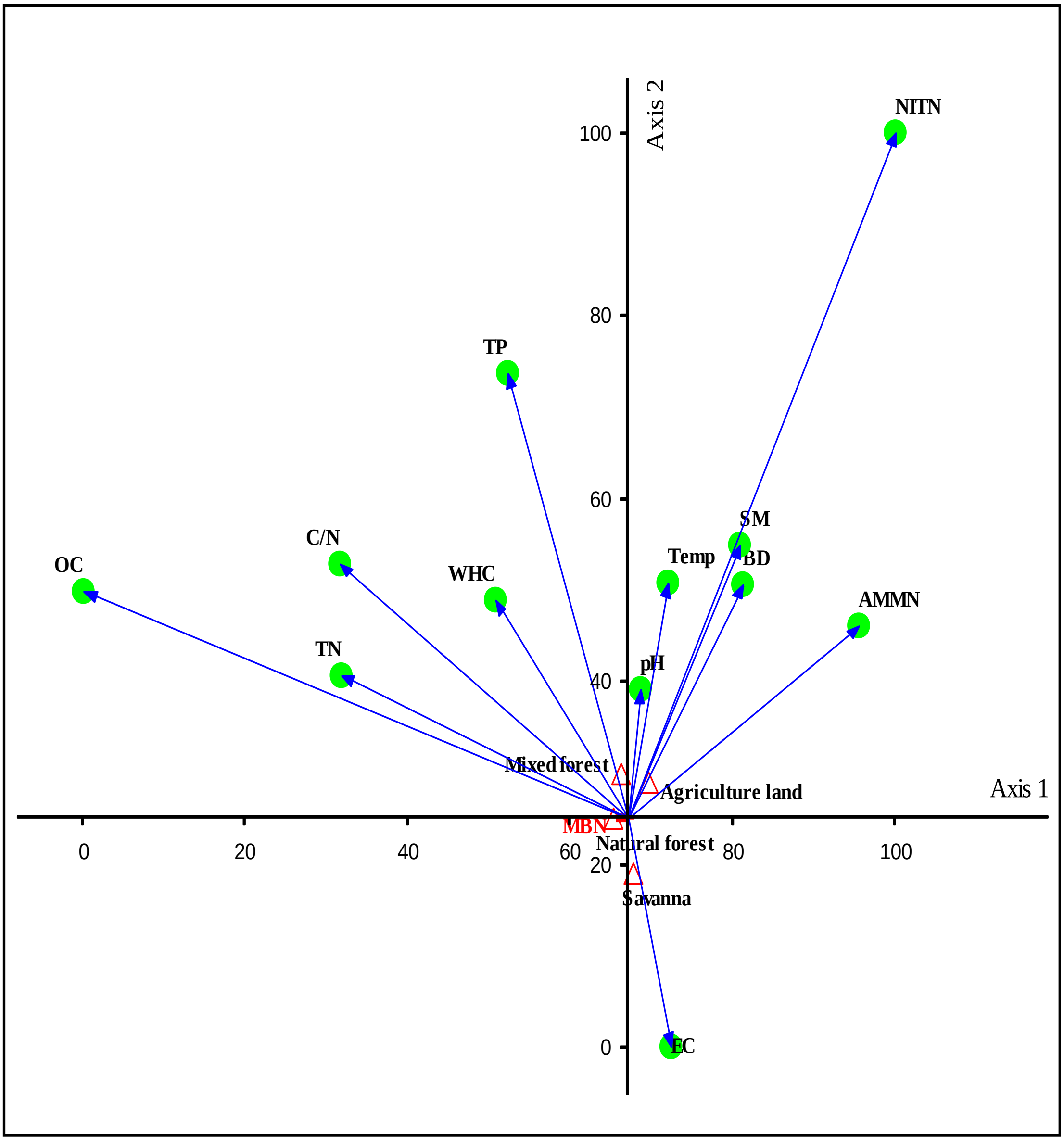


Figure 7.4. The canonical correspondence analysis (CCA) ordination showing the relationship between the SMB-N quantity and soil physico-chemical properties quantity across various land use types of Vindhyan uplands. AMMN=ammonium-N, NITN=nitrate-N, BD=bulk density, Temp=temperature, C/N= C/N ratio, OC=organic-C, SM=soil moisture, TN= total-N; TP=total-P, WHC=water holding capacity.

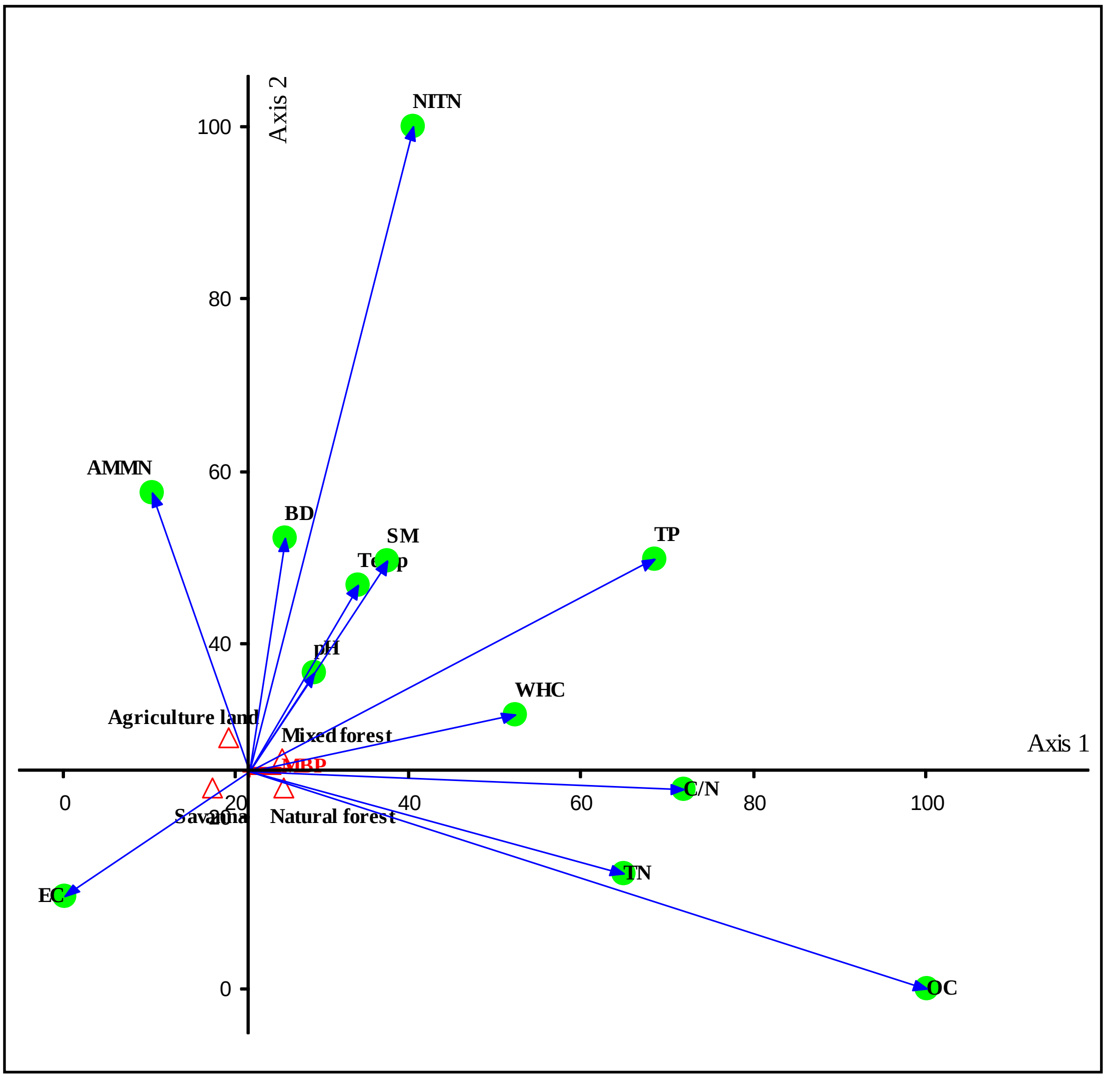


Figure 7.5. The canonical correspondence analysis (CCA) ordination showing the relationship between the SMB-P quantity and soil physico-chemical properties quantity across various land use types of Vindhyan uplands. AMMN=ammonium-N, NITN=nitrate-N, BD=bulk density, Temp=temperature, C/N= C/N ratio, OC=organic-C, SM=soil moisture, TN= total-N; TP=total-P, WHC=water holding capacity.

