IMPACT OF BIOCHAR AND CSR-BIO APPLICATION ON METHANOTROPHS, MICROBIAL BIOMASS AND PADDY YIELDS IN SALINE SOIL

THESIS

***Submitted* to**
Babasaheb Bhimrao Ambedkar University
(A Central University), Lucknow

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
LUCKNOW
प्रज्ञा शील करुणा
ESTABLISHED 1996

For the Degree of

Doctor of Philosophy
In
ENVIRONMENTAL MICROBIOLOGY

Submitted By

Chhatarpal Singh
(Enrolment No 187/10)

Under the Supervision of
Dr. Jay Shankar Singh

Department of Environmental Microbiology
(School for Environmental Sciences)
Babasaheb Bhimrao Ambedkar University
(A Central University)
Vidya Vihar, Rae Bareli Road, Lucknow-226 025
Uttar Pradesh, India

2018

CERTIFICATE

This is to certify that the Thesis entitled **"Impact of Biochar and CSR-BIO Application on Methanotrophs, Microbial Biomass and Paddy Yields in Saline Soil"** submitted by **Mr. Chhatarpal Singh** 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, Lucknow satisfies all the requirements as stipulated in the Doctor of Philosophy (PhD) regulation-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: 07/06/2018

Supervisor

7/6/18

(Head of the Department)

CANDIDATE'S DECLARATION

I, hereby declared that this Doctoral work entitled the **"Impact of Biochar and CSR-BIO Application on Methanotrophs, Microbial Biomass and Paddy Yields in Saline Soil"** submitted by me completely regular basis for the degree of **Doctor of Philosophy** in Environmental Microbiology to the Department of Environmental Microbiology, under the School for Environmental Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow is an outcome of my novel and original research work. I also declare that this thesis or any part thereof has not been submitted to any other university or institute for award of any other degree or diploma and also undertaken that the thesis is essentially free from all kinds of plagiarism.

chhatarpal singh

Date: 07/06/2018 **(Chhatarpal Singh)**

Place: Lucknow

CONTENTS

ACKNOWLEDGEMENTS

First of all I express my deep foremost thanks to my supervisor **Dr. Jay Shankar Singh**, Department of Environmental Microbiology, Babashaeb Bhimrao Ambedkar University, Lucknow for his extreme support and guidance during this doctoral work. He has devoted a good deal of attention, gave gracious advise, encouragement and has taken pains to ease out the difficulties during the entire phase of my research work. It has been pleasant and rewarding experience for me to work under his able guidance.

I avail this opportunity to express my heartfelt thanks to **Prof. Rajesh Kumar**, Head Department of Environmental Microbiology for providing infrastructure facilities, valuable suggestions and academic environment to complete this research objectives. I am deeply thankful to Prof. Ram Chandra for all kind of supports and encouragement to complete this research work. I avail this opportunity to express my heartfelt thanks to all the respected teachers, especially to Prof. N.K. Arora, Dr. V.S. Baghel, Dr. R.N. Bharagava and all newly appointed faculty of the department for their valuable suggestions.

It's my great pleasure to thank all the research scholars and seniors of the department for their continuous support and valuable guidance in every step of during the investigation as well as the preparation of thesis.

I am highly thankful to Professor Deepa H. Diwedi, Head, Departmentof Applied Plant Science of this university for providing the experimental field at Agricultural Research Farm of the university, to complete this Doctoral work.

I am also thankful to Dr. T. Damodaran, Principal Scientist, Discipline of Horticulture at Central Soil Salinity Research Institute (Regional Station), Lucknow,

Uttar Pradesh, India for providing CSR-BIO (a commercialised microbial bio-formulation) and valuable suggestions during my Doctoral work.

I wish to thankful to Dr. Ashish Yadav and his technical staff Aakaar Biotechnologies Private Limited, Lucknow to provide me lab facilities for completing the molecular analyses of soil methanotrophs community composition.

I am also thankful to soil science laboratory Biotech Park Lucknow and soil microbial laboratory Rajiv Gandhi South Campus, Banaras Hindu University Barkachha, Mirzapur, Uttar Pradesh- India, for the soil analysis. I would like to express my great full thanks to all technical staff of University Scientific Instrumentation Center (USIC) for their technical support during sample analysis.

My words will never be able to express my feeling for my family members involved and especially my parents for completion of my Ph.D. thesis who encouraged me during the moments of despair and extended all kinds of support to see my thesis completed.

I am extremely thankful to University Grant Commission (UGC) New Delhi, for providing financial assistant as fellowship during the research work.

At last, but not least, I would like to gratefully acknowledge to all the persons and institutions, those were directly or indirectly involved and helped during my Doctoral work.

Chhatarpal Singh

Date: 07/06/2018

(Chhatarpal Singh)

LIST OF ABBREVIATION & SYMBOL

%	Percentage
@	At
µmho ms^{-1}	Siemens
^0C	Degree centigrade
Al	Aluminium
ANOVA	Analysis of variance
BD	Bulk density
BLAST	Basic local alignment search tool
C	Carbon
C=O	Carbonyl group
Ca	Calcium
$CaCO_3$	Calcium carbonate
CEC	Cation exchange capacity
C-H	Methyl group
CH_4	Methane
$CHCl_3$	Chloroform
CH-OH	Organodiyl group
Cm	Centimetre
CO_2	Carbon dioxide
CSR-BIO	Central soil research-bio
Cu	Copper
DAT	Day after transplantation
DDBJ	DNA Data Bank of Japan
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxy-ribonucleotide triphosphate
dNTP	Deoxy-ribonucleotide triphosphate
EC	Electrical conductivity
EDX	Energy Dispersive X-ray
F	Forward
FAO	Food and agriculture organization
Fe	Iron
FTIR	Fourier-transform infrared spectroscopy

FYM	Farmyard manure
GDP	Gross domestic product
GHGs	Green house gases
G	Gram
GWP	Global warming potential
H	Hydrogen
H_2O	Water
H_2S	Hydrogen sulphide
HA	Humic acid
HNO_3	Nitric acid
hrs	Hours
HUR- 9-10	Hindu University Rice-9-10
IPCC	Intergovernmental Panel on Climate Change
K	Potassium
K_2SO_4	Potassium sulphate
kg	Kilogram
LSD	Least significant difference
Mg	Magnesium
$MgCl_2$	Magnesium chloride
MHB	Mycorrhizal helper bacteria
mL	Millilitre
Mm	Millimetre
Mn	Manganese
MOB	Methane oxidising bacteria
MPN	Most probable number
N	Nitrogen
N_2O	Nitrous oxide
Na	Sodium
No	Number
NCBI	NationalCentre for Biotechnology Information
N-H	Amine group
NH_4-N	Ammonium nitrogen
NMS	Nitrate mineral salts
NO_3-N	Nitrate nitrogen
O-H	Hydroxyl group
ONPG	O-nitrophenyl-beta-D-galactopyranoside

P	Phosphorus
PCR	Polymerase chain reaction
pH	Potential of hydrogen ion
pMMO	Particulate methane mono-oxygenase
Ppb	Parts per billion
Ppm	Parts per million
PSB	Phosphate solubilising bacteria
Pt	Platinum
RHB	Rice husk biochar
RNA	Ribonucleic acid
SEM	Scanning electron microscope
SM	Soil moisture
SMB-C	Soil microbial biomass-carbon
SMB-N	Soil microbial biomass-nitrogen
SMB-P	Soil microbial biomass-phosphorus
sMMO	Soluble methane mono-oxygenase
Tg	Teragram
USA	United states of America
viz.	Videlicet
WHC	Water holding capacity
WMO	World meteorological organization
WTR	Water treatment residual
yr^{-1}	Per year
Zn	Zinc
Zr	Zirconium
Mg	Microgram
µL	Microliter
Mm	Micrometre

1

Chapter-01
Introduction

CHAPTER-1

INTRODUCTION

India being an agriculture-dominant country produces more than 500 million tons of crop residues annually. The huge crop residues of rice, wheat, cotton, maize, millet, sugarcane, jute, rapeseed-mustard, groundnut, etc. are typically burnt on-farm across different states of the India. A large portion of unused crop residues are burnt every year in the fields primarily to clear the left-over straw and stubbles after the crop harvest. The problem is more severe in the irrigated paddy agriculture, particularly in the mechanized rice-wheat system of the north-west region of the country. Non availability of technically trained peoples, high cost of residue removal from the field and increasing use of combines in harvesting the crops, are main reasons behind burning of huge amount crop residues in the fields(Zhang *et al*., 2017). Burning of crop residues not only causes environmental pollution, hazardous to human health, produces greenhouse gases, but it also responsible for the loss of beneficial soil microbial community and abundance. Therefore, appropriate management strategies of crop residues to agricultural use may be assumed as a viable and sustainable option. It is need of the hour to develop recent research efforts in conservation of agriculture-based crop management technologies which may be more resource-efficient than the conventional practices.

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 food and energy demand (Timmusk *et al*., 2017). The soil fertility and agriculture productivity loss due to low precipitation, soil salinity, soil nutrient leaching and poor agricultural practices,

triggered interest to find out the ways for sustainable management of nutrient poor soils to enhance crop rather sustainable (Singh and Pandey, 2013)In general, the increase in crop productivity will lead to sustainable enhancement in the area of farming lands but also somewhat enriching the soil fertility of the existing agricultural land could be the viable option (Mekuria *et al.*, 2017). Therefore, it seems that application of amendments such as farm yard manures (FYM), microbial inoculants and biochar (consist of inherent mineral constituents) prepared from various crop residues, to nutrient poor soils could be one of the potential options to enhance the soil nutrient status, microbial diversity and agriculture productivity (Xu *et al*., 2017). It is suggested that the plant residues based biochar (10-15 t ha^{-1}), an alternative organic supplement to chemical fertilizers, could be a viable and sustainable way to enhance the crop yield (Agegnehua *et al*., 2017) in degraded nutrient poor soils (Kollah *et al*., 2015). From a long time, peoples were well interested in the use of plant residues derived biochar as soil conditioners to enhance the soil fertility and crop productivity (Zhang *et al*., 2017). It seems that the use of biochar in agriculture can reduced significantly the adverse impact of chemicalization on soil health (Kim *et al*., 2017), soil microbial biodiversity (Luo*et al*., 2017) and agriculture productivity (Agegnehua *et al*., 2017). However, information about the use of crop residues in agriculture use related to soil microbial aspects, soil fertility and crop productivity is insufficient. This work will provide some valuable information and will generate awareness about the use of rice crop residues in benefits of soil microbial aspects, soil physico-chemical properties and paddy agricultural productivity in disturbed nutrient poor soils.

It has been reported that the incorporation of rice husk biochar (RHB) derived from rice husk into the soil could significantly improve the soil physico-chemical

properties of paddy fields compared with direct application of rice husk (Knoblauch *et al*., 2011). Thus, RHB offers a good plant residues waste management to reduce air pollution due to on-site burning. As one of the crop residues in paddy field system, study shows clear evidences that biochar is highly effective for acidic and neutral soil, improve soil nutritional availability, plant growth and yield but not yet clearly know the effects of biochar amendment on nutrient poor tropical soils.

The soil microbial biomass (SMB) is not only an important labile fraction of the soil organic matter, but also a source of plant nutrients (Singh *et al*., 2009; De Souza Silva and Fay, 2012). Microbial biomass may or may not be influenced by addition of biochar to soil (Rousk *et al*., 2013). Both decreases (Dempster *et al*., 2012) and increases (Zhou *et al*., 2015) in SMB have been associated with biochar application to soil. Changes in SMB levels may well have effects on nutrient cycles or soil structure and, thus, indirectly affect plant growth (Warnock *et al*., 2007). Although soil nutrient status, seasonality, soil conditions, temperature, and other factors, are important drivers to control functioning of agro-ecosystems, SMB could be one of the vital factors affecting crop productivity cultivated in the tropical nutrient poor soils (Singh *et al*., 2010a). Recent advances in our understanding of biochar warrant an evaluation of the relationship between its impacts on the soil micro-biota and biomass. The role of biochar in soil biological processes therefore, represents a frontier in soil science research, with many unexplained phenomena awaiting exploration. While extensive research has been carried out concerning the effects of biochar application derived from plant residues on crop yields (Zhang *et al*., 2010; Wang *et al*., 2012) conversely there is very limited information on the effect of RHB application on the methanotrophs, SMB dynamics and paddy yields in nutrient poor soils. More studies on RHB application in paddy soil are obviously needed to better

understand its effects. We found that the addition of RHB can induce positive effects on soil physico-chemical properties, SMB dynamics paddy productivity and methanotrophs diversity. The RHB amendment to the paddy soil may significantly increase the abundance and diversity of methanotrophs and improve the soil fertility via enhancing the availability of inorganic-N (by N mineralization) nutrients and SMB pools as well as nitrogen mineralization in nutrient poor and saline soils.

Inorganic-N availability to crop plants is not only a primary step in soil organic N transformation, but is also, one of the most essential processes in soil N cycling (Zhang *et al*., 2017). During the paddy crop cycle more than 50% of N is absorbed by rice plants from the soil (Zhu, 1985). Inorganic-N release particularly ammonium-N, from the soil by mineralization is crucial to paddy crop N supply because paddy plants prefer ammonium-N as inorganic-N requirement. Various studies have shown the relationship between nitrogen mineralization and soil properties such as soil organic carbon, total nitrogen, pH and texture can affects N-mineralization process (Bregliani *et al*., 2010). During the process of microbial mediated N-mineralization, the organic-N is transformed in to inorganic-N (Schulten and Schnitzer, 1997). The pH and texture of the soil are important factors which affect the microbial community composition in paddy soil (Groffman *et al*., 1996; Pietri and Brookes, 2009; Dessureault-Rompre *et al*., 2010). Microorganism and some plant species used soluble organic nitrogen as labile source of N for their growth.

Increasing in loss of soil fertility and agriculture productivity due to low precipitation, soil salinity, soil nutrient leaching and poor agricultural practices has triggered the interest to find out the ways for sustainable management of nutrient poor soils to enhance crop productivity (Singh, 2018). In general the increase in agricultural yields should not be dependent on an increase in the cultivable surface

area but rather on enhanced production on existing agricultural land by enriching the soil productivity. In this context, the use of agriculture and animal based biochar may be one potential way of realizing this goal. Biochar being used as an alternative organic fertilizer additionally with chemical fertilizer for sustainable crop production in the agricultural sector have remarkable agronomic values and yield potential in degraded nutrient poor soils (Lashari *et al*., 2013). We have chosen biochar derived from rice husk to be used in this study. There is a growing interest in the use of RHB as a co-product produced from a controlled pyrolysis of dry rice husk, to enhance crop yields as well as soil conditioner (Lehmann and Rondon, 2006; Sohi *et al*., 2009; Kuppusamy *et al*., 2016). Therefore, in this way the use of chemical fertilizer can be reduced with the supplement application of biochar to reduce the need of chemical fertilizer due to bio-fortification and increases soil nutrient availability to crop plants. The soil disturbances may interferes with the agriculturally beneficial microbial community composition (Singh *et al*., 2016a; Singh *et al*., 2016b; Singh and Strong, 2016c) and thus soil nutrient cycling might be affected (Singh *et al*., 2010a). Therefore, application of biochar and microbial inoculants amendments may cause primary changes in physico-chemical properties such as organic-C, -N -P, water holding capacity, bulk density, etc. of soil. For instance, an enhancement in crops yields, mainly in salinity holding soil having low soil organic matter level have been reported (Prommer *et al.*, 2014). According to Prommer *et al.* (2014) biochar application increased total soil organic carbon but decreased the extractable organic C pool and soil nitrate. Although gross organic-N transformation rates were reduced by 50–80 %, the gross N-mineralization process remains unaffected but Ball *et al*. (2010) reported that biochar application increases the ammonia-oxidizers microbial population in soil and subsequently more than two times higher inorganic-N rates was

observed. Biochar can enhance the rate of N-mineralization by nitrogen-mediated bacterial community as well as ammonia-oxidizing bacterial community composition in paddy soil (Ball *et al.*, 2010). The higher doses of biochar application in soil accelerated also the increment of ammonium contents in soil (Singh *et al.*, 2017a). Single or combined application of biochar in combination with any organic fertilizer may increase the soil organic-N which may enhance the soil C-sequestration and thereby, could play a significant role in future environmental management planning to reduce the effect of global warming due to less CH_4 emission (Prommer *et al.*, 2014). Based on the above arguments it seems that though, a number of investigations has been conducted with reference to the management of soil productivity by the application of various amendments. But impact of RHB use, generated from paddy rice husk biochar, on soil physico-chemical conditions in paddy field condition are lacking.

Paddy is the world's most important food crop as it feeds more than half the global population however its cultivation contributes a major share in national gross domestic product (GDP) and export rises the earning in several developing countries including India. India and china, both are major rice producing countries in the world therefore both are major contributors of global CH_4 budget. The CH_4 is one of the most widespread green house gas (GHG) emitted from several other sources instead of paddy fields such as wetlands, ruminants, coal mines as well as anthropogenic activities such as leakage from natural gas systems and the raising of livestock (IPCC, 1995; Giria, 2007). In the early 19^{th} 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 (Tiwari *et al.*, 2015). The concentration of CH_4 in the atmosphere is increasing due to discrepancy in CH_4 emanation and its removal (Singh,

2011). The lifetime of CH_4 in the atmosphere is 8-12 years, but it is more efficient in trapping radiation and 23-30 times more potential than CO_2 (Tiwari *et al*., 2015). The Global surface temperature has increased by 0.8 °C in the last 100 years and CH_4 also contributed to this phenomenon as a potent GHG (Hanson *et al*., 1996). Recent Global estimates of CH_4 emission rates from wetland rice fields ranged from 20 to 100 Tg yr^{-1}, which corresponds to 6-29% of the total annual anthropogenic CH_4 emission (Neue *et al*., 1993). The CH_4 affects the chemistry and oxidation capacity of the atmosphere by influencing concentrations of tropospheric ozone, hydroxyl radicals and carbon monoxide (Wahlen *et al*., 1989). The current burden of CH_4 in the atmosphere is approximately 4700 Tg yr^{-1} (Neue *et al*., 1993). The CH_4 is produced in flooded paddy soils by a group of bacteria called as methanogens (also called marshy soil bacteria) (Sass *et al*., 1990; Sass *et al*., 2009). According to Demisie *et al*. (2015) the processes of CH_4 emission is affected by soil texture, inorganic electron acceptors, soil physico-chemical properties and methanogenic population. The flooding rice fields restricts the oxygen supply to the soil, which may results in the anaerobic fermentation of soil organic matter and consequently release of sufficient amount of CH_4 to the atmosphere. From deeper layer of flooded soil CH_4 reaches to the atmosphere by diffusion, ebullition and through arrenchyma conduits of paddy plant. Flooded paddy cultivation has been considered as a is greater CH_4 producing practice due to anaerobic soil conditions because of unique anaerobic bacteria called as methanogens (Tiwari *et al*., 2015). About 10-12% of the total global anthropogenic emissions of GHGs in 2005 were due to flooded paddy cultivation as reported by Smith *et al*. (2007). Usually CH_4 emission is based on soil conditions, which can be strongly managed by some improving soil management practices. More than 90% of the world's rice is produced in Asia however India stand in second position in a total

world rice production and share 21.4 % rice production and as well as cover 28.5 % area of total world area. Global CH_4 emissions from flooded rice paddy soils are estimated to be 40-53 Tg yr^{-1}, which account for 6-10% of the total CH_4 emissions (Wassmann *et al*., 2000). The concentration of CH_4 in 2013 has been increased 253% since pre-industrial times (World Meteorological Organization WMO), 2014). Agricultural lands cover about 40-50% of the Earth's land surface and agriculture accounted for 10-12% of total Global anthropogenic emissions of GHG (IPCC, 2007). Significantly waterlogged paddy fields are produced considerable amount of CH_4 due to several soil physico-chemicals as well as biological activity of methanogens (Yagi *et al*., 1997; Wassman *et al*., 2000; Yan *et al*., 2000; Zou *et al*., 2007). According to Linquist *et al*. (2012) the global warming potential (GWP) of GHG emissions in paddy-ecosystems was about four times higher than either wheat or maize. Nevertheless, paddy is the main food crops which are cultivated by more than 50% of the world's farmers, and it is occupied almost 155 million ha of the world's area (Kogel-Knabner *et al*., 2010). In last 3-4 decades it has been seen that the world population increased very explosively due to unrestrained growth. Therefore, it is very serious and worried full subject to satisfying the food demand to all growing population while cultivating the paddy crop. There is a big challenge to develop eco-friendly, sustainable and cost effective paddy agricultural cultivation technologies to reduce the CH_4 emissions and satisfy the calorie demand by 1.29% annually fulfil growing population by the year 2025(Cassman *et al*., 2003). It is now well accepted that flooded paddy cultivation is the substantial source of CH_4 emissions therefore, there is need to management of paddy soil due to application of suitable soil amendments that may enhance the crop productivity and minimise the emissions of CH_4 to the atmosphere.

It has been well documented that biochar can be better substitute to minimizing CH_4 emission from paddy soil. Although, various studies are presented contradictory results of biochar application in soil and not shown clear demonstration of biochar in soil such as the effects of biochar on GHG emissions has so far been inconsistent and the well developed mechanisms are still not clear. But several studies have been found that the application of biochar reduced the emission of GHG in comparison of without amended soil, resulting in biochar amended soil GHG (CH_4, N_2O and CO_2) was measured less than un-amended soil. Moreover biochar functioning potential depends their type's of feedstock, pyrolysis temperature and soil nature etc. Van Zwieten *et al.* (2009) and Smith *et al.* (2010) reported that the carbon of CO_2 was measured in biochar treated soil found same as in biochar, and found that the level of CO_2 was increased in biochar amended soil. Luo *et al.* (2011) suggested that biochar additions to soil have the potential to decompose the organic matter which is present in soil. However, this CO_2 release pathway only represents a small fraction of the C contained with the biochar and does not involve in the phenomenon of carbon sequestration in soil.

Moreover some poor agricultural practices and several human activities affecting environment in the form of climate change due to emission of GHG. Some studies have indicated that biochar can play a significant role to reducing GHG emissions directly by sequestering carbon and or indirectly by improving soil fertility and increasing methanotrophic diversity (Lehmann and Joseph, 2009; Sohi *et al*., 2010; Lehmann *et al*., 2011). But the role of biochar on GHG is a very inconsistent in paddy field due to the several variable results was found by authors on biochar and GHG in field studies. However most of the literature and current field trail studies to indicate and support the positive effect of biochar in the agricultural soil and reducing

GHG emission and enhancing the grain yields in nutrient deprived soils. Application of biochar in soil can increase or reduce the emission of CO_2 (Liu *et al*.,2011; Yoo and Kang, 2012; Wang *et al*.,2012. According to Feng *et al*.(2011) and Yoo and Kang (2012) the CH_4 emissions were reduced after biochar application in soil compared to control. But Knoblauch *et al*. (2008) reported that no significant effect was observed on the emission of CH_4 in treated soils (Zhang *et al*., 2010). According to Xiang *et al*.(2015) the earlier study based on wheat-derived biochar did not noticeably reduce N_2O emission from paddy field. Majority of authors working in the area of biochar and GHG emissions found that biochar can reduced the emission of GHG in biochar amended paddy soils (Zhang *et al*., 2010; Wang *et al*.,2012; Zhang *et al*.,2012; Singla and Inubushi, 2014). The significant role of biochar in mitigation GHG has been revealed after studying the application in field condition (Knoblauch *et al*., 2008; Zhang *et al*., 2010; Wang *et al*., 2012; Feng *et al*., 2012). Biochar may reduce the N_2O emission due to denitrification by increasing the aeration level in between soil particles and WHC (Van Zwieten *et al*., 2010a). So, these are important factors to change both the N_2O/ (N_2 + N_2O) ratio and the total nitrogen (N) due to higher aeration in side soil particles as well as higher water absorbing capacity to improve the aeration of soil (Yanai *et al*., 2007; Quin *et al*., 2015). However, studies related to biochar and N_2O emission not well clear, therefore, studies describing that the addition of biochar to soils are warranted in the field and lab conditions (Clough *et al*., 2010; Scheer *et al*., 2011). The application of biochar in soil changes the soil physical properties (soil pH, WHC, BD, moisture content of soil) and increases the soil aeration and soil N_2O emissions however, further deep investigationsare required to analyse all these soil parameters. In this way, the effects of biochar amendment on CH_4 fluxes are not properly clarify and several controversial reports are available on

CH_4 fluxes (Knoblauch *et al.*, 2011; Zhang *et al.*, 2012b). Biochar can play vital role in soil carbon sequestration and improving soil physico-chemical characteristics (nutrient retention, nutrient availability, cation exchange capacity, etc.) and soil biological properties (increasing the soil microbial biomass and community compositions) therefore, all these parameters, contributing to the agronomic performance of plants growth and yield enhancement should be investigated in details (Lehmann, 2007; Roberts *et al.*, 2010; Van Zwieten *et al.*, 2010b). The considerable roles of biochar application however, depend on various others factors such as the soil nature and the water availability and the crop type.

It has been reported that the high porosity and surface area of biochar is beneficial in enhancing the soil microbial biomass, microbial diversity, soil aeration and protect the microbes from soil predators (Chen *et al.*, 2010; Liang *et al.*, 2010). The application of biochar has been found to increase the level of soil nutrients and reduce the soil nutrients leaching (Liang *et al.*, 2006; Laird *et al.*, 2010b). Thus, the application of biochar will enhance the nutrient and water retention capacity of soil to improve the soil health (Glaser *et al.*, 2001; Major *et al.*, 2010; Van Zwieten *et al.*, 2010a). At present, several works has been carried out on the application of biochar with reference to GHG mitigation and soil health improvement, but some facts yet not clear.Therefore, comprehensive and systematic studies have to be done on soil GHG emissions/consumptions, soil improvements at different seasons with the use of different types of biochar produced fromdifferent types of feedstock. Now application of biochar, produced as a by-product of pyrolysis from the crop residues and animal waste has been proposed as a beneficial soil amendment practice for mitigating the climate change and increasing the yields of agriculture crops through long-term carbon (C) sequestration in agriculture soil (Lehmann, 2007; Laird, 2008; Roberts *et*

al., 2010). Therefore, biochar has been declared as potential by-product derived frompyrolized biomass, to reduce the CH_4 emissions from agricultural soils through direct and indirect mechanisms. The soil amended by biochar produced less quantities of CH_4 than without amended soil (Sass *et al*., 1990; Lehmann, 2007; Sass *et al*., 2009).

The upland paddy agro-ecosystems symbolize a crucial environment for the balance of GHGs by the process of CH_4 consumption (Smith *et al*., 2008) due to methanotrophs (methane-oxidizing bacteria) which can oxidize CH_4 as sole source of carbon and energy for their growth. Methanotrophs, an important group of unique bacteria that consume CH_4 as carbon and energy source, for their growth and multiplication, can play an important role in removal of atmospheric CH_4 load. Several of study has been conducted from agriculture to terrestrial ecosystems related to ecology of soil methanotrophs population dynamics. But, use of RHB, prepared from paddy plant residues on community composition and population dynamics of methane consuming bacteria from paddy field soils are scare. Therefore, this study was conducted to assess the impact of RHB application on soil methanotrophs abundance in paddy agriculture field conditions. It is assumed that biochar application in soil may improve the soil conditions, crops yieldand water holding capacity and all these will support the growth of CH_4 consuming microorganisms (methanotrophs) in soils.