The detail CCA analysis summary between SMB-C, -N and -P and soil physico-chemical properties across different land use types is given in **Table 7.2**.

Table 7.2 Details of axis summary statistics of canonical correspondence analysis (CCA) between the soil physico-chemical parameters and soil microbial biomass (SMB)-C, -N and -P quantity across various land use types of Vindhyan uplands.

Variables	Eigen-value		Variance in species data (% of variance explained)	Cumulative % explained
SMB-C	Axis 1	0.011	28.5	28.5
	Axis 2	0.028	67.9	96.4
SMB-N	Axis 1	0.011	27.2	27.2
	Axis 2	0.028	71.1	98.3
SMB-P	Axis 1	0.022	56.8	56.8
	Axis 2	0.016	41.3	98.1

The Eigen values of SMB-C and -N on ordination Axis 1 and 2 respectively, were 0.011 and 0.028. Results suggest, between SMB-C and soil physico-chemical characteristics, the ordination Axis 1 explained 28.5 % variability while Axis 2 reported 67.9% of total variation. In case of SMB-N, the ordination Axis 1 explained 27.2% variability while the Axis 2 explained 71.1% of total variation. The Eigen values of SMB-P on ordination Axis 1 and 2 respectively, were 0.022 and 0.016. The total variability for ordination Axis 1 in case of SMB-P was about 56.8% while the total variations noted for Axis 2 was 41.3%. The ordination plots 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.

Across different land uses the CCA showed that SMB-C, -N and -P contents are very closely related with soil pH, soil moisture (SM), water holding capacity (WHC), soil temperature (Temp), total-N (TN), C/N ratio and organic-C (OC). The study of Xiangmin et al. (2014) also showed a strong positive correlation between SMB and soil moisture contents across different land uses in the Changbai Mountains of Northeast China. Across different land uses it supposed that high microbial biomass may be attributed to more favourable environmental factors, such as dense vegetation covers, optimum soil moisture contents, nutrient richness (organic-C and total-N contents) and availability of greater amount of plant residues/litters (Chen et al., 2010). It expected that low soil moisture condition in agriculture and savanna ecosystems (Table 3) generally may have low organic matter levels, because a stress (low) soil moisture may negatively affect the rate of microbial decay and decomposition (Xiangmin et al., 2014) and therefore, significantly reduces the soil organic matter accumulations and SMB quantity at disturbed savanna and agriculture land uses. The results of CCA between SMB and important soil parameters of present study are in accordance with the study of Wallis et al. (2010). They performed CCA between physico-chemical variables and microbial community composition and demonstrated that especially soil organic-C content plays a significant role to govern the soil microbial community composition in KwaZulu-Natal midlands, South Africa. Several study also confirmed that soil pH, soil moisture, organic-C, total-N and -P contents were the most important factors regulating the microbial community structure and biomass under different land uses/ecosystems (Liu et al., 2014; Cao et al., 2017). Moreover, Cao et al. (2017) showed that pH was a significant factor that deciding the size and abundance of the soil microbial community/biomass. Mendes et al. (2015) confirmed that pH is strongly correlated with the bacterial community structure in south-eastern region of the Amazon. In addition, Cong et al. (2015) showed that soil temperature and soil moisture contents could have important effects on soil population and their biomass. The soil moisture status effectively increases substrate fluxes towards microbial cell surface, whereas low soil moisture availability can hinder microbial activity by decreasing the intracellular water potential and enzymatic activities (Stark and Firestone, 1995).

Conclusions

The results show that soil physico-chemical properties are good indicators of the soil quality following changes in land use/cover. The land cover changes resulted in disturbances of soil properties in agriculture and savanna soil compared to soils under natural and mixed forest. It is evident that over time recycling of inorganic and organic nutrients via litter returns significantly increases the soil organic matter contents and improves the soil methanotrophs abundance and SMB status of undisturbed natural forest. Present study also indicate that soil methanotrophs abundance and 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 which in turns affect soil methanotrophs and SMB values. These results indicate that land use practices significantly influence the soil methanotrophs population in surface soil layers (0-10 cm depths), with less significant impacts in deeper sub-soil layers (20-30 cm depths). 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 consequently showed a positive relationship with the count of soil methanotrophs numbers and BMB values. The results of this study also 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 methanotrophs community and SMB across different land uses and soil depths.Thus, SMB levels can serve as potential key ecological indicators of soil quality changes resulting from land use management practices to top and sub-soils of dry tropical region. This research recommends a plantation with suitable native broad-leaved species to restore the soil methanotrophs community and SMB levels of degraded nutrient poor soils of dry tropical Vindhyan uplands of India.

CHAPTER 8

Summary

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SUMMARY

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 LUC has been also reported to soil microbial diversity decline (HainesYoung and Potschin, 2009) and loss of soil microbial biomass in the soil of many tropical forest ecosystems (Lima et al., 2014; Qi et al., 2018). The land use management practices also influences the functional roles of soil microorganisms due to differences in quality and quantity of organic matter inputs because of variations in dominant vegetation covers (Steenwerth et al., 2002; Jin et al., 2010). The conversion of natural forest covers, savanna and grasslands into agriculture land may lead to negative impacts on soil functioning (Raiesi, 2017), soil fertility, microbial community structure (Li et al., 2015), ecosystem functioning (Singh and Gupta, 2016) and ultimate the human heaths in many ways.

The growing demand for food and energy creates various environmental problems, mainly caused by indiscriminate increasing human population (Borlaug, 2002; Mazzetto et al., 2016). The intensive land use causes may negatively affect both the environmental stability and the agricultural productivity, ignoring the soil conservation practices (Cerri et al., 2004; Foley et al., 2005). The degradation rate of natural tropical forest, which covers 7% of the earth’s surface, is ~15.4 is greatly impacting the global environmental services (Parrotta et al., 1997; Kumar and

Ghoshal, 2014). The rapid growth rate of human population caused by urbanization in northern India requires additional farmlands for the production of crops. One option is to expand the farming lands by clearing the natural forest covers, which may be correlated to further increase in serious environmental problems such as soil erosion/desertification, eutrophication, green house gases emissions and other environmental threats to northern India. Differences in the quantity and quality of substrates (organic C and N) returns caused by varying plant residue types (litter and fine roots) and the associated nutrient dynamics can be crucial drivers to influence the soil productivity (Singh and Gupta, 2018). It is also suggested that shifts in plant species composition during land use changes can influence the microbial community composition dynamics basically by changing the soil organic nutrient status (Zhang et al., 2016). Therefore, in view of above it expected that, to satisfy the future human food demands, better land use management practices such as reduced inputs of synthetic chemicals, promotion of organic farming and bio-fertilizer application, restoration of degraded land, re-forestation, etc. may be eco-friendly options for the improvement of global soil and environmental quality.

Soil physico-chemical properties

The soil, one of the main natural resources, comprises of minerals, soil organic matter microbes, water and air. The soil physico-chemical characteristics vary in space and time due to variations in topography, climate, physical weathering processes, vegetation cover, microbial activities and several other biotic and abiotic variables (Paudel and Sah, 2003). The soil development in particular area is governed by biological (vegetation, soil micro-and macro-flora) and non-biological (parent rocks, climate and topography), factors over time (Brady and Weil, 2002). The composition and nature of these components greatly influence the soil physical properties, including texture, structure, porosity and nutrient status. In turn, these soil properties may affect soil’s ability to support an ecosystems functioning.

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

Methane (CH_4), a potent greenhouse gas (GHG), contributing about 15 to 20% of global warming (Jang et al., 2006; IPCC, 2007; Dalal and Allen, 2008). Therefore, it is required to find out the more ways to increase the CH_4 sinks to reduce its atmospheric load. Besides, reaction with hydroxyl (OH^-) radicals in the troposphere and stratosphere, CH_4 oxidation in soils, mediated by methanotrophic bacteria consume about 10 and 40 Tg annually (King 1997; Schlesinger ,1997; IPCC, 2001; Mohanty et al., 2007), comprises approximately 6-10% of the total global atmospheric CH_4 oxidation (IPCC, 2001). Thus, the most reasonable way to increase the sink or consumption of CH_4 is increasing the abundance/diversity/performance of the soil CH_4 consuming bacteria. Therefore, knowledge about the ecology and responses of the CH_4 consuming bacterial diversity/population in relation to land use changes in dry tropical soils are of particular interest.

The dry tropical upland soils of Vindhyan region contributed to highest CH_4 consumption on a global scale (Singh, 2011). The soil CH_4 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. Further, a deeper insight into the methanotrophic diversity of soil is often limited by the inability to cultivate these soil microbes (Steinkamp et al., 2001). Methanotrophs or methane-oxidising bacteria (MOB) are a functional group of bacteria with proficiency of utilizing CH_4 as their sole carbon and electron source (He et al., 2012; Tiwari et al., 2015) and represent the only known biological sink for the potent greenhouse gas (GHG) CH_4 (Bodrossy et al., 2003; Tiwari et al., 2015). The MOB are ubiquitous in nature (Knief et al., 2003, Zheng et al., 2012) and has been reported from wetlands, oxic-anoxic interface and rhizospheric zone of rice field (Ferrando and Tarlera, 2009; Park and Lee, 2013), forest soil (Jang et al., 2006, Giri et al., 2007), landfill (Kallistova et al., 2007), sewage sludge (Ho et al., 2013; Park and Lee, 2013), faeces (Derrien et al., 2004), cow dung (Girija et al., 2013), cattle dung (Rastogi et al., 2009). These microbes are also exists in swamps, mud, ponds, rivers, streams, meadow soils, deciduous woods (Park and Lee, 2013), biogas reactor (Rastogi et al., 2009) stone (Kussmaul et al., 1998), sediments (Bowman et al., 1997), farmyard-manure (Singh et al., 2010), farming soil, marine sediments (Semaru et al., 2008; Park and Lee, 2013), groundwater, tundra and polar lakes (Bowman et al., 1997). The methanotrophs are found in livestock manure (Rastogi et al., 2009), anaerobic environments (McDonald et al., 2008), continental margins (Tavormina et al., 2010; 2013) and extreme environments (Trotsenko and Khmelenina, 2002; McDonald et al., 2008; Tiwari et al., 2015) possibly hot springs and alkaline soda lakes (Semaru et al., 2008) and can survive in quite cold (4 °C) (Bowman et al., 1997) as well as hot temperature (> 81 °C) (Dunfield et al., 2014).

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 CH_4 sink activity of methanotrophs too (Mosier et al., 1991; Dorr et al., 2010). Two main explanatory factors have been proposed to regulate the aerobic methanotrophs CH_4 consumption performance in soils under different land use: methanotrophic community composition and methanotrophs abundance (Malghani et al., 2016). To our knowledge, little or no investigations have previously examined the abundance of methanotrophs in dry tropical upland soils across different land uses, which acts as a strong sink of atmospheric CH_4 (Singh, 2011). The CH_4 consumption in aerobic soil by methanotrophs is typically assessed by the detection of the *pmoA* gene, which encodes the β-subunit of pMMO enzyme (Semrau 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 is 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), an important labile portion of the soil organic matter has been considered also as a source of plant nutrients in natural and agro-ecosystems (Singh et al., 2009; Silva Maganhotto De Souza and Fay, 2012). Although soil physico-chemical status, seasonality, soil temperature, and other environmental factors, are important major drivers in regulating the functioning of natural ecosystems, SMB may be one of the key ecological factors which can govern the productivity of tropical nutrient poor soils (Singh et al., 2010). The 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. As microbial adaptation and re-establishment in the form of SMB may play a major role in ecosystem responses to land use disturbances (Singh and Gupta, 2018), the long-term consequences of earlier reports on SMB levels are critical for predicting alterations in ecological and environmental functioning. The understanding of SMB levels can be used as a suitable indicator for quantifying and monitoring changes in SMB–C, –N and –P and beneficial microbial community structure as well as soil nutrient dynamics in response to anthropogenic mediated land use disturbances. 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. Therefore, we were interested to study the influence of soil conditions and soil nutrient quality (organic matter and C/N ratio) on various microbial mediated activities across different land use changes and soil depths in dry tropical region of Vindhyan region. Furthermore, abundance, diversity of CH_4 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.

Study area, meteorology and dominant vegetation cover of different land use covers

The study area is characterized by vide variations in land use covers due 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 across different land use covers

In present study the land use changes had significant effects on soil physico-chemical properties particularly in top soil (0-10 cm depth). 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 ^{o}C) was highest followed by agriculture land (44 ^{o}C), mixed forest (43 ^{o}C) and natural forest (42 ^{o}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 ≈ 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.

The soil colour was almost brownish-black of all the selected regions, except the savanna site having brownish colour. The pH of soil was variable across the land uses. The surface soil layer of agriculture (6.5), mixed (7.3), savanna (8.9), and natural forest (7.6) land was slightly acidic to alkaline in nature. The soil moisture content (0.78%) and water holding capacity (29.73%) was highest for the natural forest soil followed by agriculture land, savanna and mixed forest. However, across the all land uses, the bulk density was maximum for agriculture land soil (1.59 g cm^{-3}). The organic-C, total-N and total -P was highest in the natural forest soil compared to other land use types. The C/N ratio was in decreasing order from natural forest (25.25) to mixed forest (23.58), savanna (17.25) and agriculture land (11.55). Across various land uses the soil ammonium-N (7.46 μg g^{-1} dry soil), nitrate-N (5.93 μg g^{-1} dry soil) and soil moisture contents were greater at natural and mixed forest with top soil layer (0-10 cm depth).

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).

Soil microbial biomass (SMB) - C, -N and -P across different land use covers

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 × 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 μg 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 μg g^{-1}) in the natural forest soil, followed in decreasing order in mixed forest, savanna, and the minimum (297.65 μg 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 µg g^{-1} dry soil). The SMB- P ranged from 22.45 in the agriculture land to 48.9 µg 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 canonical correspondence analysis (CCA) between SMB-C, -N and -P and soil physico-chemical properties across different land use types was also performed. The detail CCA analysis summary between SMB-C, -N and -P and soil physico-chemical properties across different land use types is given in **Chapter 6 & 7**. The Eigen values of SMB-C and -N on ordination Axis 1 and 2 respectively, were 0.011 and 0.028. Results suggest, between SMB-C and soil physico-chemical characteristics, the ordination Axis 1 explained 28.5 % variability while Axis 2 reported 67.9% of total variation. In case of SMB-N, the ordination Axis 1 explained 27.2% variability while the Axis 2 explained 71.1% of total variation. The Eigen values of SMB-P on ordination Axis 1 and 2 respectively, were 0.022 and 0.016. The total variability for ordination Axis 1 in case of SMB-P was about 56.8% while the total variations noted for Axis 2 was 41.3%. The ordination plots 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. 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 (**Figure 6.1 A, B and C**). 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 grater in to 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 resides. 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.