Biochar can persist for long a time in soil due to presence of recalcitrant carbon which is not easily decomposed by the soil microbes due to the highly aromatic nature and hence, is considered as most important tools for carbon sequestration (Chan and Xu, 2009; Yadav *et al*., 2017). Therefore, incorporation of biochar in agriculture soil can improve physico-chemical as well as biological

properties of the soil. Biochar has lagerinternal surface area therefore; its greater surface area can work as soil microbial habitats for agriculturally beneficial microorganism. According to Xu *et al.* (2012) the application of biochar increasescrop growth yield, improved soil quality, water retention, reduced nutrient leaching and soil acidity due to their eco-friendly nature. Various studies have shown that the incorporation of biochar single and combination with organic fertilizers and microbial bio-formulations can increases the crops yields and SMB (Xu *et al.*, 2012; Singh *et al.*, 2017a). The level of SMB in soil has beenconsidered as a soil fertility index in agro-ecosystem as well as forest-ecosystem (Khodadad *et al.*, 2011). So, the greater level of SMB in soil may be an indicator of a higher soil microbial diversity. The application of biochar in agricultural soil enhances the quantity of soil microbial biomass due to the large porosity as well as surface area of the biochar which may attract the beneficial microbial population as their shelters. A longer duration sustainability of biochar in soils can also promotes the microbes in soil to survive for a longer time because of sufficient nutrient levels of biochar in soil (Yadav *et al.*, 2017). It seems that biochar application in paddy soil can provide a multidimensional role in carbon sequestration, nutrient binding capacity of soil, highest nutrient availability to crop plants in soil and reduced the emission of GHGs in the atmosphere.

Sustainable degraded agriculture land management approaches such as organic farming, novel microbial inoculation with suitable bio-inoculant carriers have been considered as key tools for combating the loss of soil fertility and crop productivity (Seneviratne *et al.*, 2011). The inoculation of bio-filmed bio-fertilisers (BFBFs), developed from agriculturally important microbes, in combination with suitable amendments, has been demonstrated to speed up the restoration of degraded land soil

fertility within short period of time (Seneviratne and Kulasooriya, 2013; Singh *et al.*, 2016). Evidently, the direct inoculation of beneficial microbial consortia in combination with suitable supporting soil amendments/carrier material can be a viable and new efficient tool to contribute significantly in enhancement of microbial density and biomass, which can help considerably to agro-ecosystem sustainability and crop productivity (Seneviratne, 2012; Singh, 2015). Therefore, in this experiment, the CSR-BIO, a commercial bio-formulation consortia prepared from agriculturally beneficial microbes (Damodaran *et al.*, 2013), was used with cow dung manure (as carrier material) and RHB as soil conditioner. We hypothesized that the addition of RHB with CSR-BIO mixture will have positive effects on soil physico-chemical properties, methanotrophs abundance, SMB levels and paddy productivity. It may also be further assumed that the application of RHB in combination with novel microbial bio-formulation mixture (CSR-BIO) would increase synergistically the soil available inorganic-N nutrients to paddy plants in nutrient deprived soils. However, the experimental evidences and answers for the above raised arguments and questions in field conditions are still to be investigated. Therefore, this study focused on impact of RHB application in combination of microbial bio-formulation-CSR-BIO (consortia of *Bacillus pumilus*, *Bacillus thurigensis*, and *Trichoderma harzianam*) (Damodaran *et al.*, 2013) on soil physico-chemical properties, paddy crop yields, SMB, and methanotrophs diversity and abundance in paddy field.

Since, application of RHB in combination with suitable commercialized microbial bio-formulation (CSR-BIO) as supporting amendment, from agriculture paddy filed conditions are lacking therefore, to find out the answers of above raised questions, the present doctoral research work has been carried out with the following objectives:

1. To assess the impact of CSR-BIO and Biochar application on soil physico-chemical properties of saline paddy fields.
2. To find out the influence of CSR-BIO and Biochar treatments on paddy yields.
3. To study the microbial biomass-C, -N and -P variations as affected by CSR-BIO and biochar amendments.
4. To assess the influence of CSR-BIO and Biochar amendments on methanotrophs abundance and diversity.

2

Chapter-02
Review of Literature

CHAPTER-2

REVIEW OF LITERATURE

The Earth surface temperature has been increased at the rate of 0.8 °C in the last one century (Hansen *et al*., 2010). The green house gases (GHGs) are the main culprits for increasing the temperature of our planet. For this serious Global problem, highest contribution of anthropogenic activities has been involved for the emission of GHGs (carbon dioxide (CO_2), nitrous oxide (N_2O) and CH_4). Further increases in these GHGs are expected and may cause adverse effects on living beings and biodiversity (Parry *et al*., 2007). Therefore, effective and sustainable tools and technologiesshould be developingto reduce the further emissions of GHG. Mismanaged agricultural practices and land use changes are dominantly responsible for the increasing concentration of GHGsin the atmosphere (Solomon *et al*., 2007a). These GHGs are responsible forthe adsorption and emissions of solar radiation within the thermal infrared range (IPCC, 2007). Several other gases such as halogens, hydrocarbons and aerosols may also increase or decrease the warming effect of GHG (Pachauri and Reisinger, 2007). The current growing problemslike global warming, climate change, drought, biodiversity loss, etc. are due to the uncontrolled emissions of GHG therefore, it urgently needs to develop some viable technologies and agricultural practices that can contribute significantly in the reduction of GHGs emissions. At present the concentration of CO_2 has reached at 396 ppm, compared to 280 ppm in pre-industrial times (Tans and Keeling, 2012). Per year CO_2 concentration is rising at the rate of 3 ppm (Tans and Keeling, 2012). Usually CO_2 is produced by different anthropogenic activities including poor agricultural practices, fossil fuel burning and land use change, etc. (Guo and Gifford, 2002; Solomon *et al*., 2007a). The persistence

of second most effective GHG, N_2O in the atmosphere is raised to 332 ppb in comparison to 270 ppb in pre-industrial times (EPA, 2012). It has been reported that the N_2O contribute every year 6% radiation on the Earth because of anthropogenic sources (Davidson, 2009; Canfield *et al*., 2010). The atmospheric concentration has been increased compared to last few decades (approximately 0.8 ppb $year^{-1}$) therefore, approximately 40% of emissions being caused by human being (Solomon *et al*., 2007b). The CH_4 is the most potent GHG and this is 25-30 times more powerful than CO_2 to contribute in global warming problems. In 2005, the average atmospheric concentration of CH_4 was measured 1.8 ppm (Le Mer and Roger, 2001; Solomon *et al*., 2007b). This trend is indicated to the imbalance between sources and sinks of CH_4 fluxes (Wuebbles and Hayhoe, 2002).

Rising temperature around the Earth's surface seems to be a Global threat to living beings at the moment. The problem is directly associated with the increasing atmospheric level of several GHGs, such as water vapour, CO_2, CH_4, N_2O, etc (IPCC, 2007). Among these GHGs, CH_4 is the second largest GHG after CO_2 (EPA, 2010; Krause *et al*., 2010; Li *et al*., 2013), present in the Earth's atmosphere with mixing ratio ~1.8 ppm. Though, the atmospheric concentration of CH_4 is extremely less than CO_2 (IPCC, 2007) but it is more efficient in trapping the sun radiation than CO_2.The Global warming potential (GWP) of CH_4 is 23-30 times more than CO_2, with a time horizon of 100 years (Solomon *et al*., 2007; Siljanen *et al*., 2011; Pandey *et al*., 2014). Further, it has been pointed out that it is 62 times potent that that of CO_2 (Houghton et al. 1995; Phillips *et al*., 2001), accounting about 15-20% of the Global warming effect (Phillips *et al*., 2001; Wuebbles and Hayhoe, 2002; Jang *et al*., 2006; IPCC, 2007; Dalal and Allen, 2008; Tiwari *et al*., 2015). Being highly reactive in nature, CH_4 affects the chemistry and oxidation capacity of the environment by influencing

the level of CO, O^- and tropospheric ozone, etc. (Cicerone and Oremland, 1998). Global atmospheric concentration of CH_4 has almost tripled since pre-industrial times (Krause *et al*., 2010) and is about ~700 to ~1800 ppb, increasing at the rate of 0.5-1% $year^{-1}$ (Tamai *et al*., 2007; Tiwari *et al*., 2015). The concentration of CH_4 in the atmosphere is increasing due to an imbalance between CH_4 production and consumption. The annual increment of CH_4 into the atmosphere was 180 Tg yr^{-1} estimated by analysing air trapped in polar ice during the 15^{th} century and 200 Tg yr^{-1} at the dawn of the 18^{th} century (Khalil and Rasmussen, 1994; Mer and Roger, 2001). But the current estimates demonstrates that the concentration of CH_4 was above 300 Tg in the 20^{th} century, and now it has been reached up to the level of 678 Tg (IPCC, 2000; Mer and Roger, 2001; Hill *et al*., 2016). The CH_4 is emanated into the environment by several natural (wetlands, termites, oceans, sediments, volcanoes, wildfires, etc.) and anthropogenic sources (rice fields, landfills, land use, eutrophication of lakes, leakage of gas, coal mines, fossil fuel combustion, petroleum industry, enteric fermentation, biomass burning, landfills, animal waste, domestic sewage, etc.) (Tiwari *et al*., 2015).

2.1 Paddy production and CH_4 emission

Paddy rice, second world largest agricultural crops is feeding by 50 % population in the world. Globally, China and India are the two major paddy producing nations in the world. It has been pointed out that in India West Bengal, Bihar, Uttar Pradesh, Punjab Haryana are the leading rice producing state of India for a long time. The total harvested area of paddy has increased from 86 to 144 million hectare during the year 1935-1985. Paddy is an annual or biannual crop of India and mainly grown at 25-30 ^0C in humid areasreceiving plenty amount of rainfall annually. Paddy cultivation is highly water demanding crop and therefore, flooded paddy cultivation area is

dominant in the country. However, some dry land rice varieties, which can be cultivated in dry regions and required very less amount of rains compared to flooded paddy varieties (Singh *et al*., 2010). In most part of the country, the paddy cultivation depends on natural rainfall, but it is also being cultivated through irrigation in those areas, receiving comparatively less amount of rainfall annually. Flooded paddy cultivation is one of the major anthropogenic sources of CH_4 emission at global scale. Every CH_4 budget assessment has shown that paddy fields are a significant fraction of the Globalemissions and an important contributor to the increasing concentration of atmospheric CH_4 (Khalil and Shearer, 2006). Annual release rates of CH_4 from paddies are estimated to range between 31 and 112 Tg per year, which contribute about 5-19% of the total Global CH_4 emissions (IPCC, 2007). In flooded paddy cultivation a large amount of CH_4 are produced via anaerobic soil conditions because of *Archebacteria* known as methanogens. Both environmental factors and soil conditions, such as organic matter content, pH, and moisture content, have an impact on CH_4 production (Yang and Chang, 1998; Liu and Wu, 2004). CH_4 production in soils can occur when organic matter is degraded anaerobically (Oremland, 1988; Conrad, 1989; Svensson and Sundh, 1992). Several bacteria that degrade organic material via a complex pathway are needed to demonstrate this phenomenon but the final step is performed by methanogens (CH_4 producing bacteria). Methanogenic bacteria can use a limited number of substrates, of which acetate and hydrogen are considered the most important ones in fresh water systems (Lovley and Klug, 1983; Goodwin and Zeikus, 1987; Yavitt and Lang, 1990; Peters and Conrad, 1996). Other substrates have never been shown to be responsible for more than 5% of the CH_4 production. Acetate and hydrogen are formed by fermentation from hydrolysed organic matter (Dolfing, 1988). Alternative electron acceptors suppress CH_4

production, which is most easily understood from thermodynamics (Zehnder and Stumm, 1988).

Application of biochar in paddy agriculture fields can be a cost effective and sustainable practice that can reduce CH_4 emission and mitigate the effect of Global warming and climate change (Singh *et al*., 2017). Biochar, namely biomass-derived char, refers to the highly aromatic substance remaining after thermal decomposition of biomass under complete or partial exclusion of oxygen. It has been reported that when biochar is applied to the soil, it acts against the decomposition process and creates aerobic soil conditions (Liang *et al*., 2006). Applying biochar to soil has been proposed as a means of long-term carbon (C) storage (Lehmann *et al*., 2006; Fowles, 2007; Lehmann, 2007) and may be a promising and revolutionary approach for capturing and sequestering C worldwide. Published results suggest that biochar application in paddy soils can play a significant role in reducing GHGs emissions (Rondon *et al*., 2005; Renner, 2007; Yanai *et al*., 2007), minimising pesticide and nutrient leaching loss (Hua *et al*., 2009; Wang *et al*., 2010; Zhang *et al*., 2010b), improving soil fertility and boosting crop yield and plant growth (Glaser *et al*., 2000; Major *et al*., 2005; Steiner *et al*., 2007; McHenry, 2009). For example, CH_4 emissions were almost completely suppressed in biochar-amended (20 g kg^{-1}) acidic soils in the Eastern Colombian Plains (Rondon *et al*., 2005). During an experiment Knoblauch *et al*. (2008) reported no significant changes in CH_4 production from a calcaric fluvisol soil amended with biochar. Another study showed that biochar amendment significantly reduced total indirect CO_2 production while increasing CH_4 emissions from paddy soil (Zhang *et al*., 2010a). To date, it appears that amounts of CH_4 emissions will depend on the physical and chemical properties of the biochar, type of the soils, microbiological circumstances, as well as water and fertilizer management

(Cai *et al*., 1997; Xiong *et al*., 2007; Zou *et al*., 2007; Zwieten *et al*., 2009). Therefore, the type and contents of soil organic matter, which directly affects the production of CH_4 (Liu and Wu, 2004), can be managed by adding both biochar and other organic matter produced from crop residues. Furthermore, the structural and chemical properties of the biochar itself may be potential driving factors affecting methanogenic activity in paddy soils. Generally, the characteristics of biochar depend mainly on the type of the feedstock and pyrolysis conditions, such as temperature, time duration, and air supply. Biochar can be produced from a wide range of biomass sources, such as shrubs, crop residues (rice husk), green waste and livestock manures. With a 10–30% annual increase in biomass accumulation, bamboo can be selectively harvested and regenerated without replanting, making it an attractive feedstock for biochar production. In addition, large quantities of crop residues, such as rice and wheat straw, produced by China, India and other leading counties annually, can be exploited for the agriculture use. It has been observed that the decomposition of the crop residues when applied directly in flooded paddy field always results in an increase in CH_4 and CO_2 evolution by enhancing heterotrophic activities (Yagi and Minami, 1990; Neue *et al*., 1996). Hence, conversion of these crop residues waste to biochar prior to their direct addition to the rice paddy can be sustainable agriculture management practices that can potentially reduce the production of CH_4. In order to provide both theoretical and practical information useful for supporting the use of biochar in agriculture, several laboratory based studies have been carried out on the effects of biochar on CH_4 and CO_2 emissions from a water logged paddy soil (Singh *et al*., 2017). It appears that biochar produced from crop resides could reduce GHGs emissions from a flooded paddy soil and invites the following research objectives (1) to compare the effects of different biochars produced from bamboo, rice and wheat

straw, rice husk and other crop residues chars, on CH_4 emissions, soil physic-chemical properties, microbial aspects and crop productivity, (2) to investigate the potential mechanisms of biocharapplication in soils that effects soil and crop plant interactions in detail.

2.2 Methanogens (CH_4 producing bacteria)

Methanogenic *Archaea* (methanogens) are strictly anaerobic microbes, plays a vital role in anoxic environments of paddy soil by performing the last step of the anaerobic decomposition of organic matter mineralization into CH_4 and CO_2 (Conard *et al*., 1999). Methanogens utilize acetate (contributes about 80% to CH_4 production) as a carbon substrate, but another substrate like H_2/CO_2 are also used and contributes to 10-30% for CH_4 production (Conard *et al.,* 1995). According to Palmer *et al.* (1993) *Methanobacteriales, Methanococcales* and *Methanomicrobiales*are important order of methanogens which showed to fix molecular nitrogen into inorganic nitrogen because they have *nif* genes. Organic matter of flooded paddy soil mainly contains plant residues material which creates anaerobic layersto the paddy soil and consequently generation of CH_4 by bacterial decomposition of organic matter (Danenberg *et al*., 1999; Dubey, 2011). The anaerobic degradation of organic matter hydrolyzes polymers, initiates fermentations form simple organic compounds, and finally CH_4 formation from H_2/CO_2, acetate, methylated compounds or alcohols and CO_2 (Yao *et al*., 2001). Methanogenesis from all these substrates requires some unique coenzymes, exclusively found in methanogens (Ludmila *et al*., 1998). At least nine methanogens-specific enzymes are involved in the pathway of CH_4 formation from H_2 and CO_2 (Shima, 1998). The acetate and H_2 are the two main intermediate precursors for CH_4 formation in paddy soils by methanogens (Yao *et al*., 1999).

Keppler *et al*. (2006) demonstrated that significant amount CH_4 produced from terrestrial plants and detached leaves under oxic conditions globally. According to experiments, they assumed that living plants and plant litter produce 62–236 Tg yr^{-1} and 1–7 Tg yr^{-1} CH_4, respectively. Natural sources are accountable for about 30% (up to160 Tg yr^{-1}) of the CH_4 flux; however the anthropogenic sources are responsible for contributing 70% (up to 375 Tg yr^{-1}) (Mer and Roger, 2001). Mostly, the atmospheric CH_4 produced biologically (70-80 %) (Mer and Roger, 2001).The CH_4 is emitted in an anaerobic environment by methanogenic decomposition of organic matter.

In global perspective, most of the atmospheric CH_4 is removed from the environment through chemical reactions with hydroxyl radicals (OH^-) in the troposphere ($CH_4 + OH^- \rightarrow CH_3^- + H_2O$), and in stratosphere CH_4 reacts with the chlorine originated from CFCs (Chlorofluorocarbons) ($CH_4 + Cl^- \rightarrow HCl + CH_3^-$.) which involve around 90% of the total Global CH_4 sinks (Schlesinger, 1997; Hutsch, 2001, Mer and Roger, 2001; Tiwari *et al*., 2015). Mer and Roger (2001) stated that if equilibrium between methanogens CH_4 emission and methanotrophs CH_4 oxidation in an ecosystem is positive, the systems may be a CH_4 source and if the equilibrium is negative the systemsmay acts as a CH_4 sink.

2.3 Methanotrophs (methane-oxidizing bacteria) and CH_4 consumption

In the global CH_4 cycle, CH_4 is consumed by chemical and biological processes. About 90% chemical destruction of CH_4 in the atmosphere occurs through reaction with free hydroxyl radicals (OH^{-1}) (Kheshgi *et al*., 1999). The only known biological sink for atmospheric CH_4 is its oxidation in aerobic soil by methanotrophic bacteria (Hutsch *et al*., 1994; Kolb *et al*., 2005). This sink activity in soil can contribute up to 15% to the total CH_4 destruction (Born *et al*., 1990).

Donald *et al.* (2005) studied the methanotrophic population in Newport bay Estuary, Southern California and concluded that this environment contains significant methanotrophic diversity. The recent advances in understanding the eco-physiological role and structure –function features of methanotrophic bacteria living in various cold ecosystems indicated that the occurrence of methanotrophs in a majority of psychrosphere site was detected (Trotsenko and Khmelenina, 2006). The study of Mohanty *et al. (2006)* from rice field and forest soils incubated in micro-cosmos and supplemented with different fertilizers and CH_4 concentrations and concluded that in both the sites, the activity as well as growth rate of CH_4-consumig bacteria was affected significantly. At present, a number of researchers have conducted molecular investigations on methanotrophs from different ecosystems (Bodelier *et al.*, 2000; Eller and Frenzel, 2001; Heyer *et al*., 2002; Hasin *et al.,* 2010).

In India, very limited investigations have been carried out to monitor site-specific methanogenesis, CH_4 consumption and the population abundance of related micro-flora from paddy agriculture soil and other terrestrial ecosystems. The concern of such studies was mainly driven by environmental aspects of estimation of CH_4, which is highly potent greenhouse trace gas due to its absorption ability of infrared radiation from the sun. The investigation on CH_4 flux measurement from dry tropical forest ecosystem indicated that the soil of these ecosystems acts as strong CH_4 sink. Plant induced spatial variation in the size of methanotrophic population in dry and flooded rice fields was reported by Dubey and Singh (2001). Late Professor A.K. Kashyap, Department of Botany, BHU was actively involved to study the environmental problems related to ecology of methanotrophs from agro-ecosystems and published the data in various international journals of repute.

Our research group reported that pyrite and farm yard manure application significantly affected the population of methanotrophs in the soil of saline rice fields (Singh *et al*., 2010a). The research report suggested that the restoration of degraded forest ecosystems or unused degraded land may significantly contribute to the recovery of methanotrophic activity in the soil and thereby the soil CH_4 sink potential (Singh and Singh, 2012). Our research group have emphasized the role of methanotrophic bacteria as only known biological sink of CH_4 and promising bacteria for environmental remediation (Singh, 2011; Pandey *et al*., 2014). Further, in a study, it has been reported that some environmental factorsuch as climate change influence the methane oxidation by methanotrophic bacteria (Singh, 2013).

Methanotrophs are Gram-negative bacteria that utilize CH_4 as their sole source of carbon and energy, play a crucial role in reducing global CH_4 load due to its CH_4 consumption characteristics. Studies on CH_4 sink measurement from various agro and natural ecosystems showed that the soils of these ecosystems exhibited a significant variation in CH_4 sink activity due to methanotrophic bacteria. Paddy soil methanotrophic communities exhibit the highest CH_4 sink activity on a global scale (Tiwari *et al*., 2015). Based on physiology, phylogeny, biochemistry, resting stage, intracellular membrane, genetic characters, ultrastructure and phospholipid ester-linked fatty acid (PLFA) analyses of 14 culturable genera (Han *et al*., 2009) of aerobic proteobacterial methanotrophs are classified as type I belongs to Gammaproteobacteria group and contain genera *Methylobacter, Methylomonas, Methylosphaera, Methylomicrobium, Methylothermus, Methylosarcina, Methylohalobius,* and *Methylosoma* while type II belongs to Alphaproteobacteria group of CH_4 oxidising bacteria and include genera *Methylocystis, Methylosinus, Methylocapsa, Methylocella*. Type-I group of methanotrophs are further subdivided

into type-Ia and type-Ib (Bodrossy *et al*., 2003; Krause *et al*., 2010). Type-I subgroup contain several culturable methanotrophs for example *Methylomonas*, *Methylosarcina*, *Methylobacter*, etc. However, *Methylocaldum and Methylococcus*comes under the subgroup Type-I or rare type-X (Hanson and Hanson, 1996; Graef *et al*., 2011; Giri *et. al*., 2014; Tiwari *et al*., 2015). Type-I methanotrophs also referred as 'high capacity-low affinity methanotrophs are adapted for high CH_4 concentrations and assimilate it through RuMP pathway whereas Type-II generally termed as 'low capacity-high affinity' methanotrophs capable of using trace quantity of CH_4 from the environment and follow the serine pathway for CH_4 oxidation (Hanson and Hanson, 1996; Tiwari *et al*., 2015). *Verrucomicrobia*, a new group of CH_4 oxidiser discovered in recent past year (Siljanen *et al*., 2011; Luke *et al*., 2011; Graef *et al*.,2011; Tiwari *et al*., 2015). Singh. (2010) reported that the work of the last 10 years has resulted in a doubling of the number of known genera and species. Currently, 18 genera of cultivated aerobic methanotrophs (*Gammaproteobacteria*) and 5 genera of *Alphaproteobacteria* represented approximately 60 different species (Singh, 2010). The CH_4, a potent GHG, has been involved in a number of chemical and physical processes in the Earth's atmosphere, including global warming. In the global CH_4 cycle, a substantial amount of CH_4 is consumed by biological processes. This biological CH_4 consumption is only known biological sink for the atmospheric CH_4 mediated by methanotrophs or methane-oxidizing bacteria (MOB) in aerobic soils, which can contribute up to 15% to the total Global CH_4 destruction (Singh, 2010).

Aerobic soils are the important biological sink for CH4 due to the presence of unique methanotrophic bacteria. Methanotrophs (methane-oxidising bacteria, MOB) are the solely biogenic sink of CH_4, play a vital function in the oxidation of significant

amount of CH_4 (Singh, 2011; Tiwari *et al*., 2015). Methanotrophs utilise CH_4 as their carbon and electron source from the surrounding environment. The estimated amount of CH_4 consumed by methanotrophic bacteria is between 10 and 40 Tg yr^{-1} and comprises approximately 6-10% of the total CH_4 oxidation of the atmosphere (Tiwari *et al*., 2015). Up to 95% of the CH_4 emitted anoxically may be consumed before destine into the atmosphere (Frenzel *et al*., 1990; Graef *et al*., 2011). Therefore, even minute alteration in consumption capacity may have global significance if key regions such as the Arctic and Antarctica are concerned (Graef *et al*., 2011). It is assumed that 10-30% of the CH_4 emitted by methanogenic bacteria in submerged conditions of paddy fields oxidised by methanotrophs linked with the roots of rice crop (King, 1997; Schlesinger, 1997; Mohanty *et al*., 2007; Tiwari *et al*., 2015).

Most studies either have determined the number of culturable methanotrophic bacteria (MB) by most-probable-number (MPN) procedures or have used microscopic techniques such as fluorescence *in-situ* hybridization (FISH) (Dedysh *et al*., 2001). Only the latter method allows targeting of different groups within the MB by using specific DNA probes (Eller *et al*., 2001). In rice field soils, for example a recent study using phospholipid fatty acid (PFLA) analysis showed that both type-II and type-I methanotrophs were abundant in rice fields over the whole vegetation period, although only the abundance of type II MB was significantly correlated to soil pore water CH_4 concentrations and rice growth (Macalady *et al*., 2002). However, the PLFA analysis and FISH cannot be applied to investigate the methanotrophic community in soils in more detail due to limited criteria for distinguishing subgroups or due to the great manual effort required (Henckel *et al*., 2000). Further, the quantitative studies of methanotrophic communities in soils are hampered by the lack of adequate methods (Kolb *et al*., 2003) and these bacteria belong to the uncultivated

fraction of soil microorganisms (Steinkamp *et al*., 2001). Therefore, molecular biology techniques such as PCR and DGGE offer some good opportunities to overcome the limitations for identification of non-cultivable CH_4 oxidizers in aerobic soil environments including dry tropical forest soils (Kolb *et al*., 2003).