Across different land uses the CCA showed that SMB-C, -N and -P contents are very closely related with soil pH, soil moisture (SM), water holding capacity (WHC), soil temperature (Temp), total-N (TN), C/N ratio and organic-C (OC). The study of Xiangmin, et al. (2014) also showed a strong positive correlation between SMB and soil moisture contents across different land uses in the Changbai Mountains of Northeast China. Across different land uses it supposed that high microbial biomass may be attributed to more favourable environmental factors, such as dense vegetation covers, optimum soil moisture contents, nutrient richness (organic-C and total-N contents) and availability of greater amount of plant residues/litters (Chen et al., 2010). It expected that low soil moisture condition in agriculture and savanna ecosystems generally may have low organic matter levels, because a stress (low) soil moisture may negatively affect the rate of microbial decay and decomposition (Xiangmin et al., 2014) and therefore, significantly reduces the soil organic matter accumulations and SMB quantity at disturbed savanna and agriculture land uses. The results of CCA between SMB and important soil parameters of present study are in accordance with the study of Wallis et al. (2010). They performed CCA between physico-chemical variables and microbial community composition and demonstrated that especially soil organic-C content plays a significant role to govern the soil microbial community composition in KwaZulu-Natal midlands, South Africa. Several study also confirmed that soil pH, soil moisture, organic-C, total-N and -P contents were the most important factors regulating the microbial community structure and biomass under different land uses/ecosystems (Liu et al., 2014; Cao et al., 2017). Moreover, Cao et al. (2017) showed that pH was a significant factor that deciding the size and abundance of the soil microbial community/biomass. Mendes et al. (2015) confirmed that pH is strongly correlated with the bacterial community structure in south-eastern region of the Amazon. In addition, Cong et al. (2015) showed that soil temperature and soil moisture contents could have important effects on soil population and their biomass. The soil moisture status effectively increases substrate fluxes towards microbial cell surface, whereas low soil moisture availability can hinder microbial activity by decreasing the intracellular water potential and enzymatic activities (Stark and Firestone, 1995).

Soil pmoA gene quantification of methanotrophs across different land use covers

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 (**Figure 5.1**). 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 (**Figure 5.2**).

The natural and mixed forest soil showed a statistically higher number of viable soil methanotrophic abundance as compared to the savanna and agriculture land (**Figure 5.3**). 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 (**Figure 5.3**). ANOVA indicated significant difference in viable methanotrophs abundance due to land uses ($P=<0.001$), soil depths ($P=<0.001$) and land uses × 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 (**Table 7.1**), nitrate-N (**Figure 7.1**) soil moisture content (**Figure 7.2**) and C/N ratio (**Figure 7.2**) and negative with BD (**Table 7.1**) and ammonium-N contents (**Figure 7.1**) 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 (**Figure 5.4**) 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 (**Figure 5.4**). 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 × depths interaction (F = 5.916; P = <0.001) (**Table 4.1**).

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 (**Figure 5.3 and 5.4**). 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 (Megonigal 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 CH_4 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 CH_4 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 NH_4^+-N inhibits the methanotrophs activity and growth in forest, grassland, arable and paddy soils (Cai and Yan, 1999) because of the molecular analogy of NH_4^+ with CH_4 molecules and consequently interfere the uptake of CH_4 by methanotrophs. The competitive inhibition of NH_4^+ to CH_4 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 NH_4^+-N content confirmed the inhibitory effect of NH_4^+-N treatments to the growth and multiplication of bacteria.

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 ago-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 conversion of natural forest and grassland ecosystems into crop lands are the important indicator of variations in the abundance and/or type of methanotrophic communities in soils (Singh and Gupta, 2016). Zheng et al. (2010) showed that methanotrophic community structure was strongly affected by land use changes. Land use change intensely influences the soil physico-chemical characteristics, particularly pH and P that may be primarily responsible for the differences in methanotrophs population density (Menyailo et al., 2008). Levine et al. (2011) demonstrated that alteration of natural forest and native lands to agriculture land led to a seven fold reduction in methanotrophs community diversity. The deforestation and land use practices changed the soil properties adversely affecting the viable soil methanotrophic population (King and Nanba, 2008; Dorr et al. 2010). A harsh change in soil characteristics in a particular ecosystem owing to land use changes could be a potential threat to adversely affect the community composition of methanotrophs (Singh and Gupta, 2016).

In conclusions, the favourable soil conditions (greater organic and inorganic nutrients, soil moisture and optimum pH) at mixed and natural forest sites exhibited higher number of CH_4 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.

The results show that soil physico-chemical properties and SMB levels are good indicators of the soil quality following changes in land use/cover. The land cover changes resulted in disturbances of soil properties in agriculture and savanna soil compared to soils under natural and mixed forest. It is evident that over time recycling of inorganic and organic nutrients via litter returns significantly increases the soil organic matter contents and improves the soil microbial community composition and SMB status of undisturbed natural forest. Present study also 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.). Since, soil parameters are indices of active soil microbial community stability that ultimately controls the ecosystem services; decline in SMB quantity reveals poor disturbed soil that can neither sustain soil microbial biodiversity nor any overlying crop/vegetation. The CCA between soil properties (soil organic–C, total–N, moisture contents, etc.) and SMB–C –N and –P levels underline the fact that variations in SMB quantity are dependent on changes in soil nutrient conditions. 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. Thus, SMB levels can serve as potential key ecological indicators of soil quality changes resulting from land use management practices to top and sub-soils of dry tropical 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.

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. The favourable soil conditions (greater organic and inorganic nutrients, soil moisture and optimum pH) at mixed and natural forest sites exhibited higher number of CH_4 consuming bacteria 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. The lower quantity of methanotrophs 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. 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 CH_4 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 CH_4 load.

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Publications and Scientific Achievements

PUBLICATIONS AND SCIENTIFIC ACHIEVEMENTS

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 CH_4 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 मे बायोचार की भूमिका। विज्ञान प्रगति **(NISCAIR) Accepted.**

Scientific Achievements

Workshop and conferences

Poster Presentation

1. AMI-2015 held at JNU, New Delhi.
2. AMI-2017 held at BBAU, Lucknow.
3. 3rd Lucknow Science congress (LUSCON-2015) held at BBAU, Lucknow.
4. International conference (ICRESE-2017) held on March 20-21, 2017 at SMVDU, Jammu.
5. International conference (ET&SD) held on 21-23 February, 2014 at BBAU, Lucknow
6. International conference (ABCD-2017) on October 27-28, 2017 at C.C.S. University, Meerut.

Workshop & Conferences attended

1. "7th Science Conclave", December, 08-12, 2014 at IIIT Allahabad (Reg. ID- SC7-BO-S-M-535091).
2. "Hands-On-Training for SEM, FTIR, FPLC and Ion Chromatography" held on February 18-20, 2015, USIC, BBAU, Lucknow.
3. Participated in the "103rd Indian Science Congress" held at University of Mysore, Mysore on January 03-07, 2016.
4. Participated in the "105rd Indian Science Congress" held at S.V. University, Tirupati on January 03-07, 2017.
5. IPRs in Agricultural research on August 30-31, 2017 at BBAU.
6. Seven days workshop "Gene cloning & Its Expression to produce GMOs" held at MUIT Lucknow on November 20th-26th, 2017.
7. 'Socio-environmental Dimensions of Rejuvenating Gomti River' on April 23rd 2018 at BBAU, Lucknow.
8. Hands-On-Workshop on Real-Time PCR held on July 17-18, 2018 at Molecular Biology Unit, IMS, BHU.