Methanotrophs are cosmopolitan in their occurrence and are worldwide vital in oxidizing the most reduced compound- a potent greenhouse gas CH_4 globally through methane monooxygenase (MMO) enzymatic activity. Soil MB, a unique group of bacteria, has been isolated from a wide variety of terrestrial environments including soils (Dedysh *et al*., 2001). Therefore, MB play a very crucial role in the reduction of the CH_4 soil into atmosphere, and it is the only terrestrial sink to reduce CH_4 in soil (McNamara *et al*., 2008).

Natural forest ecosystems are gradually being converted into farming land due to population pressure. Removal of the forest, not only leads to loss of species diversity, but also has an intense effect on the global CH_4 budget (Dorr *et al*., 2010). It has been suggested that changing forest composition and management practices result into alteration in the soil characteristics, which adversely affect the number of viable methanotrophic community (Singh *et al*., 2010a). Several workers have demonstrated that the anthropogenic activity on natural forests disturbs the CH_4 sink attribute of the soil (Dorr *et al*., 2010). However, there is no information concerning the impact of land use changes and crop residues biochar additions for CH_4 consuming bacterial abundance and diversity in paddy agriculture soils.

According to current estimate, about 10% increase in the rate of soil CH_4 uptake would be required to stabilize the present level of atmospheric CH_4 concentration, which is a potent greenhouse gas in the environment (Singh, 2011). The largest natural sink for CH_4 in the soil is due to MB, which can oxidize and

utilize the CH_4 as a source of carbon and energy (Groffman and Pouyat, 2009). All the forest management related activities such as afforestation, improved forest management and reduced deforestation can go a long way in achieving the balance in global CH_4 budget (Dorr *et al.*, 2010). McNamara *et al.* (2008) have suggested that the afforestation is a potential tool for mitigation of CH_4 emission from soils. Several study are indicative that the effect of anthropogenic disturbances (deforestation) is directly reflected on variation in net methanotrophic CH_4 sink activity due to alterations and disturbances in forest soil characteristics (Singh *et al*., 2007; Dorr *et al*., 2010). However, experimental evidences regarding the CH_4 consuming bacterial diversity of the soil due to land use changes and impact of crop residues biochars in India is still lacking.

The upland agriculture aerobic soils exhibited high uptake rates of atmospheric CH_4 at all measurement times throughout the period measured in natural forest soil (Kolb *et al*., 2005). Methane oxidation in forest soil has been intensively investigated (Knief and Dunfield, 2005), but study of CH_4 consuming bacteria in the soils due to land use changes and crop residues biochar application is minute. Abundance and diversity of CH_4 consuming bacteria in the changes of soil characteristics due to crop residues biochar amendments are relatively unclear, and subsequently investigations will be completed throughout this exertion.

2.4 Crop residues problems and its management

Every year India produces approximately 435.98 million tons of crop residues in which. These huge crop residues waste produced every year are burned out on field sites by the farmers and as a result air pollution has been experienced in Delhi and adjoining area. The crop residues waste generated every year are not managed and recycled properly because of many constrains (Murali *et al*., 2010). Agro-animal

waste is a big challenge for the proper management and conversion into further use as soil amendment in agricultural fields. Crop residues in the fields can cause considerable crop management problems if they accumulate in excess amount. The major crop residues are produced in India such as rice husk, straws of paddy, wheat, millet, sorghum, pulses (pigeonpea), oilseed crops (castor, mustard), maize and cobs, cotton and jute sticks, sugarcane trash, leaves, fibrous materials, roots, branches and twigs of varying sizes, shapes, etc. (Sugumaran and Sheshadri, 2009). According to Koopmans and Koppejan, (1997) estimated that approximately 507,837 thousand tons of agricultural crop residues were produced by India in 1997 of which 43% was rice and 23% wheat. Venkataraman *et al*. (2006) reported that, about 116 million tons of crop residues were burnt in India in 2001 which is direct indication of air pollution by increasing concentration of GHG (Gupta, 2010). According to Ministry of New and Renewable Energy (MNRE, 2009), Government of India has estimated that every year India is produced 120-150 million ton biomass. Indian Agriculture Research Institute (IARI, 2012) reported that approximately 93 million tons of agro-waste (crop residue) is burned by farmers each year in fields. Out of whichthe highest crop residue is generated in Uttar Pradesh (60 million tons) followed by Punjab (51 million tons) and Maharashtra (46 million tons) (Srinivasarao *et al*., 2013). According to farmers the crop residue waste burning at field sites provides a fast way to remove the crop residue from agricultural fields and facilitating further land preparation and cropping (Srinivasarao *et al*., 2013**)**. Thus, farmers support the burning of crop residues in the fields instead of eco-friendly recycling and conversion as soil fertility booster. Crop residue burning is a destructive phenomenon in the agricultural fields that can be harmful to soil health such as reduced the soil beneficial microbial population, imbalance the pH, lose the nutrients and many other physico-chemical and biological

properties also affected (Srinivasarao *et al*., 2013). Punia *et al*. (2008) suggested that Punjab annually is burned about 70 to 80 million tons of rice and wheat straw thereby releasing approximately 140 million tons of CO_2 in the atmosphere and two another important GHG emissions from agro-residues in which, about three fourth of CH_4 and the remaining one-fourth was N_2O. During a burning process, wheat and paddy straws alone contributes major share (approximately 42%) of GHGs. On the other hand, if crop residues are remains in soil without burning that can be degraded by soil microbes, thus the resulting higher GHG emission through biodegradation pathway in soil. Hence agro-residue based biochar can play important role to maintaining soil nutrients for the desire of higher crop production and environmental sustainability development (Izaurralde *et al*., 2001; Srinivasarao *et al*., 2012).

Biochar is a unique product, which can enhance availability of plant nutrients and significantly improves the crop yield. Biochar is produced by pyrolysis of biomass or organic materials and this practice is termed as thermal degradation of biomass such as rice straw, grass, wood, agricultural wastes and manure (which is mentioned in above paragraph) (Wu *et al*., 2015). In addition, biochar can significantly improve soil properties such as soil bulk density, pH, organic carbon, increasing available nutrients, removing heavy metals and increase soil microbial biomass and methanotrophic diversity and ultimately increasing crop yields (Milla *et al*., 2013). Thermal degradation process takes place in scarcity of oxygen at very high temperature (400-500 °C). Therefore Intense temperatures is required for the production of high-quality biochar which can be differentiated from charcoal due to its use in soil amendments for various purposes likely fertility enhancement, crop yield, to combat with pollution, etc. (Lehmann and Joseph, 2009; Johannes, 2007; Gaunt *et al*., 2008; Peter, 2007).

2.5 Biochar production and characterization

Biochar production is a thermal degradation phenomenon of organic material and biomass, using a small-scale reactor and traditional drum at 400-500 ºC with the residence time of 1 hour. **Table 2.1** showed different types of biochar produced from various sources (feedstock) (Gaunt *et al*., 2008; Peter, 2007). Ca, Si, Al and K are common elements in biochar but C, N, H and S are also determined by a dry oxidation using an elemental analyzer (Hmid *et al*., 2014). According to Gaunt *et al.* (2008) and Peter, (2007) the characterization of biochar can be completed by using following parameters such as scanning electron microscopy (SEM) equipped with an energy-dispersive X-ray spectroscopy (EDX), Fourier transform infrared spectroscopy (FTIR), volatile matter (VM), electrical conductivity (EC), total dissolved solids (TDS) analysis, water-holding capacity (WHC) and heavy metal assessment. Peter, (2007) and Milla *et al*. (2013) reported that, the sample powder is sprinkled as a thin layer on an adhesive tape placed on the brass sample holder. Excess amounts of the sample are removed with a small manual air blower. The adhered sample is then coated with gold powder using a sputtering device, FTIR spectrometer identified the sample to determine the organic functional groups present for each biomass, especially carbons. Volatile matter in biochar is determined following the ASTM D 3175-07 standard test method. A Beckman Coulter SA 3100 BET analyzer containing approximately 0.1000 g to 0.2000 g of each biochar sample is then used at a temperature of 50 ^{0}C for 60 min. Electrical conductivity and total dissolved solid analysis are theoretically the best measure to indicate the actual salinity level experienced by the plant root (Corwin and Lesch, 2003; Peter, 2007). Hence, electrical conductivity and total dissolved solids are measured using a portable conductivity meter.

Table 2.1: Physico-chemical properties of feedstocks for biochar production

Components	**Woodchip** (**Yargicoglu *et al*., 2015**)	**Grass** (**Jouiada *et al*. 2015; Mohammed *et al*., 2015**)	**Poultry litter** (**Jindo *et al*., 2012**)	**Rice Husk** (**Shackley *et al*., 2012**)	**Sugarcane bagasse** (**Carriea *et al*., 2012**)	**Wheat straw** (**Bruun *et al*., 2012; Mahinpeye *et al*., 2009**)
Soil C (%)	74.5	- -	71.47	- -	- -	- -
Ash (%)	25.4	14.7	28.53	6.5	11.9-16.4	5.9
pH	7.88	6.1	23.596	6.6	- -	6.76
EC (mS cm^{-1})	0.14	- -	3.0	- -	- -	2770
CEC (c mol kg^{-1})	- -	- -	- -	45-110	- -	- -
C (%)	51.9	42.5	38.6	41	60.4-65.3	43.7
N (%)	0.4	1.9	1.37	1.4	0.8-1.0	0.9
S (%)	- -	5.3	- -	0.1	25.4-15.1	0.283
Ca (ppm)	0.56	4.3 4	1.85	250	- -	0.18
K (ppm)	0.21	64.80	0.99	2604	- -	0.15
Mg (ppm)	0.04	2.3 4	0.19	827	- -	- -
Si (mg kg^{-1})	- -	7.44	- -	5.8	- -	0.18
P (ppm)	0.06	2.31	0.35	- -	- -	0.05

2.5.1 Biochar types

Currently varieties of feedstock being used as raw material for the making biochar, A variety of biochar from different feedstock described in **Figure 2.1**.

2.5.1.1 Grass biochar

Grass biochar is produced by a variety of grasses such as Switch grass (*Panicum virgatum* L.), Saw grass (*Cladium jamaicense*), etc. has been declared as a model bio-energy crops for the production of biochar. These are preferred due to its high-yield potential, low input requirements on marginal soils and potentially active in soil carbon sequestration and alleviation of GHGs (McLaughlin *et al*., 1998; Sadaka *et al*., 2014; Mukherjee *et al*., 2013). The switchgrass has a gross calorific value between 18-19MJ kg^{-1} as compared to hardwoods 20-21 MJ kg^{-1} (Sadaka *et al*., 2014). There were several barriers in the way of switch grass to be used as the sole source of fuel in combustors such as high moisture and ash contents in biomass, which cause ignition and combustion problems. It has been observed that, blending of biomass with coal would reduce flame stability problems and will also lead to significant reductions in CH_4 emissions. Consequently, a multitude of studies has investigated about conversion of switch grass to biochar for the safe and eco-friendly cultivation of agriculture crops (Sadaka *et al*., 2014).

2.5.1.2 Woodchip biochar

Woodchips are a medium-sized solid material made by cutting, or chipping, larger pieces of wood. Today, woodchips are being used as a raw material for the production of biochar. It has a more carbon concentration as compared to other feedstock including the highest carbon sequestration potential. Woodchips feedstock is produced; a high quality biochar at 400-500 ^{0}C, its good residential time is 2-3 hours. Woodchips absorb moisture at 15-20 ^{0}C; therefore, it requires drying before the

pyrolysis (Milla *et al*., 2013; Lai *et al*., 2013; Spokas*et al*., 2009). The *Camellia japonica* (Japanese Cedar) waste wood chips are used for biochar production by pyrolysis at either 290 or 700 ^{0}C and called biochar 290 (BC290) and biochar 700 (BC700), respectively (Lai *et al*., 2013). The percentage amount of C, N, H, and available K contents have been found about 59.1%, 0.35%, 5.73%, and 0.78 g/kg for woodchips biochar 290 (BC290) and 83.0%, 0.34%, 2.57%, and 3.90 g/kg for BC700, respectively (Lai *et al*., 2013).

2.5.1.3 Rice husk biochar (RHB)

Rice husk is the coating of rice seeds, protects the seed during the growing season, since it is formed from hard materials, including silica, carbon, magnesium and phosphorus. Today rice husk is being used as a raw material for the production of biochar in agricultural crops improvement. For making rice husk biochar, rice husk put in pyrolysis apparatus which consisted of a stainless reactor of 500 mm length with a 15 cm inside diameter. The rice husk is then heated externally by an electric furnace (5000 W) to a temperature of 600 ^{0}C obtained higher carbon and silica rich RHB (Zhanga *et al*., 2012). The application of RHB in agricultural fields, instead of synthetic fertilizers is became advantageous, because the synthetic fertilizers generate many harmful effects such as reduction of micro flora, crop yield, nutrient availability and water holding capacity, etc. (Zhanga *et al*., 2012). Current studies on cowpea, soybean and maize supported the application of biochar as a sustainable way to increase crop yields (Zhanga *et al*., 2012**)**. In Asian region due to elevated production of rice, obtained up to 560 and 112 million tons of rice straw and rice husk respectively. These residues may be important source of biochar, bio-fuels and energy production (Masulili *et al*., 2010; Zhanga *et al*., 2012). Though number of study related to biochar application on crop yields have been done. However, use of RHB

derived from rice husk in agriculture soils is almost lacking from India. In this study we have used RHB in combination with commercialized bio-formulation to find out the variations in soil physico-chemical characteristics, methanotrophs population, soil microbial biomass and paddy yields in field conditions.

2.5.1.4 Wheat straw biochar

Wheat straw is contained by lignocelluloses biomass which is the most abundant organic raw material and is being used widely for biochar production. Wheat straw is collected by a cutting machine, then shipped to the production plant and air-dried there (Zhanga *et al*., 2012). Pyrolysis of wheat straw is performed in a vertical kiln at 350-550 ^{0}C and after 1 hrs biomass of wheat straw converting into biochar. For the field study, the biochar mass originally in a particulate form is ground to pass through a 2 mm sieve, and mixed thoroughly to obtain fine granular consistency that would mix more uniformly with the soil mass (Zhanga *et al*., 2012; Wu *et al.*, 2013).

2.5.1.5 Poultry litter biochar

Poultry litter biochar (PLB) is produced by the use of chicken manure (CM) as raw material, CM is unique type of feedstock, which reduced the plant-based feedstock such as rice husk, crop residue woodchip etc. and also characteristically different from others feedstock based biochar on the basis of elemental composition (Songa *et al*., 2012; Demirbas, 2001). According to Songa *et al*., (2012) PL is a solid waste material, obtained from chicken rearing, is an alternative substitute for the production of PLB and bio-fuels, PL is a mixture of bedding materials of bird feather, hen's excreta, feed spills etc. and pyrolised these at higher temperature by thermo-chemical conversion technology whereby organic materials are heated in the absence of oxygen, PL can be readily transformed into biochar, bio-fuel for soil amendment and energy production respectively (Songa *et al*., 2012; Kim *et al*., 2009).

2.5.1.6 Sugarcane bagasse biochar (SBB)

Sugarcane industries are produced huge quantities of waste material including, bagasse (crushed cane stalks), cane trash (leaves and stalk tips removed during harvest), and filter cake, a sludge that is removed via filtration after the juice clarification step.Sugarcane bagasse is used for many purposes such as biochar production, bio-fuels, paper making and burning purpose, etc. But currently bagasse is being used on large scale for the production of biochar. Before the production of Sugarcane Bagasse Biochar (SBB) raw material (feedstock) should be dry in wet season because moisture content creates difficulties during pyrolysis; because dry feedstock has a low residential time (approximately 1-2 hours) (Eykelbosh *et al*., 2014). SBB has high concentration of carbon, silica, magnesium, etc, which is an important source of plant nutrients in saline soil (Eykelbosh *et al*., 2014).

Figure2.1. Biochar production from different feed stocks (A) poultry litter, (B) rice husk, (C) grass, (D) sugarcane bagasse, **(**E) wood chipand (F) wheat straw.

2.5.2 Applications of biochar

Biochar can increases crop productivity by improving physico-chemical and as well as biological properties of soils. These unique properties depend on the physical and chemical nature of biochar, soil conditions and the type of crop (Yamato *et al.*, 2006; Zwieten *et al*., 2010). Zhanga *et al*. (2010) showed that the combined application of biochar at 10 t ha^{-1} and conventional N fertilizers at 300 kg ha^{-1} is increased the 9% yields. However, the single use of biochar at 40 t ha^{-1} is increased the 12% yields without amendment of N fertilization. In this case, the biochar effect on rice yield was not dependent on conventional N fertilizers (Zhanga *et al*., 2010). Zhanga *et al*. (2010) reported that, biochar application can increase 10 % rice yields even though theplots were sufficiently fertile to achieve high initial yields without any synthetic fertilizer.It has been well established that biochar amendments canincrease N availability to crops and thathigh levels of soil organic carbon accumulation can enhance N efficiency and increase rice productivity during long term field studies (Pan *et al*., 2009). Biochar amended soil has a significant advantages in regarding environmental management and sustainable agriculture development. The application of biochar in soil can play important role in major areas, such as climate change mitigation, energy production, soil amendment, waste management, carbon sequestration, microbial habitat, eco-friendly fertilizers, heavy metal reducer, crops yields enhancer, reduced chemical fertilizers input and protect soil microbes from other biota.

2.5.2.1 Soil amendments and paddy productivity

The incorporation of biochar in agricultural soils produces a considerable enrichment in organic matter content and hence significantly enhances the physico-chemical properties and resulting the higher fertility and crop productivity. Biochar is

introduced as carbon rich by-product that increases the soil organic carbon, cation exchange capacity (CEC) and pH level of soil which is essential to the all plant metabolism for huger crops yield (Sohi, 2012 Singh *et al*., 2017b; Shackley *et al*., 2012). This makes the nutrients more usable for the plant growth and subsequently obtained nutrient rich higher crops yields. Application of biochar in soils contributes as carbon pool and at the same time act as eco-friendly fertilizers (Glaser *et al*., 2001; Marris, 2006). The treatment of biochar increases the total C, total N, available P, and exchangeable cation like Ca, Mg, Na, and increase K, and decreases Al in soil (Chan *et al*., 2007; Major *et al*., 2010) so plants are used these nutrients for their growth however various studies have been carried out on biochar application in agricultural soils and produced variable results (Major *et al*., 2010). According to Major *et al*. (2010) the nutrient uptake capacity of plants was increased in biochar treated soil. **Table 2.2** showed that physico-chemical properties of soil after amendment of biochar) therefore, increases soil productivity with higher availability of Ca and Mg. Biochar increases the ability of soils to keeping hold of nutrients and plant available water and as well as reduces nutrient leaching from agricultural soil (Laird *et al*., 2010) moreover, it is reduced the density of soil due to their low density nature (Laird *et al*., 2010) and in that way enhances water infiltration, plant root penetration, and better soil aeration, increase soil aggregate strength, etc. (Glaser *et al*., 2001).

Table 2.2. Physico-chemical properties of soil after biochar amendments.

Parameter	Unit	Control	Biochar
pH ($CaCl_2$)		7.5	7.4
$CaCO_3$	%	15.8	15.2
Humus	%	2.4	18.1
Total N	%	0.148	0.203
P (CAL)	mg kg^{-1}	49	84
P_{tot} (acid digest)	g kg^{-1}	5.46	5.54
Sand	%	18.3	Not determined
Silt	%	57.2	Not determined
Clay	%	24.5	Not determined
CEC	cmol kg^{-1}	22.5	20.8
Ca (CEC)	cmol kg^{-1}	20.7	18.2
Mg (CEC)	cmol kg^{-1}	1.46	1.53
K (CEC)	cmol kg^{-1}	0.36	0.99
Na (CEC)	cmol kg^{-1}	<0.04	<0.04
Al (CEC)	cmol kg^{-1}	<0.06	<0.06
Fe (CEC)	cmol kg^{-1}	<0.01	<0.01
Mn (CEC)	cmol kg^{-1}	<0.01	<0.01
H (CEC)	cmol kg^{-1}	0.002	0.002
Fe (EDTA)	mg kg^{-1}	40	67
Mn (EDTA)	mg kg^{-1}	107	128
Cu (EDTA)	mg kg^{-1}	7.2	7.1
Zn (EDTA)	mg kg^{-1}	2.3	7.5

Adapted from Prommer *et al*. (2014)

Biochar application to soils is a considerable tool to transfer more easily decomposable organic matter in soil for plant utilisation (Krishnakumar *et al.*, 2014). Biochar amended soils represent the significant impact on the improvement of root length, shoot biomass, panicle length, number of tiller per plant, crops yield and enhance the nutrient availability and carbon sequestration (Abdullah, 2009; Meyer 2011; Milla *et al.*, 2013). Biochar amended crop yields are increased up to 20 % than

non-amended crops. Yang *et al.* (2015) reported that optimum level of biochar is required for the best production of crop yield, demonstrated that 2 ton ha^{-1} or 1 ton ha^{-1} could increase the yield by 5%–15% respectively, however 4 ton ha^{-1} may increase the 20% rice yield.

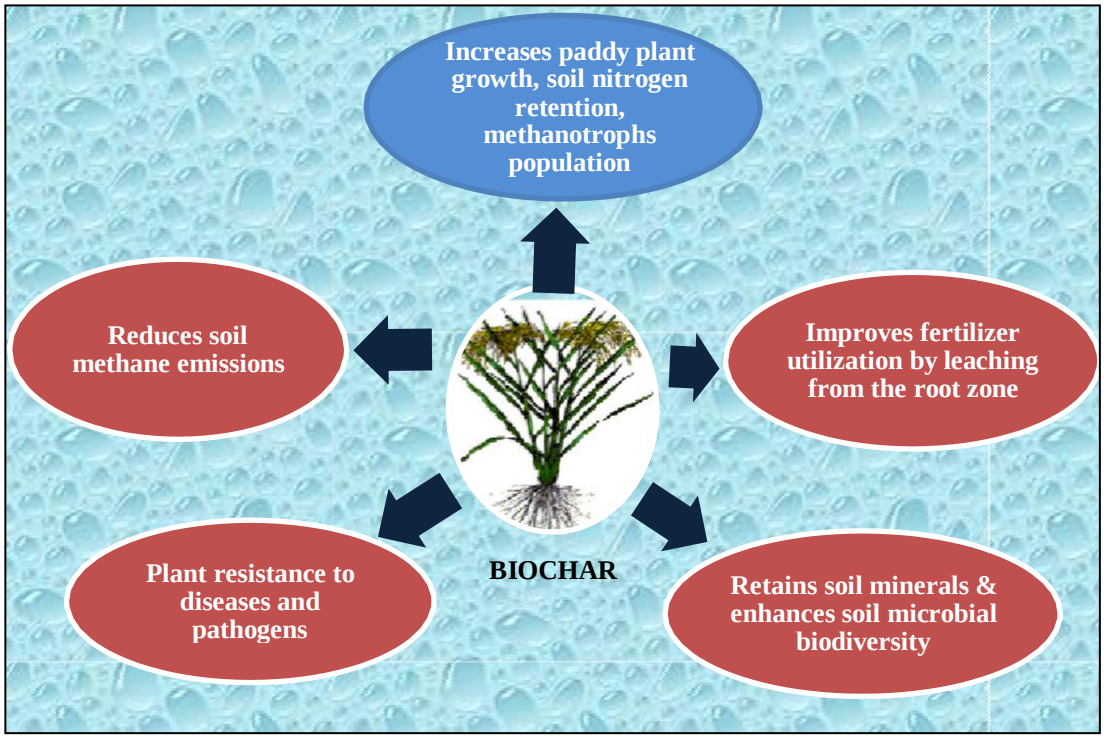

Figure 2.2. Multidimensional role of biochar in agriculturesoil

2.5.2.2 Biochar in climate change mitigation

Climate change is giant challenge on world level in the perspective of global warning because increasing concentration of green house gases into atmosphere is indicating the future challenges for all living being on earth (Singh, 2013; Singh *et al.*, 2016). Every year earth temperature is going up due to higher concentration of GHG emissions from various anthropogenic and natural sources. While anthropogenic sources are a major cause for the emission of green house gases, including wrong agricultural practices and coal mine etc. So there is the urgent need to develop sustainable and eco-friendly technique for the cultivation of agriculture all over the world, therefore the application of biochar in soil may be a strong management

practice in respect of climate change mitigation (Vantieghem, 2016; Singh *et al*., 2017). Biochar can be used as a tool to offset greenhouse gas (GHG) emissions and as a soil conditioner. The study showed that biochar application increased rice productivity, soil pH, soil organic carbon, total nitrogen but decreased soil bulk density during rice cultivation. Recent studies have confirmed that the use of biochar in paddy agriculture has capability to minimize the CH_4 production, but it's essential mechanism has not yet to be clarified. The additions of biochar in agriculture soil showed higher CH_4 consumption due to the improvement in soil aeration and porosity and also increasing the number of methanotrophs. However, further investigations are in need to evaluate the impact of biochar addition on the net CH_4 emissions and consumptions respectively, by methanogens and methanotrophs. Furthermore the highest carbon sequestration potential of biochar can reduce the GHG emissions in to atmosphere. Plants store CO_2 as organic carbon inside their tissue system by photosynthesis and during plant biomass decomposition, releases CO_2 in to the atmosphere which is an important GHG and back into the atmosphere by microbial degradation (**Figure 2.3**). Biochar can change the global carbon cycle by pyrolysis of biomass, which is stabilized carbon in form of biochar its long time existence in soil due to the recalcitrant properties against microbial degradation (Sohi, 2012; Singh and Gupta, 2016). Besides CH_4, CO_2 and N_2O also two main contributors to emission of green house gases from soil to atmosphere, especially in highly synthetic fertilizers used agriculture soil (Lehmann *et al*., 2006; Tiwari *et al*., 2015). Some authors reported, contradictory evidence to suggest that biochar amendment affects soil CH_4 emissions and most of the evidence that comes from studies in paddies soils (Zhang *et al*., 2010; Wang *et al*., 2011; Wang *et al*., 2012b). The CH_4 emissions are generally significant in saturated soils such as rice paddies but not in other more aerobic crop

soils (Le Mer and Roger, 2001). Wang *et al*. (2012b) found that soil CH_4 emissions were increased by 37% with biochar amendment in a rice paddy. Zhang *et al*., 2010; Zhang *et al*., 2012). Knoblauch *et al*. (2011) similarly observed an increase in soil CH_4 emissions from the same land use. For other crop types, three studies reported no significant effect of biochar amendment on CH_4 emissions in arable and pasture soils (Castaldi, 2011; Scheer *et al*., 2011; Wang *et al*., 2012b), whilst in Finnish agricultural soil a 96% CH_4 uptake was increased in biochar-mediated soil (Karhu *et al*., 2011; Lehmann *et al*., 2011). Increased availability of labile C substrates for methanogenic bacteria may explain increased CH_4 emissions by the addition of biochar to soil (Wang *et al*., 2012b). Methanogens produce CH_4 as a metabolic by-product of organic matter mineralisation in anaerobic conditions; the two primary pathways being via CO_2 reduction by H_2 or via acetotrophy (Le Mer and Roger, 2001). Soil methanotrophs are the only known biological sink for atmospheric CH_4, which oxidise CH_4 and produce CO_2 as a by-product (Topp and Pattey, 1997). It has been observed that the addition of biochar to increase soil methanotrophic activity. Karhu *et al.* (2011) reported that, increasing soil aeration and CH_4 uptake within an arable soil due to the biochar amendment, because methanotrophs require oxygen as a terminal electron acceptor and their activity is highest at around 60% in soil, so resulting higher CH_4 oxidation (Castro *et al*., 1995; Karhu *et al*., 2011). As previously discussed, biochar addition to soil may decrease soil albedo therefore, it has been hypothesised that increasing soil temperature and typically high pH biochar increases the pH of the soil it is added to directly in soil (Lehmann *et al*., 2011; Meyer *et al*., 2012). Likewise methanogenic activity increases in the respect of temperature (up to 40°C) and is at a maximum at close-to-neutral pH (Topp and Pattey, 1997), while soil methanotrophy increases with temperature up until 10 °C, (Castro *et al*.,

1995) and methanotrophic activity is at a maximum at a close-to-neutral pH (Topp and Pattey, 1997). However, the effect of biochar on soil temperature and soil pH has not been suggested as mechanisms to explain differences in overall soil CH_4 emissions. Additionally various studies have been exposed, the application of biochar in soils to reduce N_2O emissions also (Singh *et al*., 2010) while many study results are more indistinct in perspective to green house gases emission (Liu *et al*., 2011; Zhang *et al*., 2010). But the well defines mechanisms between biochar and potential greenhouse gases are poorly understood therefore, the detail studies in this direction are warranted.