Professional Memberships

1. Life member of Association of Microbiologists of India (AMI: **4195-2015**)
2. Life member of Indian Science Congress Association (ISCA: **L27353**)

Energ. Ecol. Environ.
https://doi.org/10.1007/s40974-018-0103-1

ORIGINAL RESEARCH ARTICLE

Land use changes: a key ecological driver regulating methanotrophs abundance in upland soils

Shashank Tiwari[1] · **Chhatarpal Singh**[1] · **Jay Shankar Singh**[1]

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Received: 23 July 2018 / Revised: 26 August 2018 / Accepted: 12 September 2018

Abstract Land use changes have been recognized as one of the key ecological drivers in regulation of methane (CH_4) consumption from dry upland soils. This study investigated the impact of land use changes and different soil depths (0–10, 10–20 and 20–30 cm) on soil physico-chemical properties and methanotrophs abundance in dry tropical region of Vindhyan uplands. Four different land use types (agriculture land, mixed forest, savanna and natural forest) were selected for a comparative study. Among the different land uses and soil depths, results indicated significantly higher soil moisture (SM), organic-C, inorganic nutrients, water holding capacity (WHC) and methanotrophs abundance at 0–10 cm depth of natural forest compared to other sites. Across different land uses, number (8.11×10^7 *pmoA* copies g^{-1} of dry soil) of methanotrophs isolated from the natural forest soil was statistically higher than the soils of other land use types. The variations in *pmoA* gene numbers across different land uses and soil depths were significant ($P < 0.001$). The *pmoA* gene numbers were positively correlated with soil C/N ratio ($R^2 = 0.9233$) and moisture ($R^2 = 0.9675$) contents. The decreasing order of *pmoA* gene abundance across different land uses was natural forest > mixed forest > savanna > agriculture land. The result suggest that land use changes (conversion of natural forest to agricultural land) alter the major soil properties (SM, WHC, organic matter, C/N ratio, etc.) and significantly reduce the methanotrophs and *pmoA* gene numbers. The land use management practices (application of bio-fertilizers in place of chemical fertilizers), especially for the degraded agricultural soils, could be beneficial option to mitigate the negative impacts on soil methanotrophs and their CH_4 sink activity in the upland soil of Vindhyan region.

Keywords Dry tropical soils · Keystone species · Land use changes · Methanotrophs · Savanna

1 Introduction

The land use changes (LUC), an anthropogenic intervention may be considered as one of the major key drivers that may affect soil characteristics, microbial diversity and functioning of terrestrial ecosystems (Tian et al. 2017; Luneberg et al. 2018). The LUC is also responsible for microbial biodiversity disturbances (Haines-Young and Potschin 2009) and loss of soil microbial biomass of tropical forest soils (Lima et al. 2014; Qi et al. 2018). The conversion of forest cover, savanna vegetation and grasslands into cultivated land may lead to negative alterations of soil functioning (Raiesi 2017), microbial community structure (Li et al. 2015a, b) and the ecosystem functioning (Singh and Gupta 2016). The soil methanotrophs diversity and their abundance may contribute significantly in the methane (CH_4) consumption (Singh and Gupta 2016). Thus, the land uses may cause long lasting impacts on the plant species composition, soil nutrient status, soil microbial properties including soil methanotrophs abundance. However, the abundance of methanotrophs in altered soil characteristics due to land uses is almost unknown in the dry tropical soils of Vindhyan plateau. Therefore, it is important to find out in details about the impacts of land

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Catena 171 (2018) 485–493

Contents lists available at ScienceDirect

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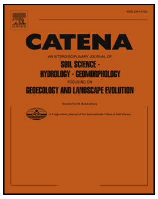

The effect of rice husk biochar on soil nutrient status, microbial biomass and paddy productivity of nutrient poor agriculture soils

Chhatarpal Singh[a], Shashank Tiwari[a], Vijai Kumar Gupta[b], Jay Shankar Singh[a,*]

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[b] Department of Chemistry and Biotechnology, ERA Chair of Green Chemistry, School of Science, Tallinn University of Technology, Akadeemia tee 15, 12618 Tallinn, Estonia

ARTICLE INFO

Keywords:
Ammonium-N
Soil microbial biomass
Nutrient poor soil
Paddy
Rice husk biochar

ABSTRACT

The study related to the effects of rice husk biochar (RHB) application on soil microbial aspects and paddy productivity in field condition is scare. Therefore, present study provides fresh insight into the effects of RHB on rice production in field conditions, with some updated information on soil microbial aspects. To study the impact of RHB and CSR-BIO (commercialized bio-formulation), on soil physico-chemical properties, soil microbial biomass (SMB) quantity and paddy productivity, four treatments were set up: control, RHB, CSR-BIO and RHB + CSR-BIO. The RHB with CSR-BIO both the amendments were applied at a rate of 10 t ha^{-1}. Across treatments, the water holding capacity, total -C, -N, -P concentrations and soil moisture content were statistically higher in RHB and CSR-BIO treated soils over the control. The highest SMB-C, -N and -P (408.66 ± 0.57, 83.33 ± 2.08 and 25.66 ± 1.52 μg g^{-1} dry soil, respectively) was recorded in RHB + CSR-BIO treated soil. Across the sampling dates, SMB-C, -N, -P and inorganic-N (ammonium- and nitrate-N) concentrations were minimum on 35 day after transplantation (DAT) (tillering stage-active growth period), and maximum on 105 DAT (maturity stage). The paddy plant growth variables (panicle length, tiller number, rice grain and paddy straw yields) were found higher in treated plots compared to untreated (control) plots, and varied significantly ($P \leq 0.001$) due to treatments. Among the various selected paddy agronomic variables, the application of RHB and CSR-BIO treatment was more pronounced to the yield of rice grains. Results indicate that an increase in the quantity of SMB due to RHB + CSR-BIO addition, improves the soil nutrient status and hence, paddy productivity in nutrient poor agriculture soils. It is suggested that RHB generation from rice husk biochar could be a sustainable crop residues waste management option to enhance the nutrient status, microbial biomass and paddy productivity of disturbed agriculture soils.

1. Introduction

India being an agriculture-dominant country produces > 500 million tons of crop residues annually. The residues of rice, wheat, cotton, maize, millet, sugarcane, jute, rapeseed-mustard and groundnut are typically burnt on-farm across different states of the India. A large portion of unused crop residues are burnt in the fields primarily to clear the left-over straw and stubbles after the harvest. The problem is more severe in the irrigated paddy agriculture, particularly in the mechanized rice-wheat system of the north-west India. Non-availability of labour, high cost of crop residues removal from the field and increasing use of combines in harvesting the crops are main reasons behind burning of huge crop residues in the fields. Burning of crop residues causes environmental pollution, hazardous to human health, produces greenhouse gases causing global warming and results in loss of beneficial soil microbial diversity and plant nutrients like N, P, K and S. Therefore, appropriate management of crop residues for agricultural use assumes a great significance. Recent research efforts have developed conservation agriculture-based crop management technologies which are more resource-efficient than the conventional practices. However, information about impact of RHB on soil nutrient status, microbial biomass and paddy productivity of nutrient poor agriculture soils is limited. This paper will provide valuable information and will generate awareness about the use of RHB generated from rice crop residues on soil microbial aspects and restoration of disturbed paddy agricultural soil productivity.

The Food and Agriculture Organization (FAO) suggested that by the year 2025, the world population (about 8.5 × 10^9 people), will require substantial enhancement in agricultural production to satisfy the demand (Timmusk et al., 2017). The soil fertility and agriculture

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https://doi.org/10.1016/j.catena.2018.07.042
Received 15 April 2018; Received in revised form 1 June 2018; Accepted 31 July 2018

Climate Change and Environmental Sustainability (April 2015) 3(1): 35-50
DOI: 10.5958/2320-642X.2015.00004.6

REVIEW ARTICLE

Methanotrophs and CH_4 sink: Effect of human activity and ecological perturbations

Shashank Tiwari[1] • **Jay Shankar Singh**[2]* • **Devendra Pratap Singh**[3]

Abstract Methane (CH_4) is the most potent greenhouse gas and contributes significantly in global warming phenomenon. The lifetime of this gas in the atmosphere is about 8 to 12 years. Therefore, its continuous increasing concentration in the atmosphere due to imbalances between genesis and oxidation is a great cause of concern. Methanotrophs has been recognized as the only efficient bio-agent responsible for CH_4 uptake at higher rate in upland arable soil. A number of environmental and anthropogenic perturbations affect the methanotrophs community composition and CH_4 sink phenomenon. The change in land-use patterns influences the methanotrophic diversity in the soil of different ecosystems. The changes in methanotrophic community composition may also have significant consequences for their potential capacity to respond to future changes in climatic conditions and the potential to restore CH_4 sink after degradation. Therefore, it is important to understand the effects of land degradation on soil methanotrophic community composition. However, the information related to this emerging environmental issue is scare. Therefore, in this article an attempt has been made to review on CH_4 consumption and methanotrophs numbers as affected by the diverse environmental and man-made perturbations.