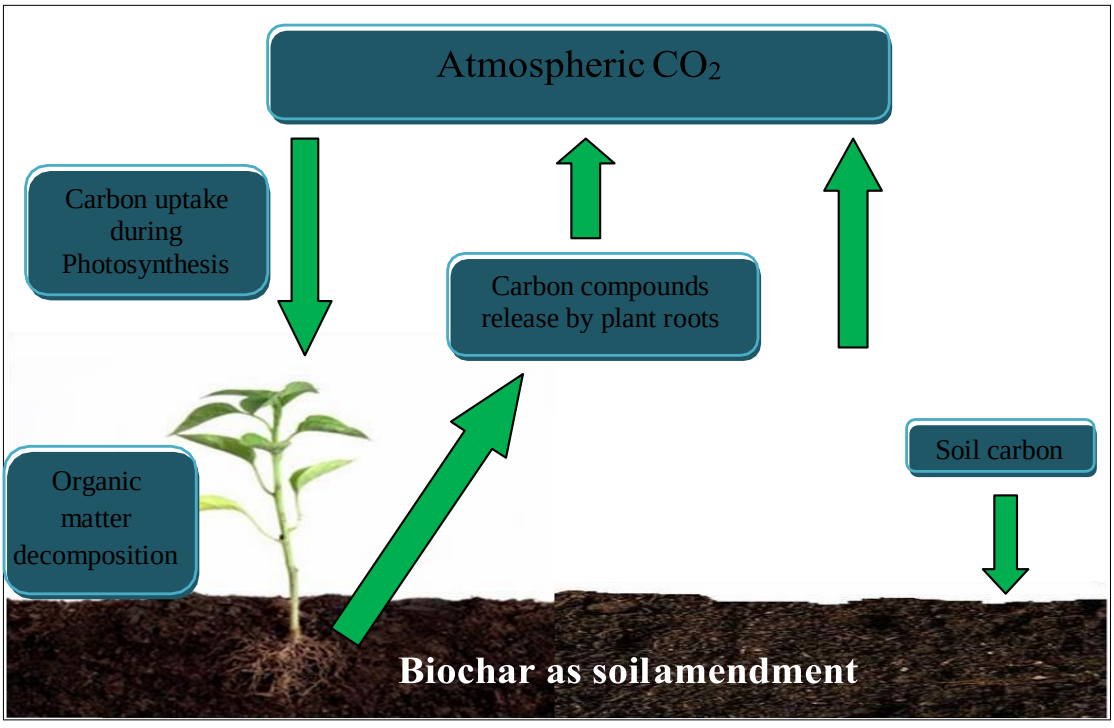

Figure 2.3. Role of biochar in soil as amendment and carbon mitigation.Modified from Cooperman *et al.* (2016).

2.5.2.3 Management of plant biomass in bio-energy production

During the pyrolysis of organic material three main components from pyrolised biomass produced are: biochar, bio oil and synthetic gas. These all the product of

pyrolised biomass are very economically feasible due to commercial uses. Bio oil and synthetic gases can be used as alternative source of energy and electricity (Vantieghem, 2016). Gasification of any organic material is produced biochar including bio-oil and synthetic gases and the use of bio-oil and synthetic gases as bio-energy. Bio-oil can be used for the transportation of vehicles (Hofstrand, 2009). When biomass of organic material is pyrolised in limited supply of oxygen produce synthetic gases including carbon monoxide, hydrogen and sometimes CO_2, all these are used with combination of various cooking gases.

The focus on management of waste materials, produced from different sources including domestic, agriculture and industries is serious concern for environmental safety. The huge amount of waste material is produced from agricultural fields all over the world but due to inadequacy of awareness most of the farmers are burned these waste in the fields and caused the different type's perilous exertion. Therefore, due to the inadequate management of these waste to leads the surface and ground water pollution but according to newly generated technology (pyrolysis) can convert the large amount of agricultural waste (biomass) in the precious form of biochar and can be used in agricultural field as soil conditioner (Vantieghem, 2016). The nutrient concentration of the biochar is increased due to the pyrolysis at above 350 °C, biochar production from waste material and application in soil is a carbon negative process. Biochar has highly aerometric and recalcitrant nature against microbial decomposition and hundreds to thousands of years can exist in the environment. Conversion of agricultural biomass in the form of biochar is a considerable method of carbon negative environment and waste management. Many studies have been reported that biochar can sequester approximately 50–80% of the carbon which is presented in the

biomass (feedstock) and provide pollution free environment (Mekuria and Noble, 2013).

2.5.2.4 Biochar in carbon (C) sequestration

The application of biochar to the soil not only works like soil fertility enhancers, but also stores the soil carbon in the form of highly aromatic recalcitrant. The conversion of organic material and agricultural waste to biochar is considered as the most valuable and eco-friendly practice for the sequestration of carbon from different plant residues in soil. Carbon sequestration is a phenomenon in which storing soil carbon with soil organic matter and thus reducing GHG from atmosphere (Warnock *et al*., 2010; Singh *et al*., 2017a). Soils are large reservoirs of carbon, which store three times more carbon. Plants absorb atmospheric carbon during photosynthesis, which is store inside their tissues and after completion of this practice, go back to soil as plant residue and contribute major role to maintain soil carbon balance. Hereby this carbon eventually returns to the atmosphereby soil microbial-mediated decomposition of plant biomass and release of CO_2 (Cooperman, 2016). Biochar based carbon sequestration potential in soils can lead to climate change mitigation practices. The carbon amount in biochar is 70-80%, which is two times higher than the plant residue (40%).

2.5.2.5 Biochar in heavy metals removal and alternatives of chemical fertilizers

Soil has very complex composition including humus, organic and inorganic matterand soil biota and contains pore spaces filled with water and air. (Singh *et al*., 2017a) Organic matter serves as a binder for mineral particles which contributing to maintain soil structure. But due to excess use of various pesticides and synthetic fertilizers for the higher production of crop yields disturbed the natural composition of soil and increasing toxicity level. Application of biochar in heavy metal contaminated soil can

be helpful to reduced the toxicity of various heavy metals to the contaminated soil (Namgay and Singh, 2010; Park *et al*., 2011). Biochar may attract various heavy metals, present in soil including cadmium (II), lead (II), copper (II), zinc (II), etc. because previous studies have shown that biochar can adsorb various toxic compounds on their surfaces from contaminated soils. So, biochar has been evaluated as a potential adsorbent of heavy metal in metal-contaminated soils. (Komkiene and Baltrenaite, 2006).

Poor agricultural practices are disturbing the natural cycles of C, N and P in agro-ecosystem (Someus, 2014). But biochar is being used to enhance the economical and ecological benefits, including soil fertility improvements, higher yields, grain quality and better soil structure and water retention. The quality or nutrients level of biochar depends on the nature of feedstock and pyrolysis temperature. Therefore, the biochar production and application strategies must be well designed. The low nutrient based feedstock-mediated biochar have inadequate potential to provide satisfactory and economical nutrient supply for reduction of synthetic fertilizers in agriculture soil (Someus, 2014). Therefore, the nutrient level of biochar is an important reservoir in soil to influencing soil fertility and crop yields. Hence, due to various beneficial properties of biochar in agriculture soil, farmers may be convinced toward the application of biochar for the improvement of barren soil and crop yields (Someus, 2014).

2.5.2.6 Soil microbial biomass and nutrient availability

Soil microbial biomass (SMB) is a key indicator of soil fertility and microbial diversity. The SMB not only responsible for carrying the nutrient cycles in paddy-ecosystems, including carbon (C), nitrogen (N) and phosphorus (P), but also play significant role in soil nutrient transformations and acting as a labile nutrient pool that

offered to plants (Liu et al., 2010). The long term application of biochar in soil can enhance the SMB-C, N and P. But biochar application rates soil type and feedstock nature also affects the soil microbial biomass (Khodadad *et al*., 2011; Zhang *et al*., 2014; Singh *et al*., 2017b). Soil microbial biomass increased due to enhancement of available soil nutrients in biochar amended soil such as dissolved organic matter, P, Ca and K etc. biochar can improve soil microbial activity, water holding capacity and pH level etc. Microbial biomass could be increased due to higher microbial diversity in biochar amended soil (Lehmann *et al*., 2011), greater surface area and porosity of biochar can be most important survival niche to soil microorganism and protect them to grazing from others soil predators, which are present in soil and store C substrates and mineral nutrients for their growth (Pietikäinen *et al*., 2000; Zhang et al., 2014) Zhang *et al*. (2014) was analysed soil microbial biomass carbon (SMB-C) and nitrogen (SMB-N) in a field experiment during a winter wheat growing season after four consecutive years cycle with the application of different treatment and resulting that biochar amendment was increased SMB-C significantly compared to the others treatment, and that the effect size increased with biochar application rate (Zhang *et al*., 2014) but further biochar application on soil SMB-N was less strong than SMB-C. However the treatment of biochar significantly increased the C/N ratio (Zhang *et al*., 2014).

Nitrogen is one of the most essential nutrients for plant growth therefore organic and inorganic form of nitrogen can act an important role in soil nutrient cycle. Nitrogen scarcities in soil influence the crop yields and biological activity. The transformation of organic nitrogen into the plant usable form (inorganic nitrogen) by microorganism can be affected with the application of various synthetic fertilizers (Singh *et al*., 2017a; Singh *et al*., 2017b). While biochar is increased the microbial

population therefore the rate of microbes-mediated nitrogen transformation become high and reduced the NH_4^+ and NO_3^- ions leaching in biochar amended soil (Rondon *et al*., 2007; Ball *et al*., 2010; Clough and Condron, 2010; Deenik *et al*., 2010).

2.5.2.7 Biochar as soil conditioners

Nitrogen (N_2) is most abundant (approximately 78.09 %) gas in the environment which is found in gaseous form and this is the major nutrient source to plant growth but plant unable to consume directly from atmosphere as nutrient source. Hence, there is the required of conversion in usable form for the plant growth by complex biological pathway. Likewise certain groups of microorganisms are contributing major role in soil nitrogen fixation (Robertson and Groffman, 2007). Gaseous N_2 can be fixed into reduced form of nitrogen (e.g. ammonia, ammonium, nitrite and nitrate) mainly by two soil bacterial species such as *Nitrobacter* and *Nitrosomonas* (Erisman *et al*., 2007; Moreira de Assumpçao, 2007). Moreover, fixed N is naturally produced by lightening and in fewer amounts from volcanoes and as well as natural fire (Levy *et al*., 1991; Galloway *et al*., 2004; Ward, 2012). However, in last few decades anthropogenic activity and wrong agricultural practices adversely affected N_2 cycle due to increased amount of reactive N_2 (e.g. ammonia, ammonium) in the atmosphere and oxidized forms N_2 (e.g., NOx, HNO^3, N_2O, NO_3, etc.) (Galloway *et al*., 2004). Hence, the application of biochar may be an effective and sustainable way in agricultural management for the enhancement of nitrogen fixation and mineralization. Mineralization is the conversion process of organic N_2 into soluble inorganic forms that plants can uptake with help of nutrient pathway (Robertson and Groffman 2007). According to Schimel and Bennett (2004) and Nasholm *et al*. (2009) certain tree species such as Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*)as well as certain crop plants can uptake organic forms of nitrogen (Gioseffi *et al*., 2012;

Paungfoo-Lonhienne *et al*., 2012). Biochar making and consequently assimilating into soil may provide carbon cultivation solutions to global climate change and increasing food requirement. Previous study showed that biochar amendment causes primary changes in soil nutrient cycles, commonly resulting in marked enhancement in crop yields, mostly in saline and unproductive soils having small soil organic contents (Prommer *et al*., 2014) However, comparable outcomes in temperate soils are changeable. Prommer *et al*. (2014) found that biochar application increased total soil organic carbon but decreased the extractable organic C pool and soil nitrate. Although gross organic N transformation rates were reduced by 50-80%, but the gross N mineralization process remain unaffected. Biochar increased ammonia-oxidizer microbial population in soil, which accelerated gross nitrification rates more than two times (Ball *et al*., 2010). Prommer *et al*. (2014) suggested that addition of any inorganic fertilizer with the combination of biochar may compensate the reduction in organic N mineralization, with plants and microbes drawing on fertilizer for growth, in turn fuelling the belowground build-up of organic Nitrogen.

Biochar have significant effects on microbial-mediated transformation of nutrients in paddy soil. Nitrification was augmented by the addition of biochar in soil (Ball *et al*., 2010) and explained by sorption of phenolics that would otherwise inhibit nitrification and an increase in ammonia and CH_4 oxidizing bacterial population (Ball *et al*., 2010). Whether observed the change in ammonia- and methane-oxidizing bacterial community composition (Ball *et al*., 2010). Changes in pH that can start similar responses in soil were not able to explain the observed changes in nitrification. Prommer *et al*. (2014) after applying biochar, ammonium level increased 0.001 mg kg^{-1} in the conventionally managed soils (about 88 mg kg^{-1} dry soils) compared with the organic soils (about 9 mg kg^{-1} dry soil). After increasing biochar application rate

ammonium contents became 66, 30 and 15 mg kg^{-1}, respectively, but does not show significant reductions from the small initial ammonium contents in the organically managed soil. Initial nitrate contents of 5 mg kg-^{1} increased over the 60-days. Studies show that single and combined application of biochar with any inorganic fertilizer may increase soil organic N in turn enhancing soil carbon sequestration and thereby could play a significant role in future soil and environmental management planning (Prommer *et al*., 2014).

2.5.2.8 Biochar application and soil mycorrhizal fungi

Biochar and mycorrhizal associations, always seen in terrestrial and paddy ecosystems, they shows ubiquitous symbiosis because potential significant in a variety of ecosystem services provided by soils, contributing to sustainable crop production and productivity, ecosystem restoration, soil carbon sequestration and mitigation of CH_4 emission (Warnock *et al*., 2007). As both biochar and mycorrhizal associations are subject to management, understanding and exploiting interactions between them may be valuable. Mycorrhizal fungi are ubiquitous key indicator in nearly all terrestrial vegetation and crop systems, showing a very high degree of specificity and mutualism, enhancing plant growth. Various type of arbuscular mycorrhizae (AM) and ectomycorrhizae (EM) are unique for their functional in different ecosystems. Biochar incorporation in soil has positive impact on mycorrhizal fungi (MF). The positive impact of biochar on mycorrhizal fungi increasesthe root colonisation in biochar amended soil (Ishii and Kadoya, 1994; Warnock *et al*. 2007). Biochar can also increase endomycorrhizal plant associations, enhancing P availability in soil (Atkinson *et al*., 2010). These potential mechanisms are nutrient availability is enhanced, or there are changes in soil physiochemical properties; alterations in other beneficial or detrimental soil microbes (e.g. mycorrhizal helper

bacteria (MHB) or phosphate solubilising bacteria (PSB); enhancement of the ability of MF to resist plant fungal pathogen infection, through enhanced root colonisation (Atkinson *et al*., 2010). After concluding variety of research papers, biochar influence the mycorrhizal population in terrestrial and paddy ecosystem (Warnock *et al.,* 2007; Ishii and Kadoya, 1994). Biochar application in soil influence the diversity of mycorrhizal fungi but still not clear that, the mechanism of biochar in mycorrhizal fungi hence, there is need for further study.

2.5.2.9 Biochar and soil methane emissions and consumptions

Biochar amendment had little effect on methanogenic *Archaeal* community compositions in paddy soils. The methanogenic activity a little increasing at the booting stage and then reduced at the growing stage of paddy (Dong *et al*., 2013). According to Dong *et al*. (2013) there are no statistically significant differences in either methanogenic or CH_4 mitigation activities in the rhizosphere of soil, between the biochar-amendment and un-amended (control) during the period of rice growing seasons.ButIn a field experiment, biochar addition, at the rate of 9 t ha^{-1}, to an agricultural soil significantly decreased CH_4 emission but made no difference to CO_2 and N_2O emissions (Karhu *et al*., 2011). But in a laboratory incubation experiment, CH_4 emission from paddy soil, after amendment with rice straw biochar at a high rate (2.5 % of the soil, w/w), was completely inhibited compared with the non-amendment control soil (Bosse and Frenzel, 1997; Liu *et al*., 2011). Feng *et al*. (2012) also reported that, amendment of wheat straw biochar, which had been pyrolysed at 300 °C and 500 °C, could significantly reduce CH_4 emission from paddy ecosystem. Liu *et al*. (2011) found that, CH_4 emission from a rice paddy field was significantly increased (compared with the un-amended control soil) in the first year, after biochar amendment but was not as distinct as in the next year. It has been observed that soil

CH_4 emission in response to the biochar amendment may vary with biochar types and properties. Most of the studies supported that, decreasing methanogenic activity in paddy soil amended with biochar and soil physico-chemical properties also improved in the response of biochar. Although use of rice straw instead of biochar in soil, the rate of methanogenesis can be enhance because readily degradable carbon in rice straw offered more substrates to methanogenesis to generate CH_4 than that in rice straw biochar. In contrast, there was no significant increase in CH_4 emissions associated with biochar amendment due to their resistance to decomposition (Liu *et al*., 2011; Liu *et al*., 2011). However, there is no considerable information about biochar with methanogenic activity a few research supported that, methanogenic activity affected by biochar such as methanogens diversity decrease with biochar amendments, while other researcher is not support. Hence there is need for detailed study in this direction.

Currently, biochar is being used as environmental and agriculturally supportive agent; hence many parts of world, applying as strong soil conditioner for the production of higher crops yields. But most important aspect is that the mitigation of CH_4 emission and stimulating the CH_4 oxidation rate in paddy soils. Reddy *et al*. (2014) reported that variation in oxidation rates and kinetics of CH_4 in soils depth was variable,therefore samples were taken from different depth of soils and examine that higher oxidation rate was found in upper layer of soil than lower depth of soil. Hence result is that higher numbers of methanotrophs communities exist in upper surface of soil after amendment of biochar. Biochar is increased the methanotrophic communities in amended soil (Feng *et al*., 2012). Methanotrophs are aerobic bacteria which are present in the upper layer of soil and tiny numbers are found in the deeper soil due to the less amount of oxygen. Oxidation rate is higher on the upper layer of

soil because methanotrophic communities exit there in large number (Feng *et al*., 2012; Reddy *et al*., 2014) Biochar has a favourable growth condition and physico-chemical properties for the higher growth of methanotrophs in paddy soil, i.e. high porosity and aeration etc. According to Zhang *et al*. (2012) biochar play significant role in green house gases emissions in paddy soil mostly methane. Graph is showing the variable rates of green house gas emission after amendment of biochar. Optimum level of biochar exposure showed a positive response in CH_4 mitigation but in excess amount the result is reciprocal.

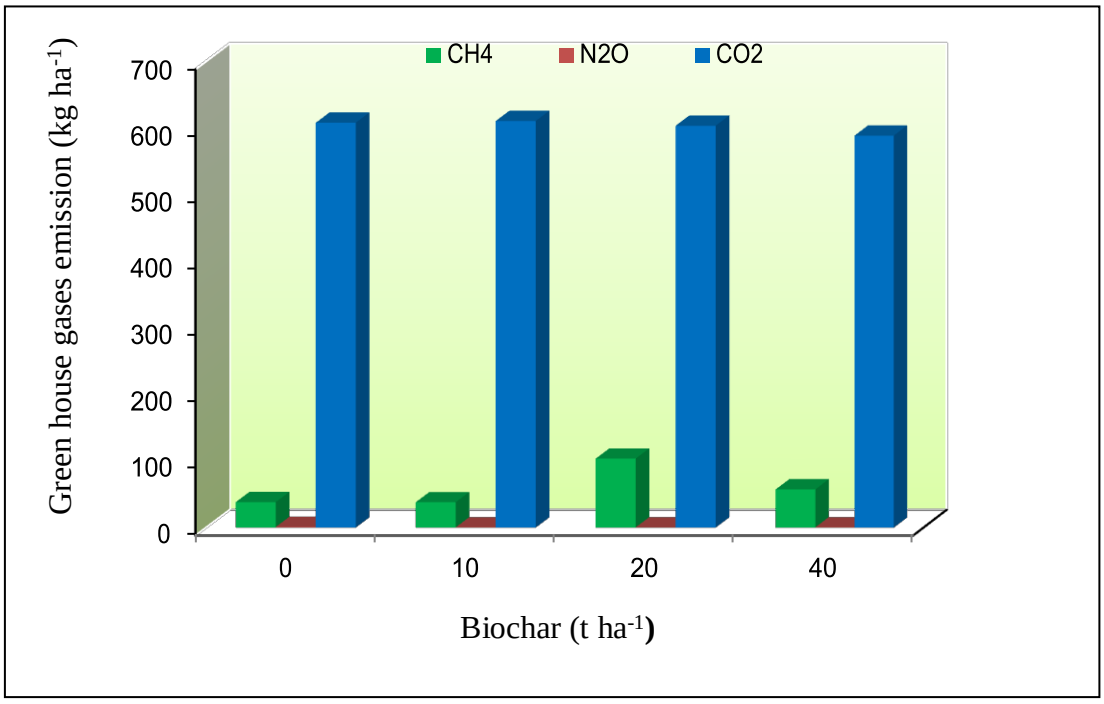

Figure 2.4. Green house gases emissions from paddy field after biochar applications. Modified from Zhang *et al.* (2012)

Although play significant role in CH_4 mitigation with promoting the methanotrophs number and reducing diversity of methanogens. Paddy is one of the largest anthropogenic sources of CH_4. **Figure 2.4** showed green house gases emission rate at different doses of biochar applied in paddy soils (Neue *et al*., 1993). Mukherjee and Lal (2013) reported that biochar amendment in soil increase the aeration and porosity therefore production of CH_4 decreases however oxidation rate increases and

later depends on both diffusion and methanotrophs activity in soil. Furthermore, the aerobic, well drained soils can be a sink for CH_4 due to the CH_4 diffusion and subsequent oxidation by methanotrophs (Fischer *et al.*, 2009). Hence two mechanisms involve here: (i) decrease the CH_4 production, and (ii) increase the CH_4 oxidation by methanotrophs may be operational in the biochar amended soil (Zwieten *et al*., 2009; Fischer *et al*., 2009; Mukherjee and Lal, 2013). According to Jien *et al*. (2013) after the incorporation of biochar in soil, was observed increasing level of microbial nitrogen and phosphorus in soil microbes at 63 and 105 days. But highest contents of microbial carbon were found at 21 days for each treated soil, which were 3200 mg kg^{-1} for 5% biochar- amended soil (Jien *et al*., 2013) which is showing that the amendment of biochar in soil supports the soil microbial growth, mostly methanotrophs which play significant role in CH_4 uptake. Therefore, an effective process to decrease CH_4 emission in paddy soil may be complete by the application of biochar (Lehmann *et al*., 2007). Previous work has shown that CH_4-oxidizing bacteria are readily enriched within landfill cover soil by exposure to the CH_4, generated from the waste material (Reddy, 2014).

2.6 CSR-BIO

Agriculture is an important source of farmer's income and foods it is contribute major share in Indian GDP (Approximately 15%), many anthropogenic factors are decreasing agricultural food production such as access use of chemical fertilizers, pesticide and insecticide, improper soil management and less irrigation etc. because food is a fundamental requirement to feeding human hunger, but increasing demand of higher food there is the need to accelerate the food production to satisfying the insistence hunger of human (Damodaran *et al*., 2011). Thus certain areas of India and world are facing various health challenges against inadequate and healthy food.

however many food agencies are working in this direction but due to lack of strong technology, food production ambulatory decreasing so all the problems are rising due to less agricultural crop production. Therefore, some sustainable and scientific technology based product (CSR-BIO, PGPR etc.) can enhance the productivity of soil and play constructive role in the higher crop production. The production of high value commercial crops is now being by all small farmers without any support of large land holders of developing countries including India (Dev and Chandrashekar, 2004). According to Damodaran *et al.* (2011) some small land holder with the desire of higher income are adopting cultivation of commercial crops using many categories of insecticide and synthetic fertilizers to get quick profit but they all are unaware from the harmful effect of its on soil health and increasing cost over the period of time. Therefore the cultivation practice of all commercial crops being by the poor and small land holder with the help of pesticide that is the environmentally harmful to all living being and also the cost of pesticides increasing the expenditure (Kamanula *et al*., 2011). Damodaran *et al.* (2011) reported that keeping this concern in consideration an eco-friendly bio-growth enhancer was developed under a strategic research project funded by National Agricultural Innovation Project of Indian Council of Agricultural Research (ICAR) with the support of World Bank. This product is produced by using microbial consortium such as CSR-B-2 (*Bacillus pumilus*), CSR-B-3 (*Bacillus thuringensis*) and CSR-T-1 (*Trichoderma harzianum*) called dynamic eco-friendly patented media and play vital role in normal and alkaline soils (Damodaran *et al*., 2013). CSR-BIO acts as a soil biomass enhancer, nutrient mobilize, soil vitalize, plant protectants against soil born diseases and growth enhancer for crops grown in normal and alkaline soils (Damodaran *et al*., 2013a). First time this technology was intervened in two blocks of Barabanki district in Uttar Pradesh, India to the small and

marginal farmers through training and demonstration (Manyong *et al*., 2001). This technology is based on low cost and as well as profitable to all farmers, therefore small farmer's can gain high crop production with low expenditure (Offermann and Nieberg, 2000). According to Damodaran *et al.* (2013) that after field demonstration of CSR-BIO in different crops like banana and tomato was found high yield with low expenditure for all over the period. The main aim of this commercialized microbial inoculums product is to enhance the income of all small and marginal land holders with low expenditure and without the use of pesticide and fungicide for the production of commercial crops.

3

Chapter-03
Experimental Site and Design

CHAPTER-3

EXPERIMENTAL SITE AND DESIGN

3.1 Description of study site and climatic conditions of the area

A field experiment for two consecutive years (2015-2016) with paddy cultivation, was carried out at Agriculture Research Farm of Babasaheb Bhimrao Ambedkar University located in Lucknow, Uttar Pradesh (India) 26^0 46' 05.51" N Lat. and $80^0$55' 39.50" E Long, with average altitude of 100-355 msl. The area experiences a marked seasonality i.e. rainy (June-September), winter (December-February) and summer (April-May). The study region had a hot sub-tropical climate with warm summers and cool dry winters. The May and June months of summer season are quite hot with a rise in temperature up to 45-48 ^{0}C. December and January months of winter season are extremely cool may goes as low as 4 ^{0}C or less. The normal period of onset of monsoon in this region is the 3rd to 4th week of June, which lasts up to end of September and receives average annual rainfall ranged from 900-1100 mm, most of which is received during the wet season of late June-October (Singh *et al*., 2011). Every year, the total rainfall (about 90%) is received during rainy months (monsoon season), however, from last few years it is highly erratic and unpredictable, causing drought spells of varying levels and durations. The soil of the experimental site is nutrient deprived (low organic C, N and P) having moderate water holding capacity, sandy and slightly saline in nature (Singh *et al*., 2011).

The average monthly and yearly temperatureand rainfall of the experimental area during the study period (July-October, 2015 and 2016) has been presented in **Table 3.1**During the study years the highest monthly temperaturein the year 2015 ranged from 26 to 31 ^{0}C and for 2016 it ranged from 31 to 37 ^{0}C. Likewise the

maximum average monthlyrainfall in 2015 ranged from was 0.05 to 6.43 mm, while in 2016 it ranged from 0.08 and 4.32 mm.It is clear from the data that the average monthly rainfall for both the study year was recorded for month of July. While the maximum monthly temperature was July (31 ^{0}C) for the year 2105 and August (37 ^{0}C) for 2016.

Table 3.1. Variations in temperature and rainfall during the study year- 2015 and 2016.

Months	Temperature (^{0}C)		Rainfall (mm)	
	Years			
	2015	**2016**	**2015**	**2016**
July	31	37	6.43	4.32
August	30	36	4.41	3.23
September	30	34	0.19	1.21
October	26	31	0.05	0.08

3.2 Experimental design and field setup

First of all the plant debris and concretes from the surface of land was removed manually on 25 June in 2015 and on 25 June during 2016. Total 12 experimental plots each having dimension of 3×2 meter was established in completely randomized block design (RBD) **Figure 3.1 and 3.2**. Four treatments i.e. rice husk biochar (RHB), CSR-BIO, and RHB+CSR-BIO, including one control plot (without any treatment) was also established in triplicate. Except control plot, RHB and CSR-BIO were applied at a rate of 10 t ha^{-1} as described respectively, by Zhang *et al*. (2010) and Damodaran *et al*. (2013). Before application, the CSR-BIO (4 kg) was incubated with 50 kg cow dung manure at 30 ^{0}C for five days. The required quantity of RHB and CSR-BIO as mentioned above were mixed thoroughly using mattock and hoes and then was applied to each experimental plot.

For this study, paddy (*Oryza sativa*)was selected as experimental crop. For this study the rice variety namely HUR 9-10 Hindu University Rice-9-10 was obtained from Department of Geneticsand Plant Breeding, Institute of Agriculture Sciences, Banaras Hindu University (South campus), Mirzapur, Uttar Pradesh. The nursery of rice cultivar was prepared on 20 June duringboththe years 2015 and 2016. After 25 days of nursery growth, thepaddy seedling was transfer to the experimental plots. Three hills (each with 2 seedlings) were transplanted on 25 July in both the years. Frequent irrigation (a water level of 6-12 cm) avoiding waterlogged condition, was provided throughout the crop cycle. At 105 day after transplantation (DAT), the matured paddy crop was harvested for the determination of selected paddy agronomic variables. The paddy agronomic variables such as panicle length (cm), tiller numbers (plant^{-1}), rice grain yield (t ha^{-1}) and paddy straw yield (t ha^{-1})was determined according to Mahamud *et al*. (2013) and Amanullah and Inamullah (2016).

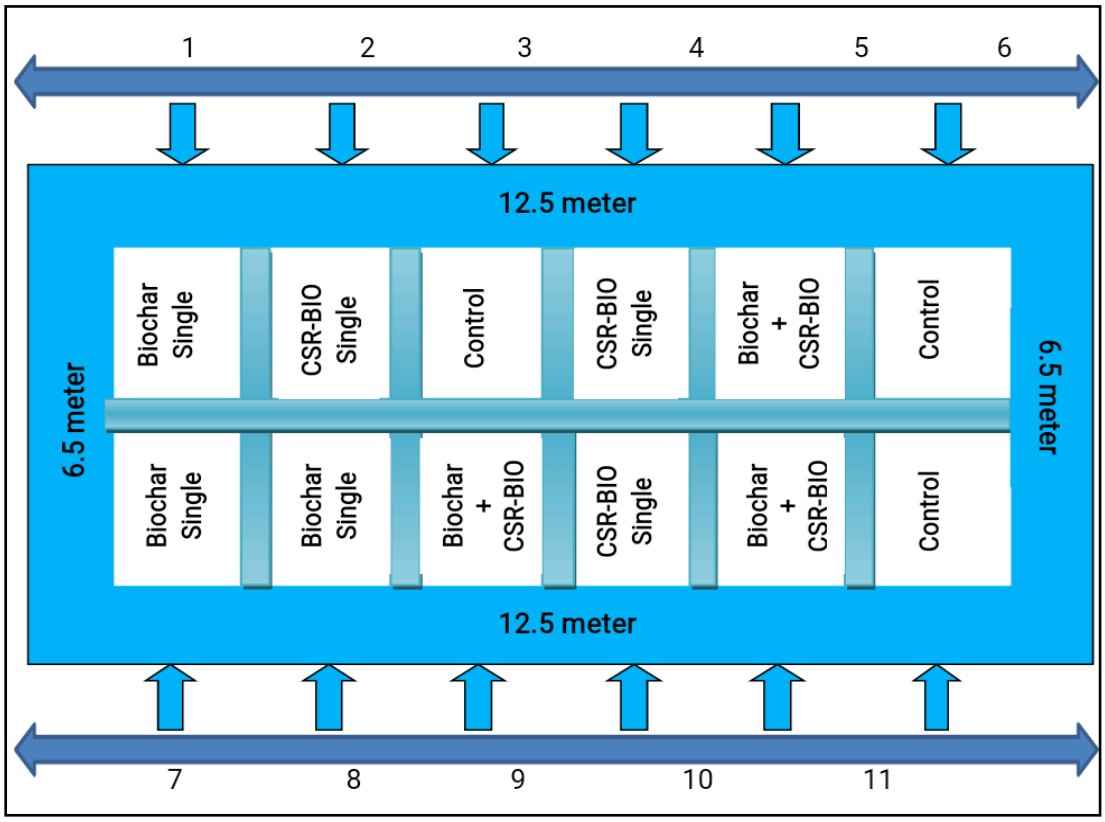

Figure 3.1. Experimental design for the cultivation of paddy crop during 2015 and 2016.

Figure 3.2. Experimental plots preparation for the transplantation of paddy seedlings (A) 2015 and (B) 2016.

3.3 Rice husk biochar (RHB) preparation and characterization

The rice husk was procured from rice mill for the preparation of RHB.The rice husk was properly dried in sun light radiation for 5-7 days to removethe moisture content. The production of RHB was carried out in limited supply of oxygen (controlledpyrolysis) in open environmental condition near the experimental site by drum method according to Srinivasarao*et al.* (2013). A drum (50 liter capacity) was partially filled with the completely dried rice husk and burning process was continued for 40 minutes at 250-300 ^{0}C (proposed temperature) to obtain high quality biochar (Milla *et al*., 2013). Thereafter, drum was left for cooling and dark-black colour RHB was harvested in air tight plastic bags for further application in experimental fields (**Figure 3.3**). Sopreparation of RHB from the rice husk by this method may be eco-friendly and cost effective to all small and marginal farmers who cannot afford high-scale pyrolysis plants for the production ofbiochar.

Figure 3.3. Preparation of RHB from rice husk by traditional drum method. Preparation of biochar during low temperature RHB pyrolysis processfrom the rice husk by this method may be cost effective and of good quality.

The surface morphology of rice husk (RH) and RHB was analysed to using scanning electron microscopy (SEM), with MODEL: JSM-6490LV, MAKE: JEOL, Japan). Dried sample was mounted on aluminium stub using double sided carbon tape and excess amounts of the sample were removed with a small manual air blower. The adhered sample was sputter-coated with palladium coater (auto fine coater JFC-1600 JEOL, Japan) and then transferred into the JEOL sample chamber for analysis (Prakongkep *et al*., 2013). The accelerating voltage was set at 15 kV; 200 and 500 times magnification were selected. The SEM image of RH (**Figures 3.4A and B**) was shown higher surface bulge than RHB (**Figure 3.6**) due to the higher volatile matter and tars but after the pyrolysis of RH at the higher temperature to remove the all

volatile matter, thus was obtained dark hollow RHB. Therefore, 20 µm pores size (**Figure 3.6-D**) was measured by the SEM analysis, which indicated that the large microbial community may attract inside the porous region of RHB.The porosity of RHB is depends on pyrolysis condition because during the pyrolysis, temperature was increased so at that time pores size also increased because micro pores were filled with tars ordecomposition products, which could easily be volatilizedwith increasing temperature.

The elemental or mineralogical studies of RH and RHB were carried out by scanning electron microscopy equipped with energy dispersive X-ray spectroscopy (SEM-EDX) under the different charring conditions were obtained following elemental composition such as Si, O, C (**Figure 3.5**) and C, O, Mg, Si, K, Ca, Zr and Pt respectively (**Figure3.7**). The maximum content of Si was found in RH therefore, the biochar produced from rice husk has a bettersoil conditioner(Liu *et al*., 2016).

Table 3.2: Nutrient analysis of RHB and cow dung manure of CSR-BIO applied as amendments to the paddy soil.

Amendments	**Available mineral nutrients (mg kg^{-1})**								
	Ash content (%)	**pH**	**N**	**P**	**Mg**	**Si**	**Ca**	**K**	**S**
RHB	49.42	8.2	1.12	0.98	184	168	225	176	0.19
Manure	ND	5.9	0.98	0.56	0.08	ND	125	0.45	0.09

ND = Not determined

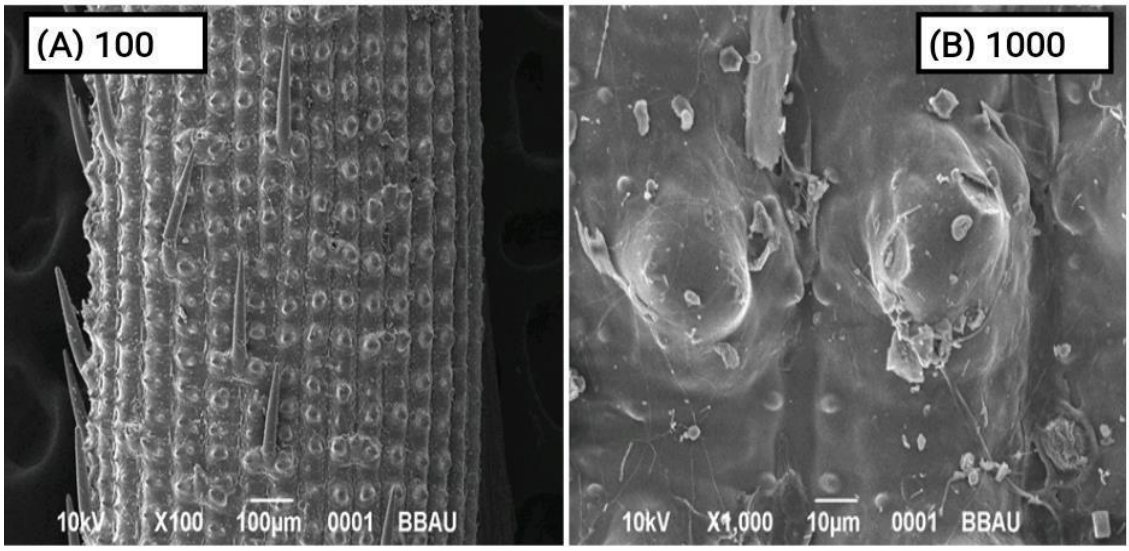

Figure 3.4. SEM images of rice husk (RH) at different magnifications.

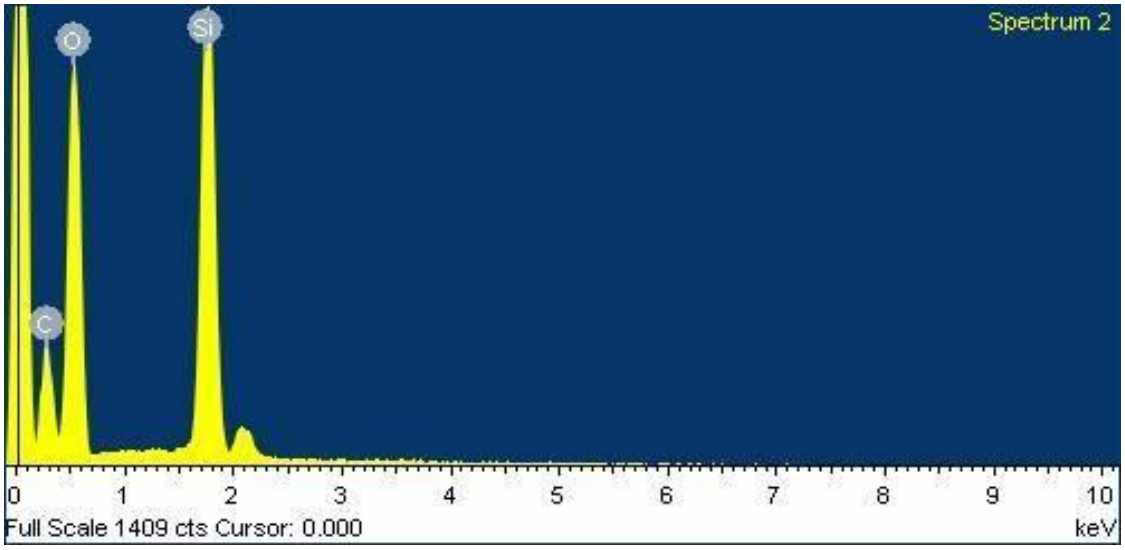

Figure 3.5. Elemental analysis of RH by SEM-EDX.

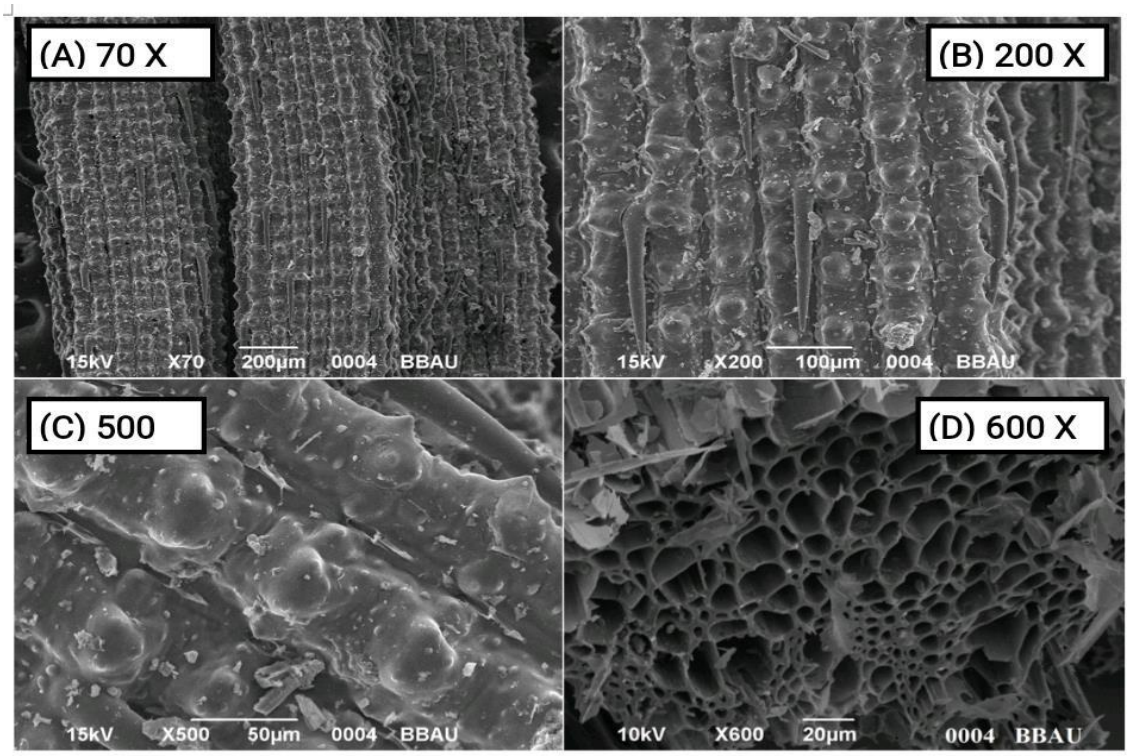

Figure 3.6. SEM Images of RHB at different magnifications

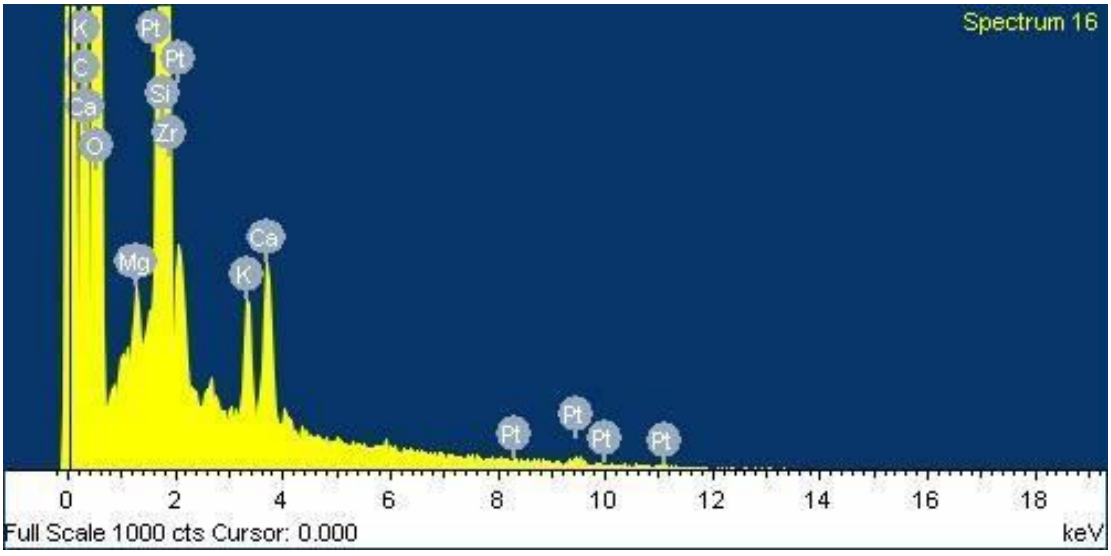

Figure 3.7. Elemental analysis of RHB by SEM-EDX.

3.3.1 Fourier-Transform Infrared Spectroscopy (FTIR) analysis of RH and RHB

The functional groups of RH and RHB compounds were categorized byFTIR. The FTIR is routinely used to determine the changesin various functional groups of molecule. **Figure 3.8** and **Figure 3.9** are depicting the FTIR spectra of the RH and

RHB respectively, producedfrom heating temperatures. In this study, we observed large number of functional groups in RH and RHB. All the functional groups were detected by vibration (stretching and bending) frequency of molecule with the absorption of infrared radiation therefore the study demonstrated a similar trend with a wavenumber/frequency, primarily RH functional groups were analysed (**Figure 3.8**). The O-H Stretching frequency were ~3700-3100cm^{-1} (peak at 3832.7 cm^{-1}), and similarly O-H stretching frequency ~3200-3550cm^{-1} (peak at 3427.7 cm^{-1}), C-H stretching frequency ~2900-3000 cm^{-1} (peak at 2920.1cm^{-1}), C-H stretching frequency were ~2850-3000 cm^{-1} (peak at 2854.0cm^{-1}), C=O stretching frequency ~1620-1680cm^{-1} (peak at 1637.4 cm^{-1}), N-O stretching frequency ~1515-1560cm^{-1} (peak at 1515.0 cm^{-1}), C=C stretching frequency ~1400-1600 cm^{-1} (peak at 1430.7 cm^{-1}), C-N stretching frequency ~1340-1250 cm^{-1} (peak at 1366.5 cm^{-1}) C-H stretching frequency ~1000-1300cm^{-1} (peak at 1161.4 cm^{-1}), C-O stretching frequency ~1050-1150 cm^{-1} (peak at 1059.5 cm^{-1}), C-H stretching frequency ~690-900cm^{-1} (peak at 794.7 cm^{-1}), C-Br stretching frequency ~750-500 cm^{-1} (peak at 592.6 cm^{-1}), all the above functional groups were shown approximate absorption frequency by chemical compounds were presented in RH and similarly RHB also shown different functional groups by the absorption of infrared radiation (**Figure 3.9**) such as O-H stretching frequency ~3650-3200 cm^{-1} (peak at 3404.5 cm^{-1}),and similarly,C-H group stretching frequency ~2960-2850 cm^{-1} (peak at 2949.5 cm^{-1}), C=O stretching frequency ~1750-1680 (peak at 1699.2 cm^{-1}), C=C bending frequency ~1700-1500 cm^{-1} (peak at 1589.1 cm^{-1}), N-H bending frequency ~1550-1640 cm^{-1}(peak at 1509.7 cm^{-1}), C=O stretching frequency ~1395-1440 cm^{-1} (peak at 1436.8 cm^{-1}), CH-OH stretching frequency ~1085-1125 cm^{-1}(peak at 1099.1 cm^{-1}), RCH=CR stretching frequency ~ 790-840 cm^{-1} (peak at 800.7 cm^{-1}), R-Br stretching frequency ~ 500-680 cm^{-1} (peak

at 664.2 cm-^{1}). Overall, the FTIR spectra displayed different functional groups among RH and RHB. Thus all the above functional groups were found in FTIR analysis of RH and RHB.

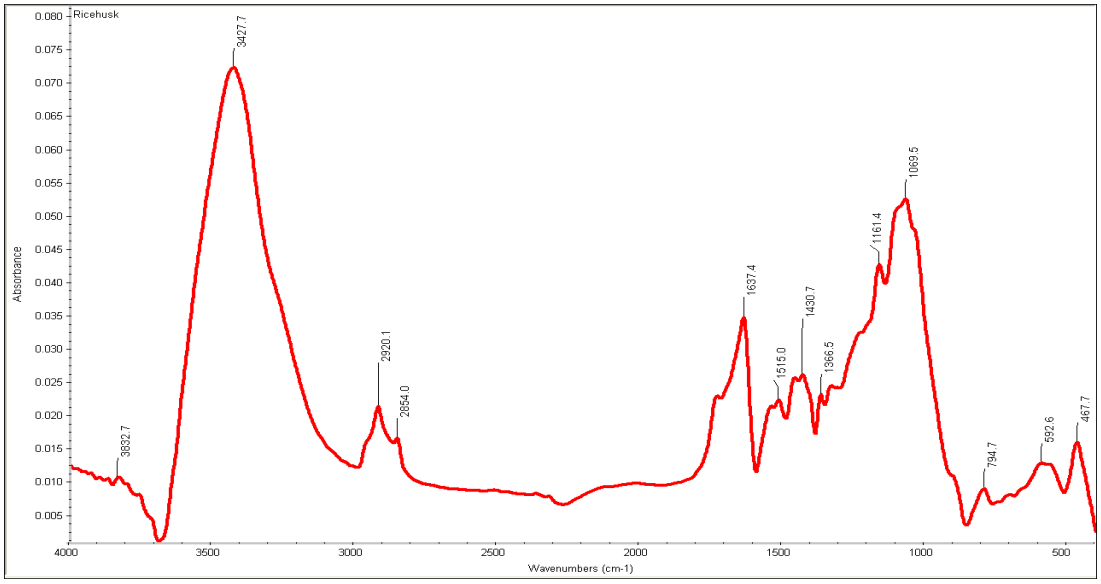

Figure 3.8. FTIR analysis of RH for the detection of functional groups.

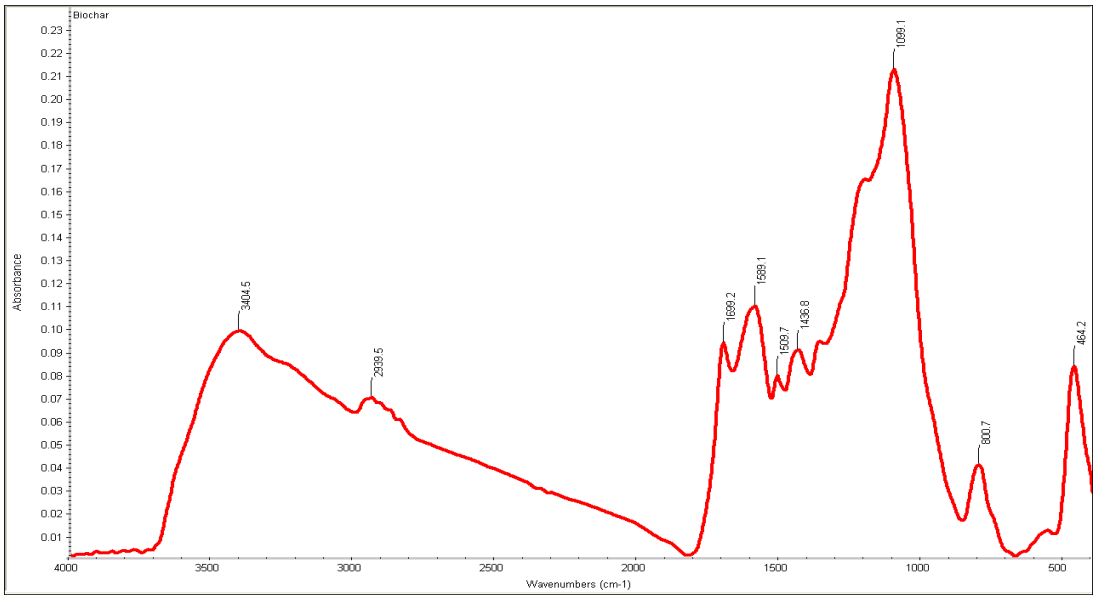

Figure 3.9. FTIR analysis of RHB for the detection of functional groups.