Keywords Global warming, Methane, Methanotrophs, *Verrucomicrobia*, Land uses

1. Introduction

Methane (CH_4), a potent greenhouse gas (GHG), absorbs infrared terrestrial radiation thus trapping heat (Cicerone and Oremland, 1988) is 23 times more potent than CO_2 (He *et al.,* 2012b) contributing 15 to 20% of global warming (Bouwman, 1990; Hutsch, 2001; Intergovernmental Panel on Climate Change, 2001; Jang *et al.,* 2006; Intergovernmental Panel on Climate Change, 2007; Dalal and Allen, 2008). The concentration of CH_4 in the atmosphere is increasing due to discrepancy in CH_4 emanation and its removal. In the early 19th century, the atmospheric concentration of CH_4 was 700 ppb, but the current concentration is 1750 ppb and has shown a 1% yr^{-1} increase rate over a century (Intergovernmental Panel on Climate Change, 2001).

In global perspective, most of the atmospheric CH_4 is oxidized through chemical reactions with hydroxyl radicals (OH^-) in the tropospheric region, which comprises approximately 90% of the total Global CH_4 sinks (Schlesinger, 1997; Intergovernmental Panel on Climate Change, 2001; Hutsch, 2001). Aerobic upland soils are the second largest biological sink for CH_4 due to methanotrophic bacteria. Methanotrophs (methane-oxidising bacteria, MOB), the only biological sink of CH_4, play an important role by consuming significant amount of CH_4 into the atmosphere (Singh, 2011). The amount of CH_4 oxidised by soil methanotrophic bacteria is estimated to be between 10 and 40 Tg yr^{-1} comprises approximately 6-10% of the total CH_4 oxidation of the atmosphere (Intergovernmental Panel on Climate Change, 2001). About 10-30% of the CH_4 produced by methanogens in rice field is consumed by aerobic methanotrophs associated with the roots of paddy crop (King, 1997; Schlesinger, 1997; Intergovernmental Panel on Climate Change, 2001; Mohanty *et al.,* 2007). The numbers of methanotrophs in the soil of paddy fields across different countries is given in Figure 1.

There are several abiotic and biotic factors which affect the activities of CH_4 oxidising bacteria and also creating deep impact on the global CH_4 budget. The ecological distribution, diversity, and CH_4 oxidation activity of methanotrophs are affected by soil characteristics, pH, temperature, moisture, tillage, no-tillage and land use changes (Cole *et al.*, 1997; Kessavalou *et al.*, 1998; Knief *et al.*, 2005; Horz *et al.*, 2005; Mohanty *et al.*, 2007; Ussiri *et al.*, 2009; Singh *et al.*, 2010;

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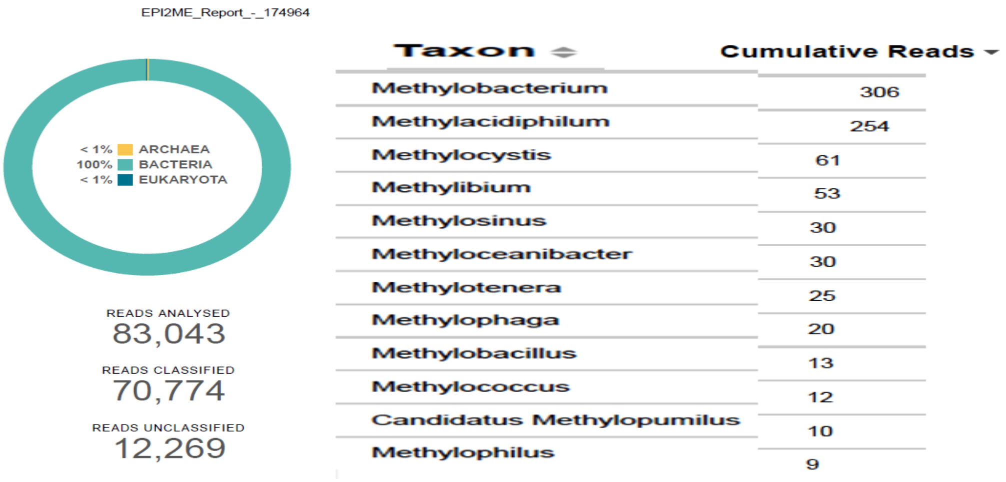
*Corresponding author E-mail id: jayshankar_1@yahoo.co.in

Appendix

Methanotrophic community structure

Agriculture land



EPI2ME_Report_-_174964

< 1% 🟨 ARCHAEA
100% 🟩 BACTERIA
< 1% 🟦 EUKARYOTA

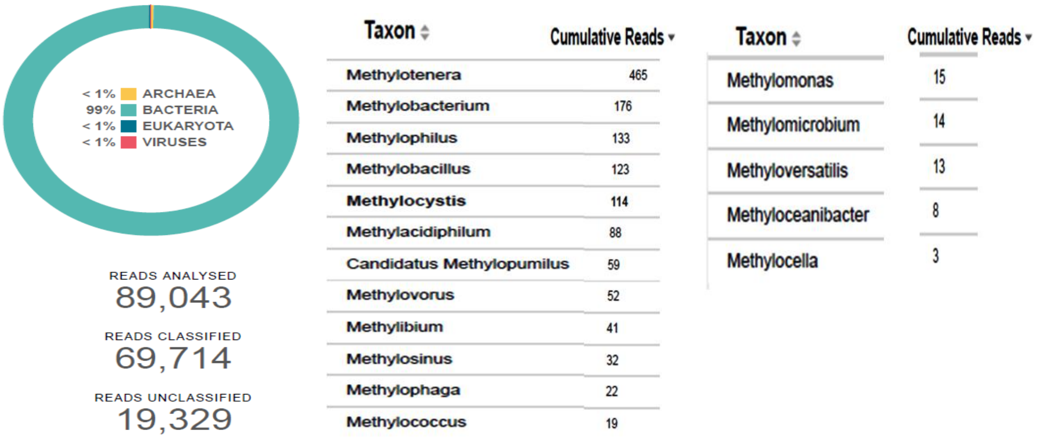
READS ANALYSED
83,043

READS CLASSIFIED
70,774

READS UNCLASSIFIED
12,269

Taxon	Cumulative Reads
Methylobacterium	306
Methylacidiphilum	254
Methylocystis	61
Methylibium	53
Methylosinus	30
Methyloceanibacter	30
Methylotenera	25
Methylophaga	20
Methylobacillus	13
Methylococcus	12
Candidatus Methylopumilus	10
Methylophilus	9