3.4 CSR-BIO (a commercial microbial bio-formulation)

A novel microbial bio-formulation known as CSR-BIO (**Figure 3.10**), developed by Central Soil Salinity Research Institute (CSSRI), Lucknow, India under a strategic research project funded by National Agricultural Innovation Project of Indian Council of Agricultural Research (ICAR) with the support of World Bank, was also used in this experiment. The CSR-BIO is prepared by using consortia of microbes and CSR-T-1 (*Trichoderma harzianum*), CSR-B-2 (*Bacillus pumilus*), and CSR-B-3 *(Bacillus thuringiensis)* cultured on dynamic eco-friendly patented culture media (Damodaran *et al*., 2013). The CSR-BIO is used as a nutrient mobilizer, soil conditioner; pathogen suppressor and growth promoters for crops cultivated in nutrient poor and disturbed soils. This is a low cost and profitable technology to all poor and marginal farmers, those cannot afford to high cost technology based product. CSR-BIO was produced in solid and liquid forms to improve the physico-chemical and as well as biological properties of normal and alkaline soil that gain higher crop production. This technology particularly dedicated to all small and poor landholders. They cannot generate high income with low expenditure (Damodaran *et al*., 2013).But we have used solid form,before the application of CSR-BIO in paddy field, properly incubated with cow dung at 30 °C for 7-8 days. Thus 4 Kg CSR-BIO was incubated with 50 Kg cow dung manure (**Figure 3.11**) at 30 ^{0}C for 7-8 days and after completion of incubation, 10 t ha^{-1} blend was applied in paddy soil (Damodaran *et al*., 2013).

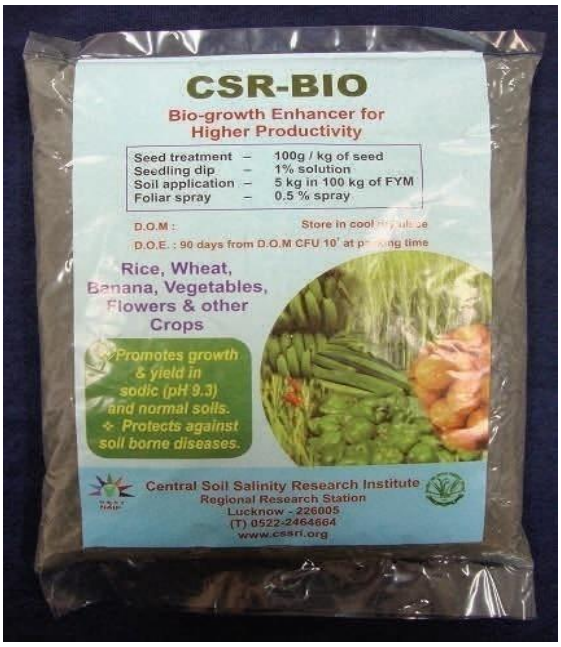

Figure 3.10.A packet of CSR-BIO procured from CSSRI, Lucknow.

Figure 3.11. The CSR-BIO was mixed with the cow dung manure to enhance the viability and survivability of inoculants and was incubated at 30 °C for 7-8 days before application in the paddy fieldsas described by Damodaran *et al*. (2013).

4

Chapter-04

To Assess the Impact of CSIR-BIO and Biochar Application on Soil Physico-Chemical Properties of Saline Paddy Fields

CHAPTER- 4

TO ASSESS THE IMPACT OF CSR-BIO AND BIOCHAR APPLICATION ON SOIL PHYSICO-CHEMICAL PROPERTIES OF SALINE PADDY FIELDS

4.1 Introduction

Soil salinity is a massive problem for the reduction of agriculture production (Singh *et al*., 2010). In the hot and dry regions of the world the soil salinityis one of major problems, responsiblefor soil stress environment and constrains the agriculture production. The salinity problems are due to the mismanagement of agricultural practices such as inadequate irrigation management and water logging, both are major factor to increase the salinity of normal soil and affects 20% of agricultural land worldwide (Glick *et al.*, 2007). Therefore, application of sustainable and eco-friendly soil improving materials in the saline and disturbed soils can be helpful strategies for increasing the soil health and production of agricultural crops.

In general, the increase in crop productivity will lead to sustainable enhancement in the area of farming lands but also somewhat enriching the soil fertility of the existing agricultural land could be the viable option (Mekuria *et al*., 2017). Therefore, it seems that application of amendments such as farm yard manures (FYM), microbial inoculants and biochar (consist of inherent mineral constituents) prepared from various crop residues, to nutrient poor soils could be one of the potential options to enhance the soil nutrient status, microbial diversity and agriculture productivity (Xu *et al*., 2017). It is suggested that the plant residues based biochar (10-15 t ha^{-1}), an alternative organic supplement to chemical fertilizers, could be a viable and sustainable way to enhance the crop yield (Agegnehua *et al*., 2017)in degraded nutrient poor soils (Kollah *et al*., 2015). From a long time, peoples were

well interested in the use of plant residues derived biochar as soil conditioners to enhance the soil fertility and crop productivity (Zhang *et al*., 2017). It seems that the use of biochar in agriculture can reduced significantly the adverse impact of chemicalization on soil health (Kim *et al*., 2017), soil microbial biodiversity (Luo*et al*., 2017) and agriculture productivity (Agegnehua *et al*., 2017). However, information about the use of crop residues in agriculture use related to soil fertility improvements and its effects on crop healthare scare. This work will provide some valuable information and will generate awareness about the use of rice crop residues in benefits of soil physico-chemical properties and paddy agricultural productivity in disturbed nutrient poor saline soils.

Many past studies have been carried out on the importance of biochar in different agricultural soils but significant results not found, such as nutrient status of the amended soils including CEC, pH, nutrient content, vegetative growth, C sequestration potential and as well as GHG emissions (Mukherjee and Lal, 2013). But, now biochar has been widely accepted as a soil fertility enhancer in all soils because many current studies are showing positiveeffects of biochar on soil nutrient status, C sequestration, GHG emissions and microbial community or soil biota (Glaser *et al*., 2002; Fowles, 2007; Lehmann *et al*., 2006; Lehmann *et al*., 2011; Singh *et al*., 2017), which are related to physico-chemical and as well as biological properties of soils (Berglund *et al*., 2011). But distinct feedstock mediated biochars do not have the same properties thus their characteristics are based on feedstock type, pyrolysis temperature, rate of heating slow versus fast pyrolysis) and duration of charring, etc. (Zimmerman *et al*., 2010; Mukherjee, 2011; Mukherjee *et al*., 2011; Zimmerman *et al*., 2011). Moreover the application of biochar as an amendment also depends on soil types. The characteristics feature of biochar are highest content of

carbon, large elemental composition and essential plant nutrients, level, large surface area and microspore can play vital role to soil microorganism as shelter or habitat. Large surface area and porosity depends on the pyrolysis temperature and volatile matter, present in the feedstock. It has been reported that the volatile matter of feedstock can be removed at higher temperature; therefore, higher temperature can generate large surface area and porosity inside the biochar because lower temperature does not remove tars and volatile compounds which can block pore spaces resulting in lower surface area and porosity so this effect is diminished. However, biochar is produced at higher temperature (>650 ℃) as the volatile matter is remove, thus found higher porosity and surface area (Braida *et al*., 2003; Rutherford *et al*., 2004; Mukherjee *et al*., 2011). These conditions of biochar can alters the soil physico-chemical and as well as biological properties of soil such as pore size distribution (PSD), bulk density (BD), water holding capacity (WHC), penetration resistance (PR), pH, electrical conductivity (EC), carbon, nitrogen, phosphorous, ammonium ions, nitrate ions, etc. According to Mukherjee and Lal (2013) biochar have different surface area even when it is produced from same feedstock due to differences in production conditions and more specifically the final pyrolysis temperature and time. Currently, various studies are conducting on application of biochar on physico-chemical and biological properties of soils (Glaser *et al*., 2002; Lehmann *et al*., 2011; Ding *et al*., 2016; Lu *et al*., 2014; Nelissen *et al*., 2015). Therefore, due to the improvement of soil properties the level of soil nutrients, water and crop productivity is increased. The physical and chemical properties of RHB can play key role to understand functions and mechanism of biochar in the improvement of soil's fertility (Ding *et al*., 2016). Asai *et al.* (2009) reported that biochar has higher porosity, and it could retain water in small pores and thus increase water holding capacity to assist

water infiltrate from the ground surface to the top soil through the large pores after heavy rain. Peake *et al*. (2014) reported that biochar application could increase available water capacity by over 22 %. Moreover, the formation and stability of soil aggregates could increase the crop production and the prevention of soil erosion (Amezketa, 1999). The capacity of soil aggregation increased ranging from 8 to 36 % after the application of RHB (Lu *et al*., 2014). Peake *et al*. (2014) and Nelissen *et al*. (2015) reported that biochar can increase the soil compactness by ver 10 %, decrease bulk density from 1.47 to 1.44 mg m^{-3}. Overall, the improved physico-chemical properties of soil such as bulk density, water holding capacity and aggregation ability, may increase the retention of both water and nutrients, which benefited to soil fertility directly and increases EC, pH, C, N, P, etc. (Ding *et al*., 2016). Soil pH is one of the important factors which decide the microbial mediated soil nutrients mobilization and can change the forms of soil nutrients (Ding *et al*., 2016). The ion exchange capacity is an important characteristic feature of soil which influences the soil structure stability, nutrient availability, soil pH, soil responses to fertilisers and other ameliorants (Hazleton and Murphy, 2007), retains water and nutrients in soils. Laird *et al*. (2010) found that the biochar amendments can significantly increased cation exchange capacity by 4 to 30 % in comparison to un-amended soils (Ding *et al*., 2016). Biochar application can be a best agricultural soil management practice for all types of soils.So this could be a low expenditure strategy for poor and marginal farmers whocannot afford highly expensive agro-fertilizers for agriculture. Therefore,use of biochars will be beneficial for future to satisfy the high food demand against huge growing human population to enhance the agriculture yields.

Furthermore, it has been reported that the incorporation of rice husk biochar (RHB) derived from rice husk into the soil could significantly improve the soil

physico-chemical properties of paddy fields compared with direct application of rice husk (Knoblauch *et al.*, 2011). Thus, RHB offers a good plant residues waste management to reduce air pollution due to on-site burning. As one of the crop residues in paddy field system, study shows clear evidences that biochar is highly effective for acidic and neutral soil, improve soil nutritional availability, plant growth and yield but not yet clearly know the effects of biochar amendment on nutrient poor tropical saline soils. The RHB, a co-product produced from a controlled pyrolysis of dry rice husk, can be used as a crops productivity enhancer as well as soil conditioner in agriculture. The application of biochar in soils is based on its properties as it improves the physico-chemical and biological properties of soils such as water holding capacity, soil nutrients retention and plant growth, nitrogen transformations, carbon sequestration and reduced green house gases emissions, particularly methane (CH_4) and nitrous oxide (N_2O). Most of the study has been conducted on the soil organic nitrogen dynamics. However, application of rice husk biochar and its impact on N-mineralization in paddy field condition is still lacking.

Nitrogen (N) mineralization is not only a primary step in soil organic N transformation, but is also, one of the most essential processes in soil N cycling. During the paddy crop cycle more than 50% of N is absorbed by rice plants from the soil.Inorganic-N release particularly ammonium-N, from the soil by mineralization is crucial to paddy crop N supply because paddy plants prefer ammonium-N as inorganic-N requirement. Various studies have shown the relationship between nitrogen mineralization and soil properties such as soil organic carbon, total nitrogen, pH and texture can affects N-mineralization process. During the process of microbial mediated N-mineralization, the organic-N is transformed in to inorganic-N. The pH and texture of the soil are important factors which affect the microbial community

composition in paddy soil. Microorganism and some plant species used soluble organic nitrogen as labile source of N for their growth.

Biochar amendment causes primary changes in physico-chemical properties of soil. For instance, an enhancement in crops yields, mainly in saline soil having low soil organic matter level has been reported. Biochar application increased total soil organic carbon but decreased theextractable organic C pool and soil nitrate. Although gross organic-N transformationrates were reduced by 50–80 %, the gross N-mineralization process remains unaffected but biochar application increases the ammonia-oxidizers microbial population in soil and subsequently more than two times higher inorganic-N rates was observed. Biochar can enhance the rate of N-mineralization by nitrogen-mediated bacterial community as well as ammonia-oxidizing bacterial community composition in paddy soil. The higher doses of biochar applicationin soil accelerated also the increment of ammonium contents in soil. A number of studies have been conducted from agro-ecosystem to terrestrial and forest ecosystems regarding ecology of methanotrophs population dynamics. But to date, uses of rice husk biochar on community composition and population dynamics of methane consuming bacteria from paddy field soils are missing. Therefore, this study focuses on impact of RHB in combination of microbial bio-formulation-CSR-BIO on soil physico-chemical properties and N-mineralization in paddy agriculture field conditions.

4.2 Material and methods

The details of experimental design and field preparation, paddy nursery establishment and transplantation, RHB and CSR-BIO treatment application, have already been described in **Chapter 3.**

4.2.1 Soil physico-chemical analyses

From each treatment plot soil samples (in triplicate) were collected and brought to laboratory for comparative analysis of soil physico-chemical properties. The soil samples were passed through a 2 mm mesh sieve and stored at 4 °C until analyses. The soil texture was analysed using sieves of different mesh sizes (Piper, 1944). Soil pH and EC was determined through a suspension sample with a soil (air-dried) to water ratio of 1:2.5 and measured with pH/conductivity meter (Model no: HI 5522; made in HANNA) according to Pansu and Gautheyrou, (2006) and Nigussie *et al*. (2012). Soil moisture was measured by oven-drying the soil samples at 105 ^{0}C for 48 hours as described by Black (1965). The water holding capacity (WHC) and bulk density of soil were estimated according to Piper (1944). Total-C was analysed by Walkley and Black rapid titration method (Walkley, 1947). Total-N and -P were measured by the method of Jackson (1958) as well as the estimation of ammonium-N and nitrate-N by potassium chloride solution (ISO, 14256-1) method.

For determination of inorganic-N contents (ammonium- and nitrate-N) and N-mineralization rates, soil samples were also collected in triplicates at 0, 35, 65 and 105 days after transplantation (DAT) of paddy plants. The net N-mineralization was calculated as the differencebetween the concentration of soil inorganic-N (ammonium- and nitrate-N) values after and before the incubation of soil samples and expressed in g^{-1} dry soil day^{-1} as described by (Singh *et al*., 2011).

4.2.2 Statistical analyses

The data recorded on different parameters were subjected to the analysis of variance (ANOVA) to find out the difference between different treatments. In cases where differences were found significant, means were compared for differences using least

significant difference (LSD) test at < 0.05-0.001. Statistical computer software IBM SPSS Statistics Version 20 was applied for computing both the ANOVA and LSD.

4.3 Results and discussion

4.3.1 Soil physico-chemical properties

Impact of rice husk biochar (RHB) and CSR-BIO treatments on physico-chemical properties of two consecutive years (2015-2016) paddy cultivation in nutrient poor soil have been given in **Table 4.1**. Compared to treated plots, maximum electrical conductivity (EC) (5.4 ± 0.15 and 5.7 ± 0.35 μmhoms^{-1} respectively, for 2015 and 2016) and pH (8.4 ± 0.20 and 8.5 ± 0.37 respectively, for 2015 and 2016) was noted for untreated (control) plot. For 2015 and 2016, across different treatments thegravimetric soil moisture (GSM) and water holding capacity (WHC) were highest (25.21 ± 0.16 and 26. 23 ± 0.17 % and 76.6 ± 0.57 and 77.8 ± 0.58 %, respectively) in RHB + CSR-BIO treated plots. Compared to treated plots, bulk density (BD) was lowest in control plot (1.19 ± 0.06 and 1.25 ± 0.08 gm cm^{-3}respectively, for 2015 and 2016). During both the years, among different treated plots the values of total-N, -C and -P were lowest (0.05 ± 0.005 and 0.05 ± 0.008, 0.61 ± 0.02 and 0.61 ± 0.05, 0.12 ± 0.009-0.12 ± 0.009 g kg^{-1} dry soilrespectively, for 2015 and 2016) in control plot than the treated soils. ANOVA revealed that studied soil physico-chemical characteristics such as EC (F=40.77; N=4; P= <0.001), pH (F=68.03; N=4; P= <0.001), SM (F=399.60; N=4; P= <0.001), WHC (F = 10.57; N= 12; P= < 0.005), BD (F=5.41; N=4; P= 0.025), total-N (F=116.25; N=4; P= <0.001), total-C (F=44.54; N=4; P= <0.001), and total-P (F=15.33; N=4; P= <0.01) varied significantly due to treatments (**Table 4.1**).

Table 4.1. Impact of rice husk biochar (RHB) and CSR-BIO amendment on physico-chemical properties of soil. The values for the each parameter for each year are average means of three replicates ±SE.

Treatments	Year	Parameters							
		EC (µmho ms^{-1})	**pH**	**Gravimetric soil moisture (%)**	**Bulk density (gm cm^{-3})**	**WHC (%)**	**Total-N (g kg^{-1} dry soil)**	**Total-C (g kg^{-1} dry soil)**	**Total-P (g kg^{-1} dry soil)**
Control	2015	5.4 ± 0.15	8.4 ± 0.20	19.23 ± 0.04	1.19 ± 0.06	60.57 ± 1.00	0.05 ± 0.005	0.61 ± 0.02	0.12 ± 0.009
	2016	5.7 ± 0.35	8.5 ± 0.37	19.35 ± 0.09	1.25 ± 0.08	60.68 ± 1.00	0.05 ± 0.008	0.61 ± 0.05	0.12 ± 0.009
RHB	2015	4.4 ± 0.20	7.1 ± 0.05	24.56 ± 0.11	1.14 ± 0.01	75.3 ± 0.57	0.12 ± 0.025	1.54 ± 0.26	0.14 ± 0.008
	2016	4.1 ± 0.19	7.1± 0.02	25.65 ± 0.12	1.11 ± 0.01	76.2 ± 0.64	0.13 ± 0.027	1.58 ± 0.27	0.15 ± 0.007
CSR- BIO	2015	4.8 ± 0.13	7.3 ± 0.20	23.60 ± 0.33	1.13 ± 0.05	70.2 ± 1.06	0.11 ± 0.015	1.59 ± 0.04	0.13 ± 0.012
	2016	4.9 ± 0.14	7.4 ± 0.24	24.56 ± 0.34	1.14 ± 0.06	71.2 ± 1.07	0.12 ± 0.016	1.62 ± 0.05	0.13 ± 0.015
RHB + CSR-	2015	4.4 ± 0.15	7.2 ± 0.05	25.21 ± 0.16	1.12 ± 0.03	76.6 ± 0.57	0.13 ± 0.015	1.75 ± 0.03	0.15 ± 0.013
BIO	2016	4.3 ± 0.16	7.1 ± 0.06	26. 23 ± 0.17	1.11 ± 0.02	77.8 ± 0.58	0. 14 ± 0.016	1.79 ± 0.03	0.15 ± 0.016
One-way	2015	F=40.77	F=68.03	F=399.60	F=5.41	F=6.73	F=116.25	F=44.54	F=15.33
ANOVA		N=12	N=4	N=4	N=4	N=4	N=4	N=4	N=4
		P=<0.001	P=<0.001	P=<0.001	P= 0.025	P=0.014	P=<0.001	P=<0.001	P=<0.01
	2016	F=35.12	F=69.05	F=406.63	F=6.03	F=7.05	F=118.21	F=46.53	F=17.21
		N=12	N=4	N=4	N=4	N=4	N=4	N=4	N=4
		P=<0.001	P=<0.001	P=<0.001	P=<0.001	P=<0.001	P=<0.001	P=<0.001	P=<0.001

Owing to the physical properties (large surface area and micro-pores) (**Figure 3.6, Chapter 3**) and elemental composition (N, P, K, O, Si, Mg, etc.), the application of RHB had the potential to alter the soil physico-chemical properties of disturbed paddy agriculture soil. The application of biochar to the soil had been noted for changes in both physical and chemical soil properties such as BD, WHC, pH, and cation exchange capacity (Dong*et al.*, 2015; Joseph *et al.*, 2009), soil aggregation (Major*et al.*, 2010), and soil compactness (Mukherjee *et al.*, 2011 and Chintala *et al.*, 2014). The addition of RHB to the paddy fields changes the soil properties due to intrinsic properties of biochar i.e. the surface charge, density, elemental composition and larger pore size distribution which are reliant on the nature of feedstock and low temperature pyrolysis conditions. Therefore, the chemical and physical properties of biochar applied to the soil directly influenced the microbial mediated available nutrient supply to crop plants (Luo *et al.*, 2017). However, the relationship between biochar and the soil micro-biota, and their implications on different soil processes in relation to crop productivity has not been described adequately and the exact underlying mechanisms are not well understood yet.

In this study, the occurrence of various opened pores on the rough and irregular surface of RHB is resulted due to evaporation of volatile substances during pyrolysis. The various pore shapes and sizes of RHB in present investigation ranged from 1.0 µm to > 20.0 µm, larger than the study of Dong *et al.* (2015) (>10.0 µm). As RHB pyrolysis during present experiments was performed at low temperatures (250 to 300˚C), higher ash content, elemental contents, pore size and surface area in RHB, might be expected (Major*et al.*, 2010). Owing to unique physical properties (large surface area and micro-pores) and elemental composition, application of RHB has the potential to alter the soil physico-chemical properties of paddy agriculture soil (**Table**

4.1). The addition of biochar brings about changes to both soil physical and chemical properties such as soil BD, WHC and pH (Chintala *et al*., 2013), cation exchange capacity (Joseph *et al*., 2009), aggregation (Major *et al*., 2010) and soil compactness (Mukherjee *et al*., 2011; Chintala *et al*., 2014). The changes in soil properties due to addition of RHB in mediated by the inherent properties of biochar, e.g., the surface charge, density and pore size distribution, which are dependent on the nature of feedstock and pyrolysis conditions. Therefore, the soil which is directly influenced by the chemical and physical properties of biochar may ultimately affect soil-plant-microbe interactions (Quilliam *et al*., 2013; Vimal *et al*., 2017). The relationship between biochar and the soil biota, and their implications on different soil processes have yet not been adequately described. At the moment, there is a wide gap in our knowledge of interactions between the soil biota and biochar. This calls for systematic and strategic investigation of soil-biochar dynamics to evaluate the potential consequences of widespread application of a seemingly wonderful product. Biochar can act as a soil conditioner by improving the soil physical properties. However, the underlying mechanisms are not yet well understood.

4.3.2 Soil ammonium-N, nitrate-N and N-mineralization

Across different treatments, inorganic-N (ammonium- and nitrate-N) levels and N-mineralization were minimum in control plots and maximum in RHB +CSR-BIO treated plots in both the years (**Table 4.2**). Across different sampling dates, ammonium-N and nitrate-N levels were noted minimum on 35 DAT (tillering stage) and maximum on 105 DAT (maturity stage) in both the years (**Figure 4.1 and Figure 4.2**). Across different sampling dates, N-mineralization was noted minimum on 0 DAT and maximum on 105 DAT (maturity stage) in both the years (**Figure 4.3**). ANOVA

indicated significant difference in ammonium-N, nitrate-N and N-mineralization due to sampling dates for 2015 (P = < 0.001) and 2016 (P = < 0.001) (**Table 4.3**).

Table 4.2. Average values of available inorganic–N (ammonium-and nitrate-N) and N-mineralization in rice husk biochar (RHB) and CSR-BIO treated paddy field across different treatments. The values are means of threereplicates ±SE derived from **Figure 4.1** and **4.2** for the two consecutive years 2015 and 2016.

Treatments	**Year**	**Parameters**		
		Soil ammonium–N (µg g^{-1}dry soil)	**Soil nitrate–N (µg g^{-1}dry soil)**	**N-mineralization (µg g^{-1}dry soil)**
Control	2015	5.7 ± 0.20	4.43 ± 0.15	1.16 ± 0.09
	2016	5.8 ± 0.42	4.46 ± 0.41	1.04 ± 0.05
RHB	2015	4.2 ± 0.10	3.33 ± 0.15	1.55 ± 0.02
	2016	4.3 ± 0.11	3.34 ± 0.19	1.71 ± 0.04
CSR-BIO	2015	4.2 ± 0.10	3.23 ± 0.05	1.77 ± 0.08
	2016	4.4 ± 0.18	3.25 ± 0.08	2.03 ± 0.07
RHB + CSR-	2015	4.83 ± 0.05	3.5 ± 0.10	2.01 ± 0.05
BIO	2016	5.84 ± 0.07	4.6 ± 0.14	2.45 ± 0.08
One-way	2015	F=15.56	F=10.17	F=20.72
ANOVA		N=12	N=12	N=12
		P=<0.001	P=<0.002	P=<0.001
	2016	F=23.76	F=11.23	F=23.87
		N=12	N=12	N=12
		P=<0.003	P=<0.001	P=<0.001

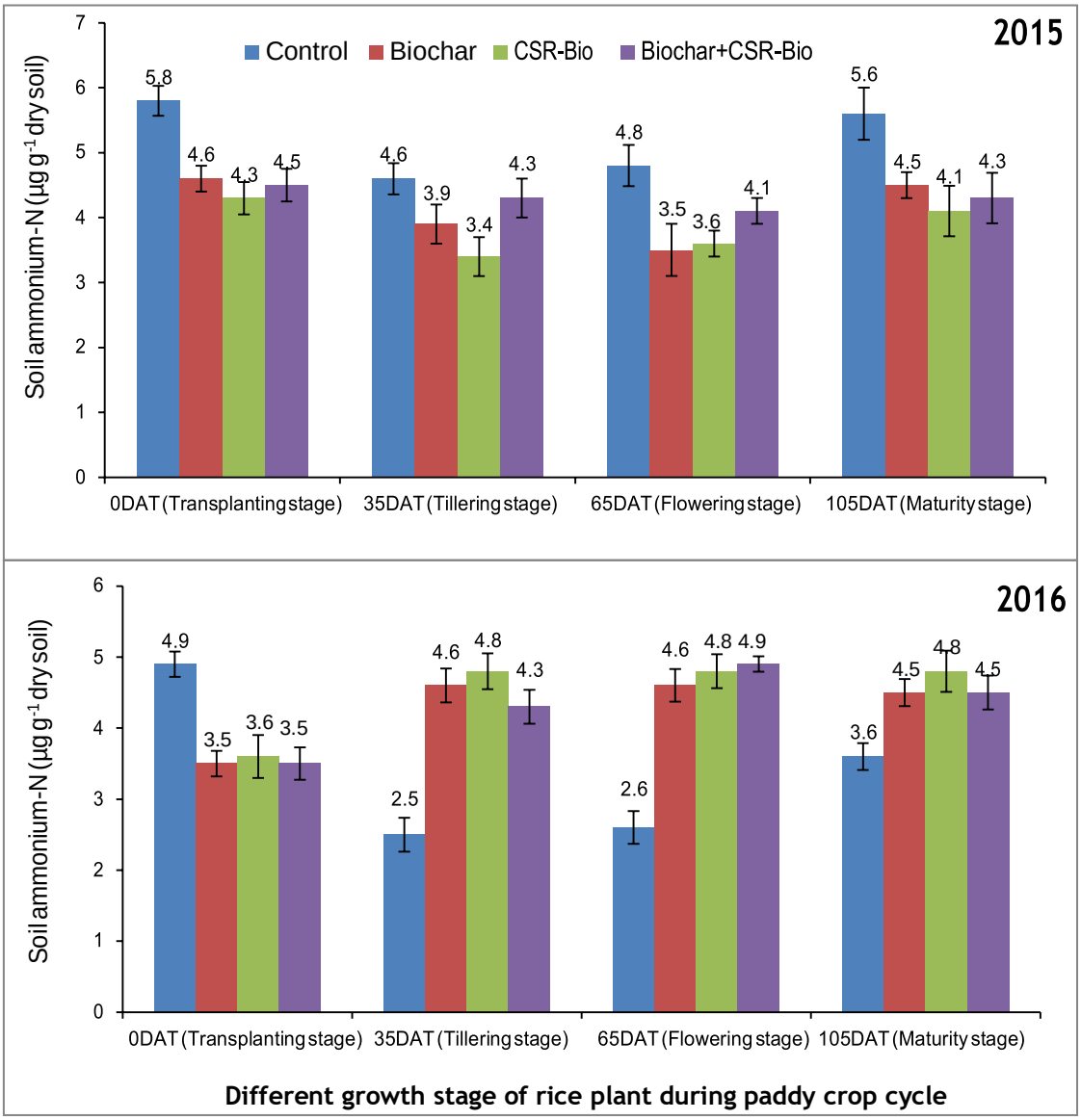

Figure 4.1. Variations in quantity of soil ammonium-N (µg g^{-1} dry soil) at different sampling dates after transplantation (DAT) during paddy crop cycle for the year 2015 and 2016. Vertical line on each bar represents ± 1SE. ANOVA to find out impact of sampling dates on available soil ammonium–N across different treatments has been given in **Table 4.3**.