Natural forest



< 1% 🟨 ARCHAEA
99% 🟩 BACTERIA
< 1% 🟦 EUKARYOTA
< 1% 🟥 VIRUSES

READS ANALYSED
89,043

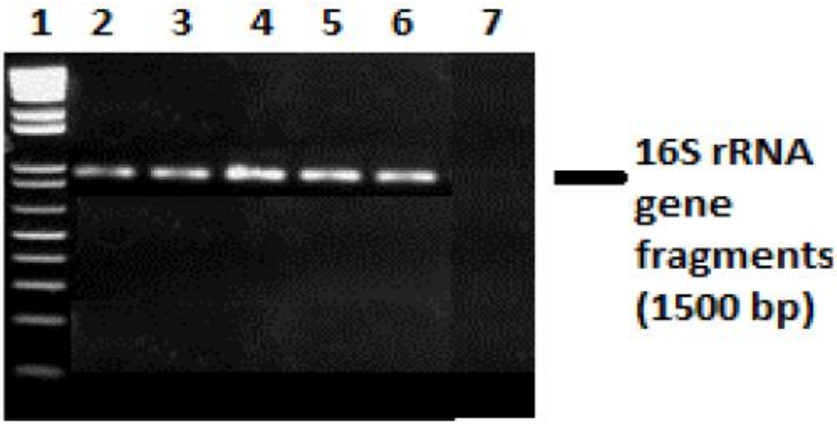
READS CLASSIFIED
69,714

READS UNCLASSIFIED
19,329

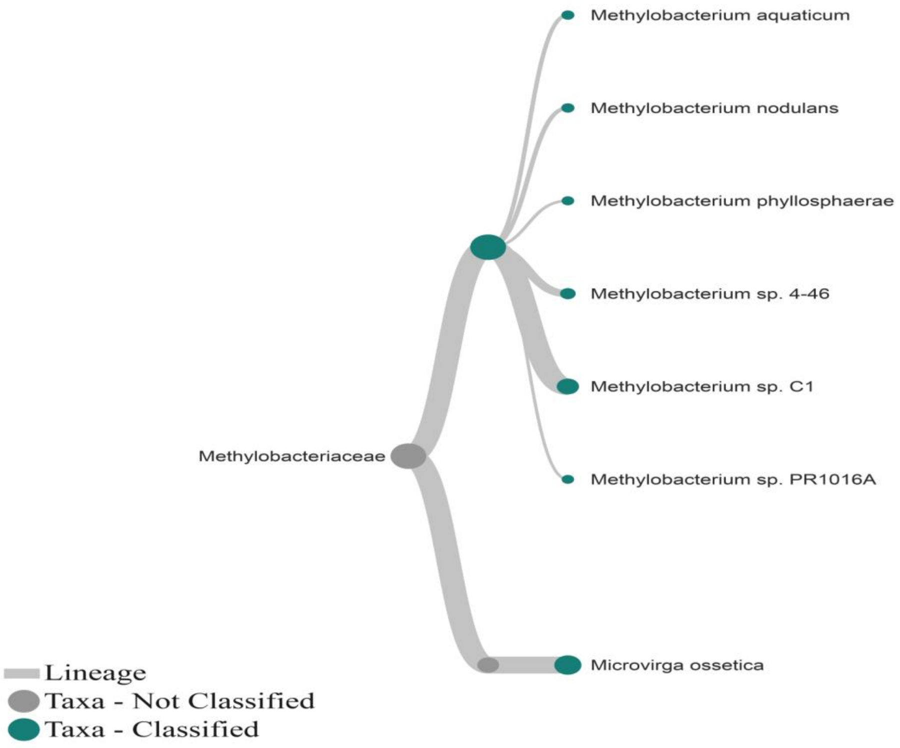
Taxon	Cumulative Reads
Methylotenera	465
Methylobacterium	176
Methylophilus	133
Methylobacillus	123
Methylocystis	114
Methylacidiphilum	88
Candidatus Methylopumilus	59
Methylovorus	52
Methylibium	41
Methylosinus	32
Methylophaga	22
Methylococcus	19

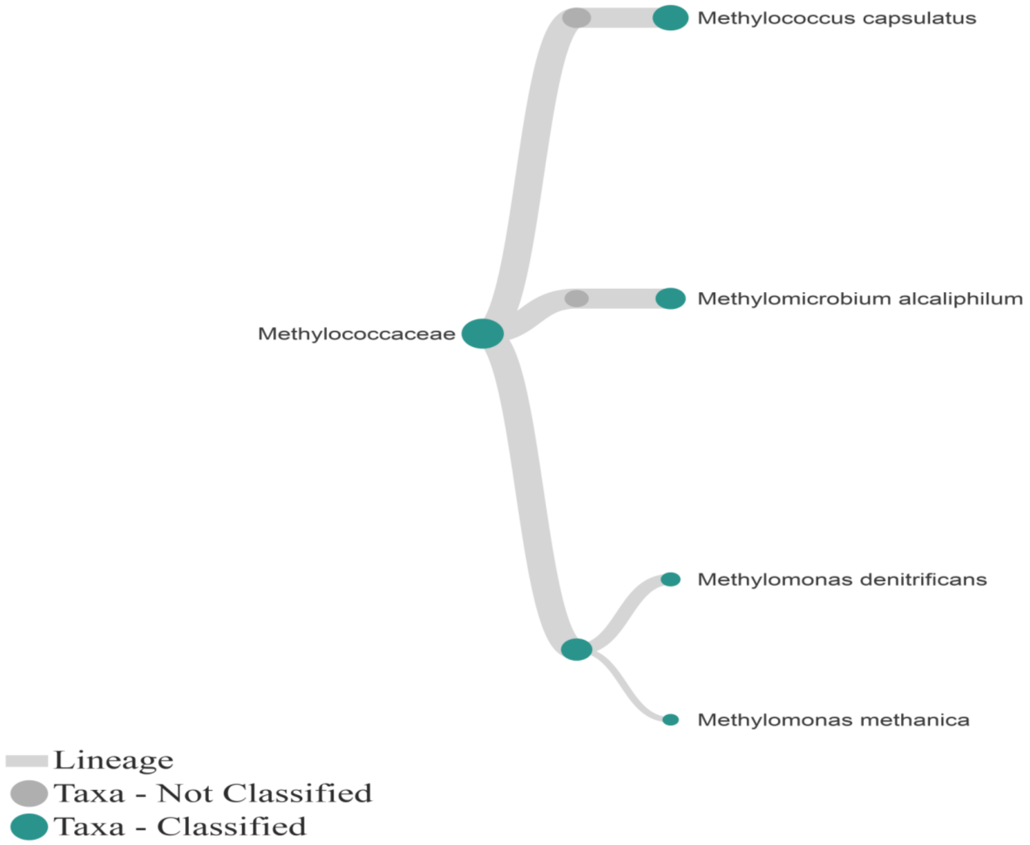
Taxon	Cumulative Reads
Methylomonas	15
Methylomicrobium	14
Methyloversatilis	13
Methyloceanibacter	8
Methylocella	3

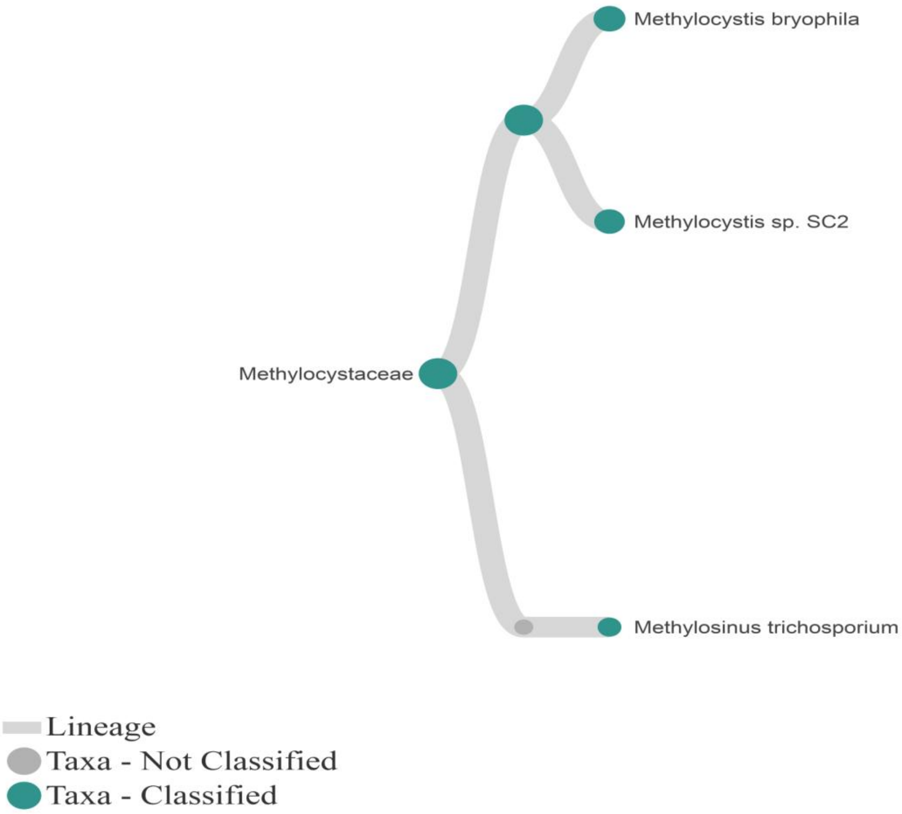
Gel Image



Phylogenetic Tree



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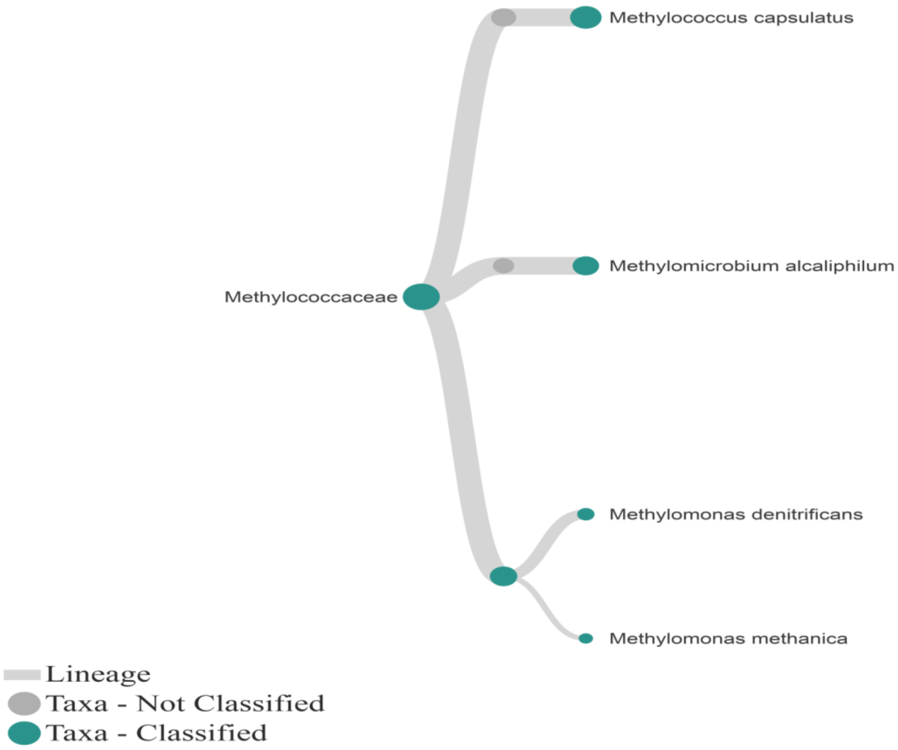
Methylocystis sp. SC2
Methylocystaceae
Methylosinus trichosporium
Lineage
Taxa - Not Classified
Taxa - Classified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Methylocystis sp. SC2
Methylocystaceae
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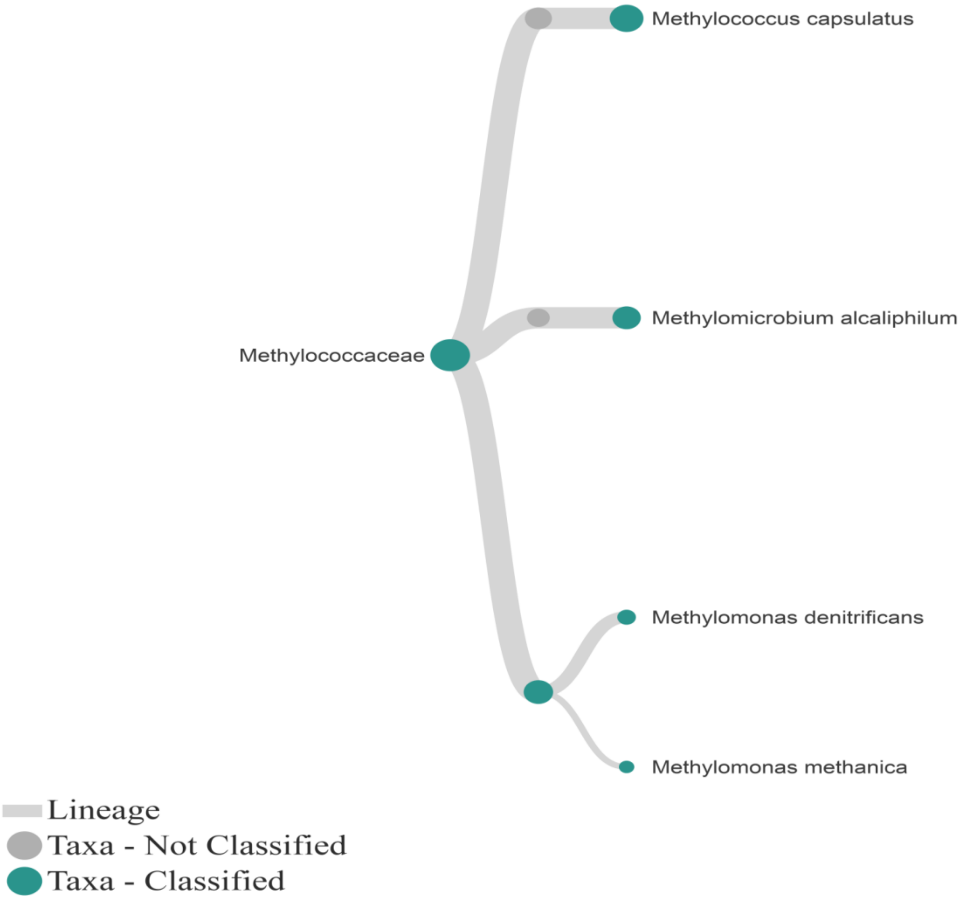
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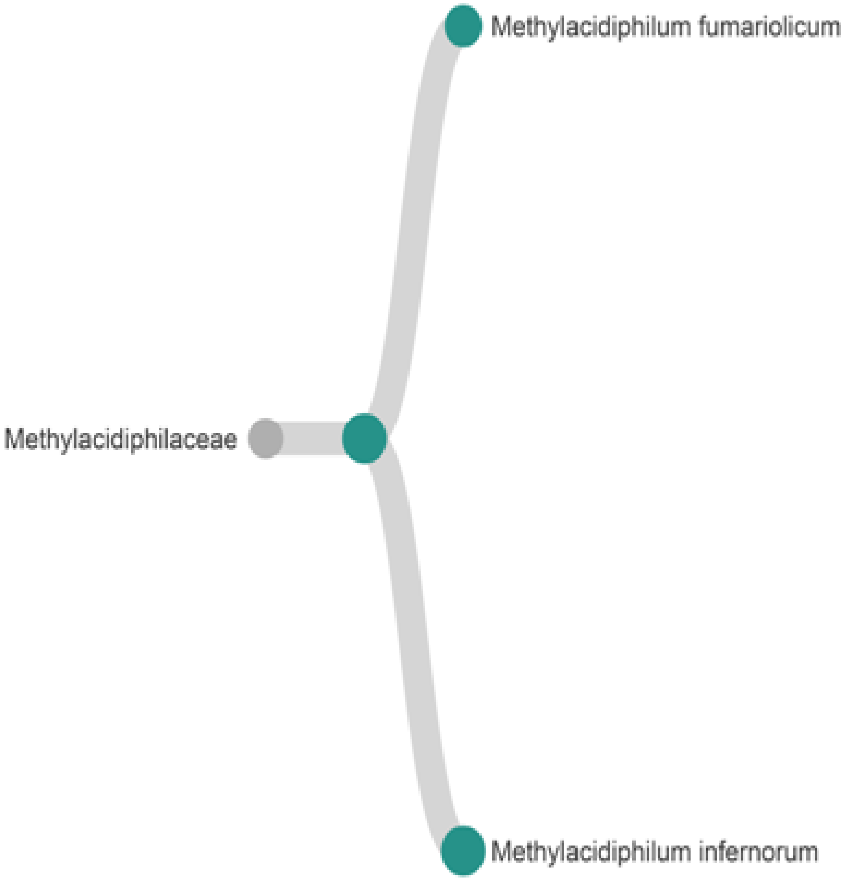
Taxa - Classified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Methylacidiphilaceae
Methylacidiphilum infernorum