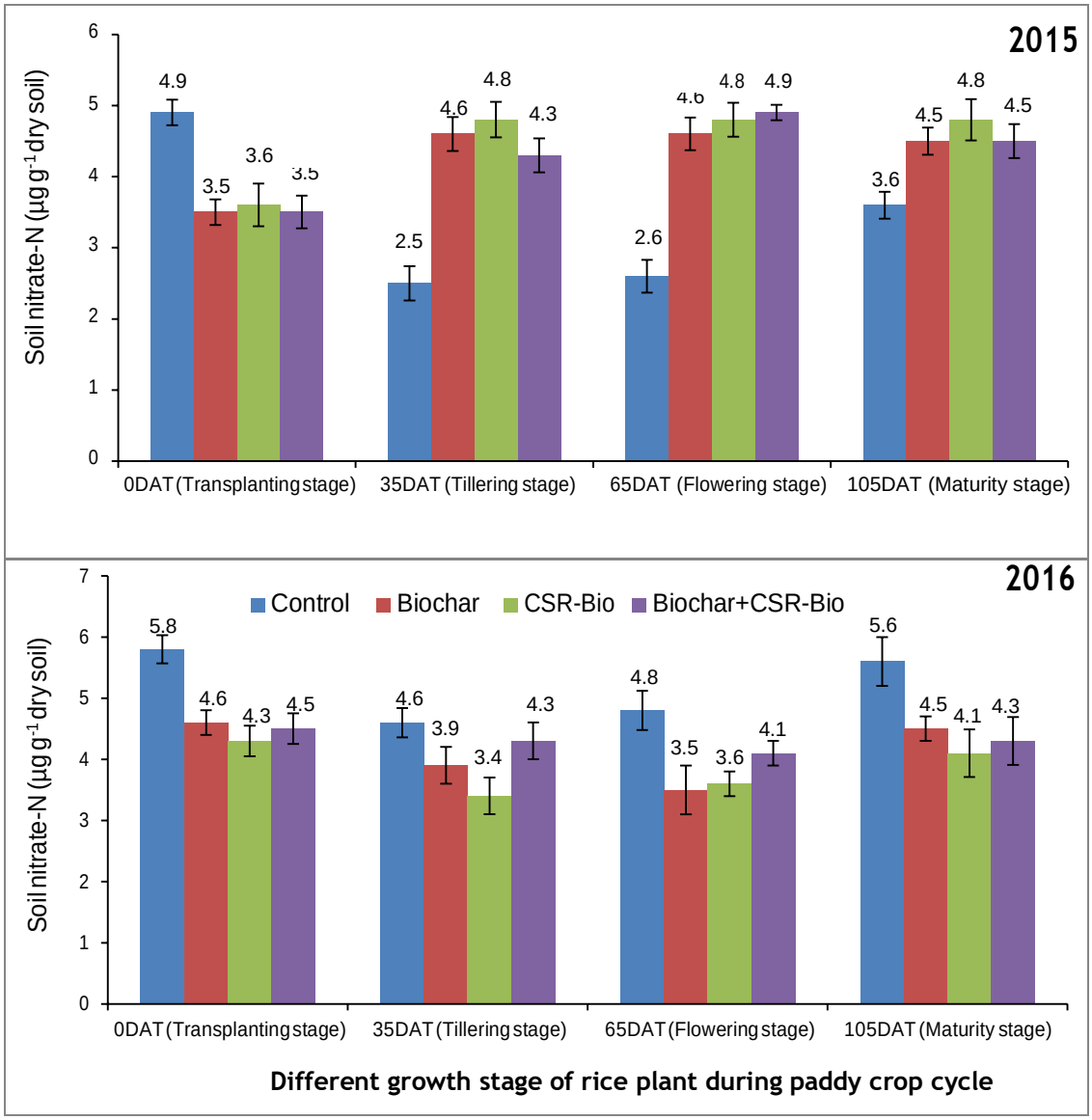

Figure 4.2. Variations in quantity of soil nitrate-N (µg g^{-1} dry soil) at different sampling dates after transplantation (DAT) during paddy crop cycle for the year 2015 and 2016. Vertical line on each bar represents ± 1SE. ANOVA to assess the impact of sampling dates on available soil nitrate–N across different treatments has been given in **Table 4.3**.

The higher level of available ammonium- and nitrate-N in soils of control plots compared to treated plots in present study could probably be due to reduced rates of N-mineralization, as biochar inhibits ammonification (DeLuca *et al.*, 2009)and also nitrification (Granatstein *et al.*, 2009). It has been also demonstrated that biochar application inhibits growth of soil micro-flora involved in soil mineralization and

nitrification soil N (Pratiwi and Shinogi, 2016) and therefore, low levels of inorganic-N in this study, might be expected. This could have occurred through some toxic agents present on the surface of the biochar (Kim *et al*., 2007) that inhibit the generation of available ammonium and nitrate-N, and also, the growth and multiplication of the concerned micro-flora (Singh *et al*., 2016; Singh, 2013; Singh 2014 and Singh, 2016). Since the paddy plants prefer inorganic-N (preferably ammonium-N) for N source, therefore, a greater demand for N-nutrients by effective paddy plant growth in RHB treated soils might have lower the ammonium-N, and perhaps this could be the reasons for the of negative relationship between paddy plant growth variables and inorganic-N values. As the data presented in this study, are based on short-term durations a more in-depth and long-term *in-situ* study are required to decipher the importance of various mechanisms responsible for low inorganic-N availability in RHB treated, nutrient poor paddy soils.

The application of rice husk biochar treatment on nitrogen mineralization across different sampling dates and years has been given in **Figure 4.3**. Across different treatments and study days, the highest nitrogen mineralization rate in both the years was observed in RHB+CSR-BIO amended soil on 105 days (1.83± 0.05 µg g^{-1}dry soil) in comparison to control showing lowest (1.26 ± 0.05 µg g^{-1} dry soil). ANOVA revealed significant variation in soil N-mineralization rate due to treatments. The higher rate of nitrogen mineralization in RHB+CSR-BIO amended soil could be due to improved soil conditions and CSR-BIO microbial consortium which was also applied in soil as a supporting amendment. Biochar also provides favourable conditions and space for the colonization of agriculturally beneficially micro-flora present in the soil. Microbial mediated N-mineralization of organic matter may depend on the amount and type of organic matter present in the. The microbial consortium of CSR-BIO applied in combination with biochar in this experiment may convert organic matter into ammonium-N and therefore, a higher rate of N-mineralization in such treatments might

be expected in paddy soils. Further, the porous characteristics of biochar not only provide a safe habitat for microbial growth and multiplication in soil but it also protects from soil predators such as protozoan's and nematodes. The highest nitrogen mineralization on 105 days could be due to increase in numbers of N-mineralizing microbes at this stage.

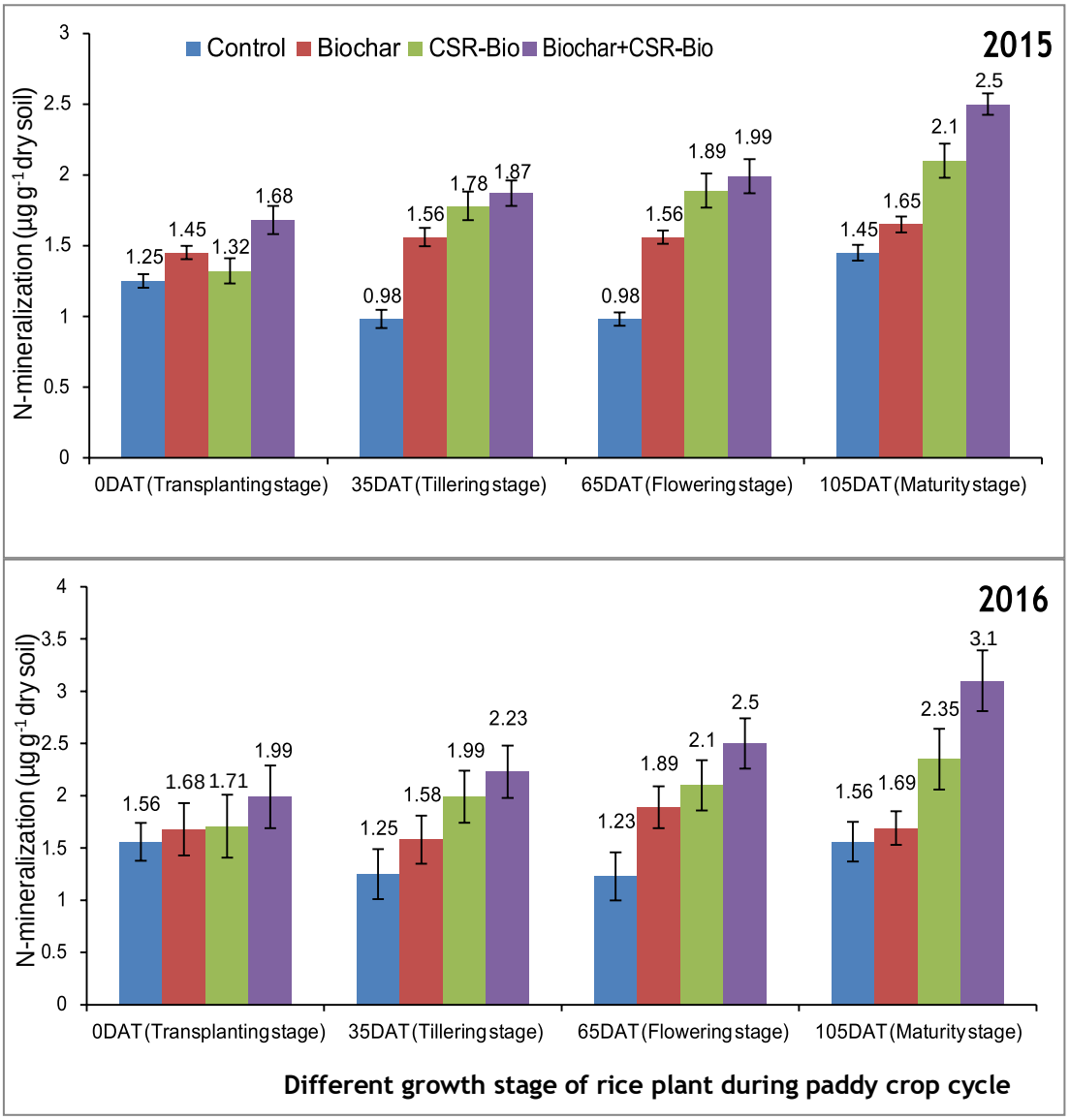

Figure 4.3. Variations inN-mineralization (µg g $^{-1}$dry soil) at different sampling dates after transplantation (DAT) during paddy crop cycle for the year 2015 and 2016. Vertical line on each bar represents ± 1SE. ANOVA to assessthe impact of sampling dates on N-mineralization across different treatments has been given in **Table 4.3**.

Table 4.3. One-way ANOVA for the impact of sampling dates on available inorganic–N (µg g^{-1}dry soil) and N-mineralization (µg g^{-1}dry soil) across different treatments. Pooled data across different sampling dates, derived from **Figure 4.1, 4.2** and **4.3** were considered for this analysis. Total number of samples analysed for each parameter was N=48 (4 treatments × 3 replicates × 4 sampling dates).

Year	Parameters	F	P
2015	Ammonium – N	14.96	<0.001
	Nitrate– N	08.79	<0.005
	N-mineralization rate	03.79	<0.001
2016	Ammonium – N	17.34	<0.001
	Nitrate–N	11.23	<0.003
	N–mineralization	07.56	<0.001

For each parameter, results of one-way ANOVA are reported, showing the significance of sampling dates.

4.4 Conclusions

The results showed that compared to treated plots, maximum electrical conductivity (EC) and pH, respectively was noted for untreated (control) plot in both the years. Across different treatments gravimetric soil moisture (GSM) and water holding capacity (WHC) were highest in RHB + CSR-BIO treated plots and lowest in control plots for each year. Compared to treated plots, bulk density (BD) was lowest in control plot. During both the years, among different treated plots the values of total-N, total-C and total-P were lowest in control plot than the treated soils. ANOVA revealed that studied soil physico-chemical characteristics such as EC, pH, SM, WHC, BD, total-N, C and -P varied significantly due to treatments.Across different treatments, inorganic-N (ammonium- and nitrate-N) levels and N-mineralization were minimum in control plots and maximum in RHB + CSR-BIO treated plots in both the years. Across different sampling dates, ammonium-N and nitrate-N levels were noted minimum on 35 DAT (tillering stage) and maximum on 105 DAT (maturity stage) in both the years. Across different sampling dates, N-mineralization was noted minimum on 0 DAT and

maximum on 105 DAT (maturity stage) in both the years. ANOVA indicated significant difference in ammonium-N, nitrate-N and N-mineralization due to sampling dates for 2015 ($P = < 0.001$) and 2016 ($P = < 0.001$).

This study demonstrates that RHB and CSR-BIO application improves the soil physico-chemical conditions that in turn enhance the availability of inorganic-N (ammonium- and nitrate-N) and rate of soil N-mineralization in paddy soil. The long term use of RHB may beneficial to enhance the soil nutrient status of nutrient deprived and saline soils. Thus, the plant residues after plant harvest such rice husk can be converted into RHB and in combination with CSR-BIO or other PGPR bio-formulations/amendments could be important strategies for enhancing the rate of soil N transformation and beneficial available N nutrients in poor paddy agriculture soil. This experiment was carried out only with the application of RHB and CSR-BIO, but othersuitable organic amendments, green manures, farm yard manure (FYM) derived from crops residues may be used for restoration of soil fertility of disturbed soils.

5

Chapter-05

To Find Out the Influence of CSR-BIO and Biochar Treatment of Paddy Fields

CHAPTER- 5

TO FIND OUT THE INFLUENCE OF CSR-BIO AND BIOCHAR TREATMENTS ON PADDY YIELDS

5.1 Introduction

Paddy, the world's third largest cereal crop after wheat and corn (Binod *et al*., 2010), is the basic food for nearly 50 % population on the Earth (Soest, 2006). Paddy is staple food crop of India and is cultivated on about 0.44 million hectare land, almost throughout the year. Annually approximately 1.1 million tons of rice is being produced and on the basis of production it occupies second position in the world. Almost 90% world rice cultivated and consumed in Asian countries (Hayashi, 2013). In these countries, paddy is the main food crop and is usually consumed two or three times a day, due to the good source of carbohydrates in medium and low-income countries (Lai *et al*., 2017). Fageria *et al*. (2003) reported that rice production must be increased almost 60 % up to 2025 which will be more than current productivity in order to fulfil the needs of an ever growing global population. The quantum of production has increased from 0.3 million ton in 1966 to nearly 1.1 million ton in recent years (Zhou *et al*., 2014).Soil nutrients play important role in cropping system, in which N is an essential macro nutrient because it is required by paddy in large amount and is more needed than any other nutrients during vegetative growth (Samonte *et al*., 2006). The application of N based chemical fertilizers for paddy production is justified through high grain yield and profitable farming; however excessive application of chemical fertilizers is not only costly but could detrimentally affect our environment (Anonymous, 2000). The more application of N based chemical fertilizers caused serious environmental concerns such as emission of nitrogen oxides (NOx), ammonia volatilization, leaching of nitrate and other reactive N species in ground and surface water resulting in water pollution and

eutrophication of streams and lakes (Golloway *et al*., 2008; Gupta *et al*., 2008; Velmurugan *et al*., 2008; Weligama *et al*., 2010; Kumar *et al*., 2012). Therefore, the current survey in many state of India found that Haryana have been highly affected by nitrate pollution to the ground water (Handa, 1986; Rawat and Singh, 2010).

As it has been demonstrated that the large application of N fertilizers cause a potentially alarming situation of environmental health hazards and indicates an urgent need to reduce excess use of these harmful fertilizers. There are many alternatives to adopted from last decades, e.g. organic and eco-friendly fertilizers. Hence, for the sustainable agriculture, the use of crop plant residues based biochar should be assessed not only for the higher agricultural production but also for climate change mitigation (Brentrup *et al*., 2008, Singh *et al*., 2017). The application of biochar single and along with microbial consortium in agricultural soil to improve physico-chemical as well as biological properties of soil and consequently contribute towards sustainable and higher productivity of paddy crops. Overall crop residue mediated biochar is a sustainable and profitableagriculture practice to all small farmers because after harvesting of paddy crops, huge quantity of crop residues are burnt on the field sites, resulting several problems of air pollution, loss of soil nutrients and beneficial microbial diversity (Lai *et al*., 2017). The conversion of rice husk and rice straws into biochar is well supported by the many researcher and beneficiaries because it is reduced the application of several chemical fertilizers as well as promotes the use of cost effective and recycled agricultural waste such as rice straw and rice husk (FAO, 2004). After pyrolysis of rice husk at 600 ^{0}C, formed Si-C bond and in this complex, C showed distinct chemical properties due to the higher Si content, therefore, C prevents from microbial degradation and exist for long time thus completing the carbon requirement of crops in soil (Parr *et al*., 2006; Guo and Chen, 2014; Jindo *et al*., 2014). According to Jaafar (2014) and Lone (2013) biochar application improves the physico-chemical and

biological properties of soil, suppressing soil-borne pathogens and acting as soil conditioner in nutrient poor soil. Liu *et al*. (2011) also reported that the rice straw biochar application altered the soil properties and decreased the CH_4emissions. Further, Kamara *et al*. (2015) proposed that rice straw and rice husk mediated biochar can enhance the crops yield through early transplantation of seedling (Lai *et al*., 2017). However, Kyaw (2016) found significant differences in rice yield by the application of rice husk and rice straw biochar comparison of un-amended crops.

The crop productivity loss due to low precipitation, soil salinity, soil nutrient leaching and poor agricultural practices, triggered interest to find out the ways to enhance crop rather sustainable (Singh and Pandey, 2013). In general, the increase in crop productivity will lead to sustainable enhancement in the area of farming lands (Mekuria *et al*., 2017). Therefore, it seems that application of biochar to nutrient poor soils could be one of the potential options to enhance paddy productivity (Xu *et al*., 2017). The plant residue biochar (10-15 t ha^{-1}), an alternative organic supplement to chemical fertilizers, could be the viable and sustainable way to enhance paddy yields (Agegnehua *et al*., 2017) in degraded nutrient poor soils (Kollah *et al*., 2015). From a long time, peoples were well interested in the use of biochar derived from plant residues, as soil conditioners to enhance soil physico-chemical properties and crop productivity (Zhang *et al*., 2017). The use of biochar in agriculture as the possible option to enhance agriculture soil fertility, the adverse impact of chemicalization on soil fertility (Kim *et al*., 2017) and agriculture productivity can be reduced significantly (Agegnehua *et al*., 2017). The role of biochar application in soil biological processes therefore, represents a frontier in soil science research, and needs detail investigations. It has been reported that compared to direct addition of rice husk, the incorporation of biochar derived from rice husk into soils could significantly improve the soil physico-chemical properties such as soil moisture content, WHC, BD, available-N nutrients, etc.

in the paddy fields (Knoblauch *et al*., 2011). Thus, generation of RHB from rice husk and other crop residues after crop harvesting, may be considered as the very viable crop residues waste management strategy to avoid the on-site burning of crop residues which has been considered as one of the key issues related to air pollution and the loss of soil microbial community and biomass. Study indicated that biochar application was good for acidic and neutral agriculture soil improvements (Agegnehua *et al*., 2017); however, the effect of biochar amendment to nutrient poor tropical soils is still not investigated. Though, investigations have been carried out concerning the application of several organic and inorganic amendments on the paddy crop yield (Zhang *et al*., 2010; Xu *et al*., 2017; Kim *et al*., 2017 and Luo*et al*., 2017), however, information is scare for the use of crop residue mediated RHB and microbial inoculants (CSR-BIO) on paddy yields in the field conditions. Therefore, in this study we investigated the impact of RHB derived from rice husk on paddy agronomic variables for two consecutive years 2015 and 2016 in the disturbed soil of dry tropical farming land.

5.2 Material and methods

The details of experimental design and field preparation, paddy nursery establishment and transplantation and RHB + CSR-BIO treatment application, have already been described in **Chapter 3**. For this study the paddy (*Oryza sativa*, Hindu University Rice-9-10 variety) was used as the experimental crop. The dry land rice variety, selectedfor present experiment was obtained from Department of Geneticsand Plant Breeding, Institute of Agriculture Sciences, Banaras HinduUniversity (South campus), Mirzapur, Uttar Pradesh.

Beside all the amendments (RHB and CSR-BIO), a blanket application of NPK fertilizer was also served as basal dose for paddy crop growth establishment. The experimental plots were given 30 kg K_2O and 60 kg P_2O_5 ha^{-1} respectively, in the form of potassium chloride (KCl) and single super phosphate ($CaH_6O_8P_2$). During the

experiment, total nitrogen (N) in the form of urea (CH_4N_2O) was given 150 kg ha^{-1}. During the crop cycle, this N was applied at three different times, i.e. 50 kg N ha^{-1} on transplanting day (July 15, 2015); 50 kg N ha^{-1} on 35^{th} day after transplantation (tillering stage), and 50 kg N ha^{-1} on 65^{th} day after transplantation (flowering stage).

The paddy nursery was shown on 20 June for the years 2015 and 2016 and after 25 days the seedling weretransferred to the experimental plots. Twenty-five-day-old seedlings (3 seedlings of equal heights per hill) of paddy were transplanted manually at a depth of 4-5 cm at a spacing of 12 cm 26 cm to three replicate plots. Frequent irrigation (a water level of 6-12 centimetres) avoiding waterlogged condition, was provided throughout the crop cycle. In both the years, at 105 day after transplantation (DAT), the matured rice plants were harvested, and then selected agronomic variables were determined. The paddy agronomic variables such as panicle length (cm), tiller numbers ($plant^{-1}$) and rice grain yield (t ha^{-1}) was determined according to Mahamud *et al*. (2013) and Amanullah and Inamullah (2016).

5.2.1 Statistical analyses

The data recorded on different paddy growth parameters were subjected to the analysis of variance (ANOVA) to find out the difference between different treatments. Statistical computer software IBM SPSS Statistics Version 20 was applied for present statistical analyses.

5.3 Results and discussion

Impact of RHB and CSR-BIO applications on paddy plant variables such as panicle length, tiller number, rice grain and paddy straw yield for two consecutive years (2015 and 2016) are given in **Table 5.1.** All the selected paddy agronomic parameters were found greater in treated plots (maximum in RHB + CSR-BIO treatment) compared to untreated (control) plot (**Figure 5.1 and 5.2**). ANOVA showed significant difference (P=< 0.001) in paddy agronomic variables due to treatments. The percent increase in

panicle length, tiller number, rice grain yield and paddy straw yields in RHB + CSR-BIO treated plot was comparatively than other treatments for both the years (**Table 5.1**). Further, it was interesting to note that among the various selected paddy agronomic variables the influence of RHB treatment was more efficient for rice grain yield (**Figure 5.3 and 5.4**). It has been well documented that biochar applications can increase availability of N nutrients to crops (Chan *et al*., 2008). The high levels of soil organic carbon accumulation in RHB amended plots (**Table 4.1 Chapter 4**) in present study can enhance N efficiency and increase paddy productivity in nutrient poor soils of tropical regions. The higher rice grain yield in present study is in conformity to the results of Dong *et al.* (2015) who also reported higher rice yields owing to RHB amendments. Though, the effect of biochar on the selected paddy agronomic variables (tiller numbers and panicle length) is difficult to elucidate because there is insufficient information based on the present experiment or previous studies (Pratiwi and Shinogi, 2016). However, the positive effects of RHB treatments on paddy yields in present study may be attributed to the nutrients directly available to the paddy plants by the RHB because of having sufficient trace elements (**Table 3.2 Chapter 3**). Further, the increase in paddy yield witnessed is basically due to increase in nutrient mobilization to the paddy crop plants from the rhizosphere soil which has been enabled by the inoculated microbial consortia present in CSR-BIO to harness the available nutrients from the soil strata in field condition.

Table 5.1. Impact of rice husk biochar (RHB) and CSR-BIO amendments on paddy plant growth variables (panicle length, tiller number) and productivity (rice grain and dry paddy straw yields) for two consecutive years. Total number of samples analysed for each year was N=12 (3 replicates × 4 treatments).

Treatments	Year	Paddy agronomic variables			
		Panicle length (cm)	Tiller number plant^{-1}	Rice grain yield (t ha^{-1})	Paddy straw yields (t ha^{-1})
Control	2015	17.66 ± 0.57	15.66 ± 0.57	2.57 ± 0.05	6.12 ± 0.33
	2016	17.83 ± 0.28	16.21 ± 0.21	2.59 ± 0.05	6.42 ± 66
RHB	2015	25.93 ± 0.40 (46.83 %)	27.66 ± 0.56 (76.63 %)	4.55 ± 0.05 (77.04 %)	8.15±1.11 (33.17 %)
	2016	27 ± 1 (51.43%)	28.66 ± 0.57 (79.13%)	4.87 ± 0.07 (88.03%)	8.62 ± 1.09 (34.27 %)
CSR-BIO	2015	24.50 ± 0.86 (38.73 %)	25 ± 0.59 (59.64 %)	3.65 ± 0.15 (42.02 %)	6.88 ± 1.03 (12.42 %)
	2016	25.08 ± 1.01 (40.66%)	26.66 ± 0.57 (66.63%)	3.93 ± 0.05 (51.74%)	7.41 ± 1.08 (15.42 %)
RHB +CSR-BIO	2015	26.66 ± 0.57 (50.96 %)	28.33 ± 0.53 (80.91 %)	5.68 ± 0.03 (121.01 %)	10.42±1.03 (70.26 %)
	2016	29.33 ± 1.52 (64.50%)	30.66 ± 0.57 (91.63%)	5.91± 0.07 (128.19%)	10.95±1.06 **(**71.18 %)
One-way ANOVA	2015	F=220.62; N=12; P=<0.001	F=267.65; N=12; P=<0.005	F=560.32; N=12; P=<0.001	F=312.56; N=12; P=<0.001
	2016	F=118.92; N=12; P=<0.007	F=340.02; N=12; P=<0.003	F=243.78; N=12; P=<0.001	F=321.78; N=12; P=<0.001

Values in parenthesis indicate % increase in agronomic parameters due to treatments over control.

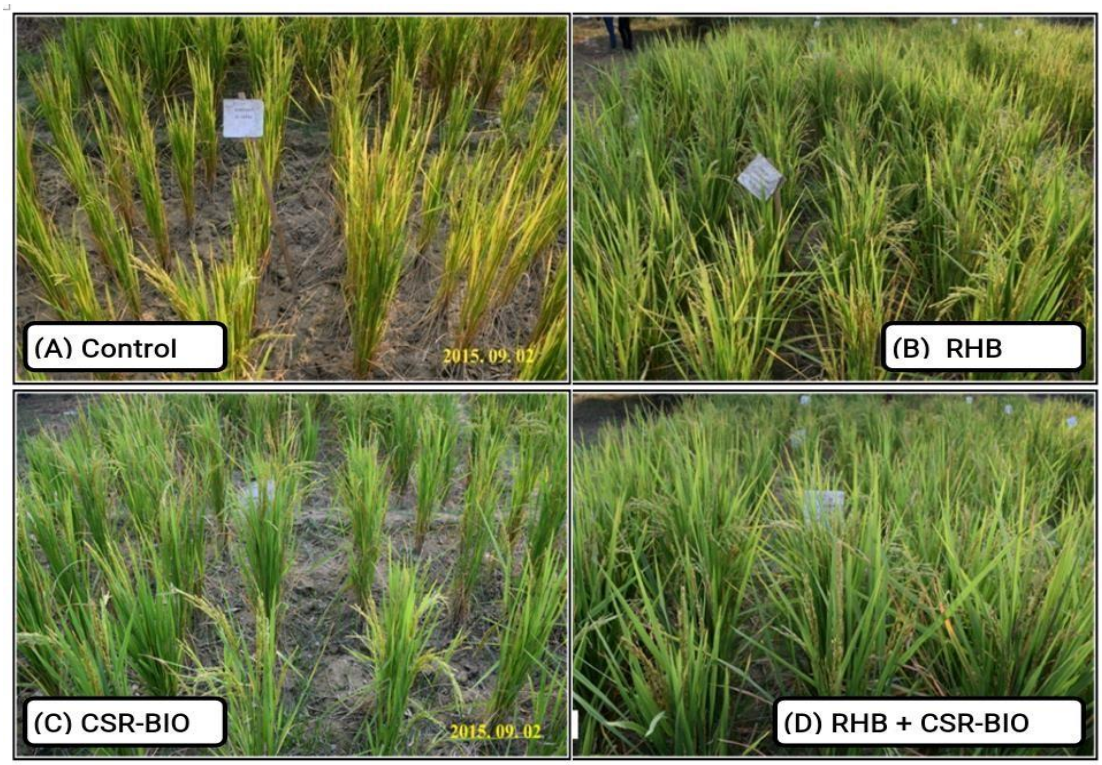

Figure 5.1. Variations in vegetative growth and agronomic variables of paddy plants due to different treatments. The photograph for each plot was taken almost from the same distance on October 05, 2015. The four treatments are: **(A)** Control (untreated), **(B)** Rice husk biochar (RHB), **(C)** CSR-BIO and **(D)** RHB + CSR-BIO.

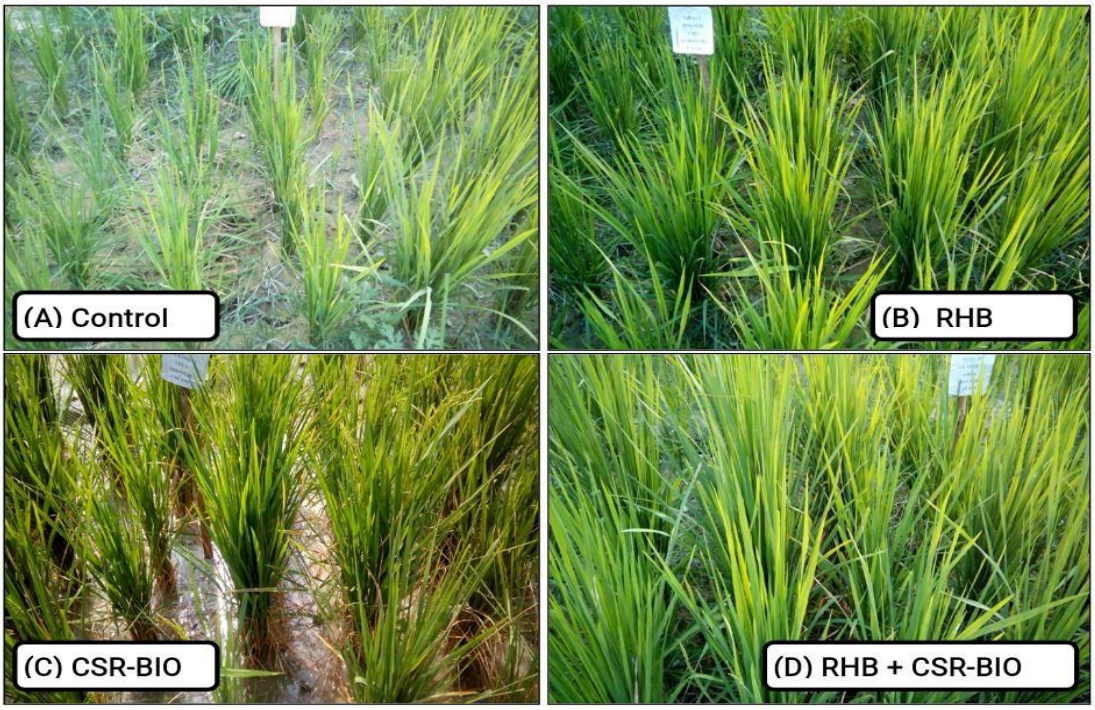

Figure 5.2. Variations in vegetative growth and agronomic variables of paddy plants due to different treatments. The photograph for each plot was taken almost from the same distance during October 2016. The four treatments are: (A) Control (untreated), (B) Rice husk biochar (RHB), (C) CSR-BIO and (D) RHB + CSR-BIO.

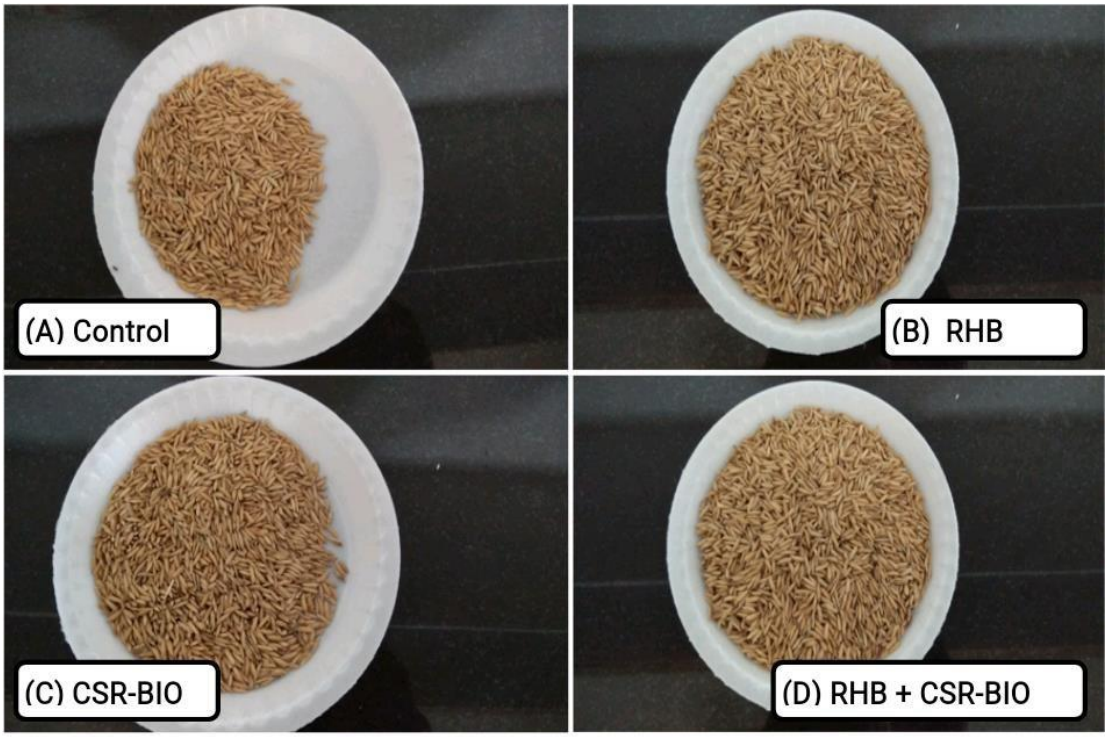

Figure 5.3. Differences in rice grain yields among the treatments for the study year 2015.

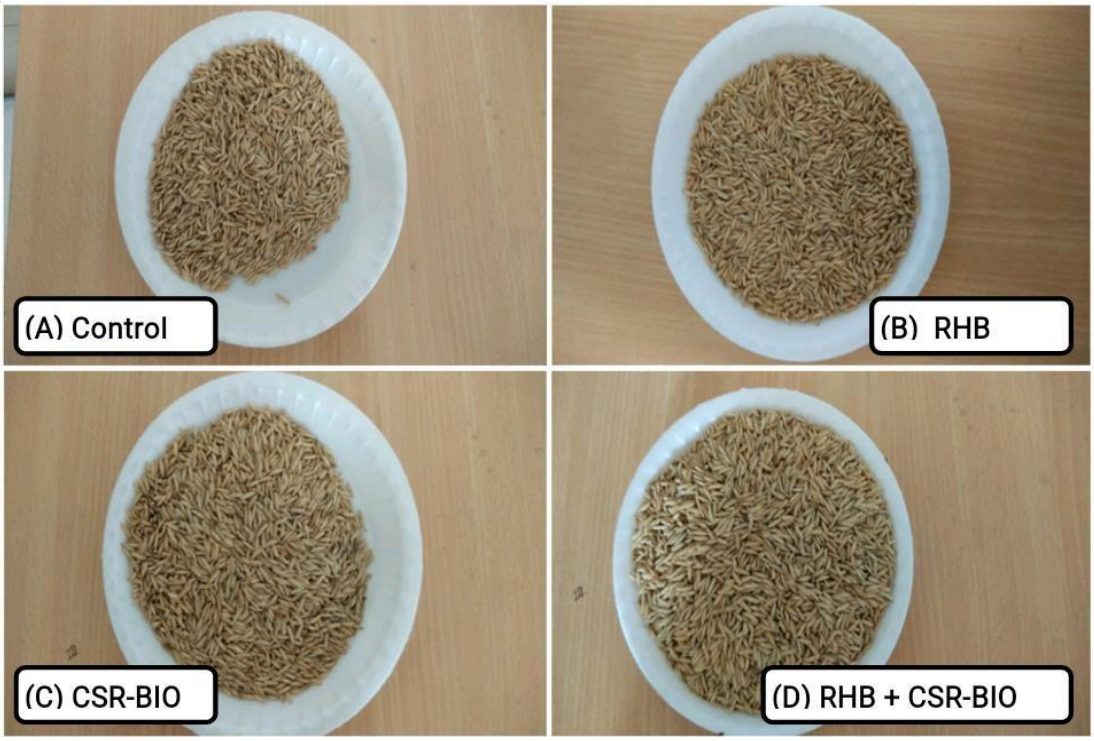

Figure 5.4. Differences in rice grain yields among the treatments for the study year 2016.

5.4 Conclusions

The results suggest that variation in paddy agronomic variables (variables such as panicle length, tiller number, rice grain and paddy straw yield) due to RHB and CSR-BIO treatments for two consecutive years (2015 and 2016) are statistically significant. All the selected paddy agronomic parameters were found greater in treated plots (maximum in RHB + CSR-BIO treatment) compared to untreated (control) plot. It was interesting to note that among the various selected paddy agronomic variables the influence of RHB treatment was more effective for rice grain yield.The paddy plant growth parameters (number of tillers and panicle length) and yields (rice grain and paddy straw) enhancement, following to RHB and CSR–BIO application, could be attributed to the synergistic effects of combined amendments on soil nutrients availability to paddy crop plants in nutrient poor soils. The RHB generation from paddy rice husk and its application with beneficial microbial inoculants may be a sustainable crop residues waste management option to enhance the nutrient status and paddy productivity of nutrient poor agriculture soils.

6

Chapter-06

To Study the Microbial Biomass-C, -N, and -P Variation as Affected by CSR-BI0 and Biochar Amendment

CHAPTER- 6

TO STUDY THE MICROBIAL BIOMASS-C, -N AND -P VARIATIONS AS AFFECTED BY CSR-BIO AND BIOCHAR AMENDMENTS

6.1 Introduction

The soil microbial biomass (SMB) quantity and its turnover rate of an ecosystem are considered widely as the soil fertility index that governs the ecosystem functioning and plant productivity (Singh *et al*., 2016a). Apart from paddy yield reduction, soil disturbances may interferes with the beneficial microbial community composition (Singh *et al.*, 2016a,b) and thus, SMB are also affected (Singh *et al*., 2010). The SMB, a living component of agro-ecosystems, soils also acts as available nutrients for plant uptake during nutrient release after death and decay of the microbial communities (Singh *et al*., 2009). Therefore, any changes in SMB values are recognised as an important indicator of soil nutrient dynamics and agro-ecosystems productivity (Warnock *et al*., 2010). Although, the soil nutrient status, seasonality, soil conditions, temperature and other factors are important drivers in controlling the functioning of an agro-ecosystems (Singh *et al*., 2016a), SMB could be also one of the vital factors that may affect the crop productivity of nutrient poor soils (Singh *et al*., 2010). The reports related to the affects of biochar addition to the SMB levels are not very clear (Rousk *et al*., 2013).

The application of rice husk biochar in agricultural soil is a sustainable and cost effective practisefor small and large scale agricultural cultivation. The key feature of biochar in agricultural soil, to maintain the efficiency of carbon content due to long term stable nature and displayed recalcitrant properties against microbial degradation (Lehmann and Joseph, 2009) because recalcitrant carbon is highly resistant to microbial attack and eventually less CO_2 is released back to the

atmosphere (Shackley *et al*., 2009; Azeem *et al*., 2010). Biochar functioning and properties are affected by pyrolysis temperature and high stability of biochar arises from the change in the chemical structure of the cellulose, hemicelluloses and lignin which take place at > 300 ^{0}C hence these properties are provide long residential time (100-1000 of years) in soil (Verheijen *et al*., 2009). Several short and as well as long term field studies have been carried out on the useful effect of biochar including SMB, soil microbial diversity, nutrient retention (Liang *et al*., 2006) or change in soil pH (Rousk *et al*., 2010), soil water retention, reduction of greenhouse gases emission and nitrate leaching, adsorption of toxic metals and agrochemicals (Spokas *et al*., 2009; Sohi *et al*., 2010) therefore most of the studies are supported and promoted the use of biochar in agricultural and environmental management which is ultimately leads to increasing the productivity of soil (Zwieten *et al.,* 2010). Likewise RHB can improve soil biological properties viz. soil microbial biomass, abundance and communities composition may beincreased with the application of biochar (Pietikäinen *et al*., 2000; Yin *et al.,* 2000; Kim *et al.,* 2007; O'Neill *et al.,* 2009; Liang *et al.,* 2010; Grossman *et al.,* 2010; Jin, 2010). Therefore these changes affects microbial community structures and nutrient cycling (Rillig and Mumme 2006; Steiner *et al*., 2008) and ultimately increase the soil microbial diversity and improve the plant growth (Warnock *et al.,* 2007; Azeem *et al*., 2010). Application of RHB is increased the soil microbial biomass due to the presence of labile C fractions (Bruun *et al.,* 2011; Zimmerman *et al*., 2011). Feedstock types and application rates of biochar can play significant role in improvement of soil microbialbiomass (Lehmann *et al*., 2011). SMB can be influence by the improvement in soil physico-chemical properties such asenhanced soil nutrients availability (C, N, and P), adsorption of toxic compounds, improve water holding capacity, maintain soil moisture and pH

level so all of these parameters influence the activity of microorganisms in biochar amended soil (Lehmann *et al.,* 2011). RHB has porous nature that can attract highest soil microbial population and protect them from various soil predators such as nematodes,protozoa, etc. and provide shelter to all soil beneficial microorganisms. Therefore, higher numbers of microbial population can reside inside the RHB pores (Pietikäinen *et al.,* 2000) for several soil nutrients on their surface (Saito and Muramoto, 2002; Warnock *et al*., 2007).

Study indicated that RHB application is good for acidic and normal agriculture soil properties improvements (Agegnehua *et al*., 2017) likewise, ample investigations have been carried out concerning the application of plant derived biochar on crop yields (Zhang *et al*., 2010; Kim *et al*., 2017; Luo *et al*., 2017; Xu *et al*., 2017) but limited information is available on RHB application on SMB dynamics and paddy yields. Therefore, in this objective we investigated the impact of CSR-BIO and RHB derived from rice husk on SMB quantity in paddy soil. The RHB amendment to the paddy soil will significantly improve the soil fertility via enhancing the availability of inorganic-N nutrients and SMB pools in nutrient poor and saline soils.

It has been proposed that SMB acts as a major source of plant nutrients in nutrient limited dry tropical deciduous forest soils (Singh and Gupta, 2017). Further, the quantity of SMB in agro-ecosystem can be considered as an index of soil fertility (Singh *et al*., 2007). It is noteworthy to mention that the SMB in natural and disturbed ecosystems acts as reservoir of important labile pools of C and mineral nutrients after their death and decay. Since, the soil of present agro-ecosystem is nutrient poor and disturbed in nature, addition of RHB to soil may provide better soil environment for the growth and multiplication of beneficial microbial abundance (SMB), present in

CSR-BIO (commercialized bio-formulation). The greater quantity of SMB in RHB+CSR-BIO treated soil, after their death and decay may be providing plenty amount of available nutrient pools to soils. Alterations in physico-chemical properties of paddy soils due to RHB and CSR-BIO application may correspond to variations in the SMB values. However, the experimental evidences for above arguments from filed conditions are lacking. Therefore, the main focus of this objective was to investigate the effect of biochar with or without microbial inoculants on SMB-C, -N and -P during two consecutive years 2015-2016during the paddy crop cycles.

6.2 Material and methods

The details of experimental design and field preparation, paddy nursery establishment and transplantation and RHB + CSR-BIO treatment application, have already been described in **Chapter 3**.

The soil samples collected on days 0, 35, 65 and 105 after paddy seedling transplantation, were analyzed for the SMB-C, -N and -P. The SMB-C, -N and -P (μg g^{-1} dry soil basis) 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 purpose (Srivastava and Singh, 1988). The 0.5 mol L^{-1} K_2SO_4 (1:4 soil: extract) was uses for the soil extraction for about 30 minutes. Similar extraction procedures were also adopted for no-fumigated soil samples. MB-C and MB-N in 0.5 mol L^{-1} K_2SO_4 soil extract was determined by dichromate digestion (Vance *et al*., 1987). The SMB-P was estimated as inorganic-P (Pi) in 0.5 mol L^{-1} $NaHCO_3$ extract of fumigated and non-fumigated samples by ammonium molybdate-stannous chloride method according to Sparling *et al*. (1985).

6.2.1 Statistical analyses

The data recorded on different parameters were subjected to the analysis of variance (ANOVA) to find out the difference between different treatments and sampling dates. Statistical computer software IBM SPSS Statistics Version 20 was applied for all the statistical analyses.

6.3 Results and discussion

The RHB and CSR-BIO applications to paddy soil in present investigation exhibited variations in soil physico-chemical properties (**Table 4.1, Chapter 4**), which correspond to strong differences in SMB-C, -N and -P due to treatments and sampling dates during two consecutive years (2015-2016) paddy cycle. Across the treatments highest quantity of SMB-C, -N and -P (410.68, 84.42and 27.86µg g^{-1} dry soil, respectively) was observed in RHB + CSR-BIO treated plots and variations in SMB-C, -N and-P due to treatments were significant (P = < 0.001) (**Table 6.1**). A significantly higher SMB-C, -N and -P in RHB and CSR-BIO treated soil could probably be due to the higher microbial multiplication rate or microbial abundance in biochar-amended soils (Steiner *et al*., 2008). However, little direct evidence is available for nutrient-related effects of biochar on microbial community compositions and biomass (Warnock *et al*., 2010). Warnock *et al*. (2007) suggested that biochar pores may act as a shelter place or microhabitat for microbial colonization, where they are protected from being grazed upon by their natural predators (microbial feeding biota). A greater microbial population, under nutrient limiting conditions, may be increased by slightly greater microbial nutrient availability, either due to biochar-driven improvements in nutrient retention on the RHB surface or due to nutrients that are released by the biochar (Lehmann *et al*., 2011). Though, the porous structure of biochar, its high internal surface area and its ability to adsorb soluble organic matter,

gases and inorganic nutrients are likely to provide a highly suitable habitat for microbes to colonize, grow and reproduce (Warnock *et al*., 2010) therefore, a higher microbial community and SMB in RHB treated soil (**Table 6.1**) resulted in this study. A greater microbial colonisable surface area due to biochar amendments may increase the microbial biomass is also shown for sediments and coarse-textured soils (Yamamoto and Lopez, 1985). Rillig *et al*. (2010) reported that biochar application in soils could stimulate the spore germination of fungi that may be another mechanism potentially leading to increased microbial populations and consequently higher SMB values. In RHB treated soils, the large biochar surface area (**Figure 3.6, Chapter 3**) (Liang *et al*., 2006; Downie *et al*., 2009) and greater WHC, biochar may retain moist pore spaces that provide favourable moisture levels for microbial communities in dry tropical soils of present study.

Table 6.1. Variations in quantity of soil microbial biomass-C, -N, -P in rice husk biochar (RHB) and CSR-BIO treated paddy field. The values for the each parameter are average means of three replicates ± SE. Total number of soil samples analysed for each parameter and year was N=12 (3 replicates × 4 sampling dates).

		Parameters		
Treatments	**Year**	**SMB – C (µg g^{-1}dry soil)**	**SMB – N (µg g^{-1}dry soil)**	**SMB – P (µg g^{-1}dry soil)**
Control	2015	223.00 ± 1.00	13.66 ± 1.52	11.33 ± 0.54
	2016	223.65 ± 14	13.81 ± 1.14	11.43 ± 0.41
RHB	2015	307.33 ± 2.51	53.66 ± 1.52	25.66 ± 0.57
	2016	309.56 ± 2.86	53.78 ± 1.89	25.75 ± 0.61
CSR-BIO	2015	326.66 ± 1.52	55.66 ± 1.52	26.33 ± 2.08
	2016	326.71 ± 1.52	55.94 ± 1.65	26.48 ± 2.09
RHB +	2015	408.66 ± 0.57	83.33 ± 2.08	26.66 ± 1.52
CSR-BIO	2016	410.68 ± 0.64	84.42 ± 2.09	27.86 ± 1.78
One-way	2015	F=6969.72;	F=24.32;	F=19.82;
ANOVA		N=12;	N=12;	N=12;
		P=<0.001	P=<0.001	P=<0.001
	2016	F=2536.54;	F=36.54;	F=56.25;
		N=12;	N=12;	N=12;
		P=<0.004	P=<0.001	P=<0.001

Across different sampling dates, the SMB-C, -N and-P was recorded minimum on 65 DAT (tillering stage) and maximum on 105 DAT (maturity stage), respectively given in **Figures 6.1, 6.2 and 6.3**. ANOVA indicated significant differences (P = < 0.001) in SMB-C, -N and-P quantity due to treatments and sampling dates in both the years 2015 and 2016 (**Table 6.2**).

Table 6.2. One-way ANOVA to assess the impact of sampling dates on soil microbial biomass-C, -N and -P (µg g^{-1}dry soil) across different treatments was applied. Pooled data across different sampling dates for each parameter from the respective **Figure 6.1, 6.2** and **6.3** were considered for this analysis. Total samples analysed for each parameter was N= 48 (4 Treatments × 4 sampling dates × 3 replicates).

Year	Parameters	F	P
2015	SMB–C	05.08	<0.003
	SMB–N	34.57	<0.001
	SMB–P	44.10	<0.001
2016	SMB–C	04.36	<0.001
	SMB–N	32.25	<0.001
	SMB–P	47.23	<0.005

For each parameter, results of one-way ANOVA are reported, showing the significance of sampling dates.

The low levels of SMB-C, -N, and -P in this study on 65 DAT (tillering stage) could be due to a greater demand of inorganic N nutrients by the paddy crop plants during the panicle and tiller initiation stage (active paddy crop growth period) that may limit the nutrient availability to soil microbes and consequently, lower SMB-C, -N and -P. This situation reflected a negative relationships between inorganic-N (ammonium and nitrate-N) and SMB-C, -N and-P (**Table 6.3**).

Table 6.3. Pearson's correlation (2-tailed) between soil microbial biomass (SMB) and inorganic-N nutrients (data were taken from **Table 4.2**, **Chapter 4**) in RHB and CSR-BIO treated paddy agriculture soil. Total number of soil samples analysed was N = 12 (4 treatments × 3 replicates).

Parameters	**SMB-C**	**SMB-N**	**SMB-P**	**Nitrate-N**
SMB–N (µg g^{-1}dry soil)	0.989^{**}			
SMB–P (µg g^{-1}dry soil)	0.796^{**}	0.851^{**}		
Nitrate –N (µg g^{-1}dry soil)	-0.689^{*}	-0.754^{**}	-0.970^{**}	
Ammonium –N (µg g^{-1}dry soil)	-0.498^{NS}	-0.596^{*}	-0.883^{**}	0.925^{**}

NS= Not significant; *Significant at P = < 0.05; **Significant at = P < 0.001.

The higher soil SMB-C, -N and -P on 105 DAT (crop maturity stage) values in present study could be due to the reduced demand of inorganic-N nutrients by the matured crop (less active paddy crop growth period) and so, nutrients might be easily available to microbes and consequently a higher SMB. Furthermore, the situation of higher SMB levels at paddy crop maturity sampling date (105 DAT) may arise because of the higher microbial N-immobilization, as available-N nutrients demand by matured plants probably be extremely reduced.These situations make easy availability of nutrients to micro-flora and consequently a higher SMB build-up. In view of the above arguments, when the pooled data across different sampling days were considered for correlation analysis, a negative relationship between SMB and inorganic nutrient parameters might be expected (**Table 6.3**).

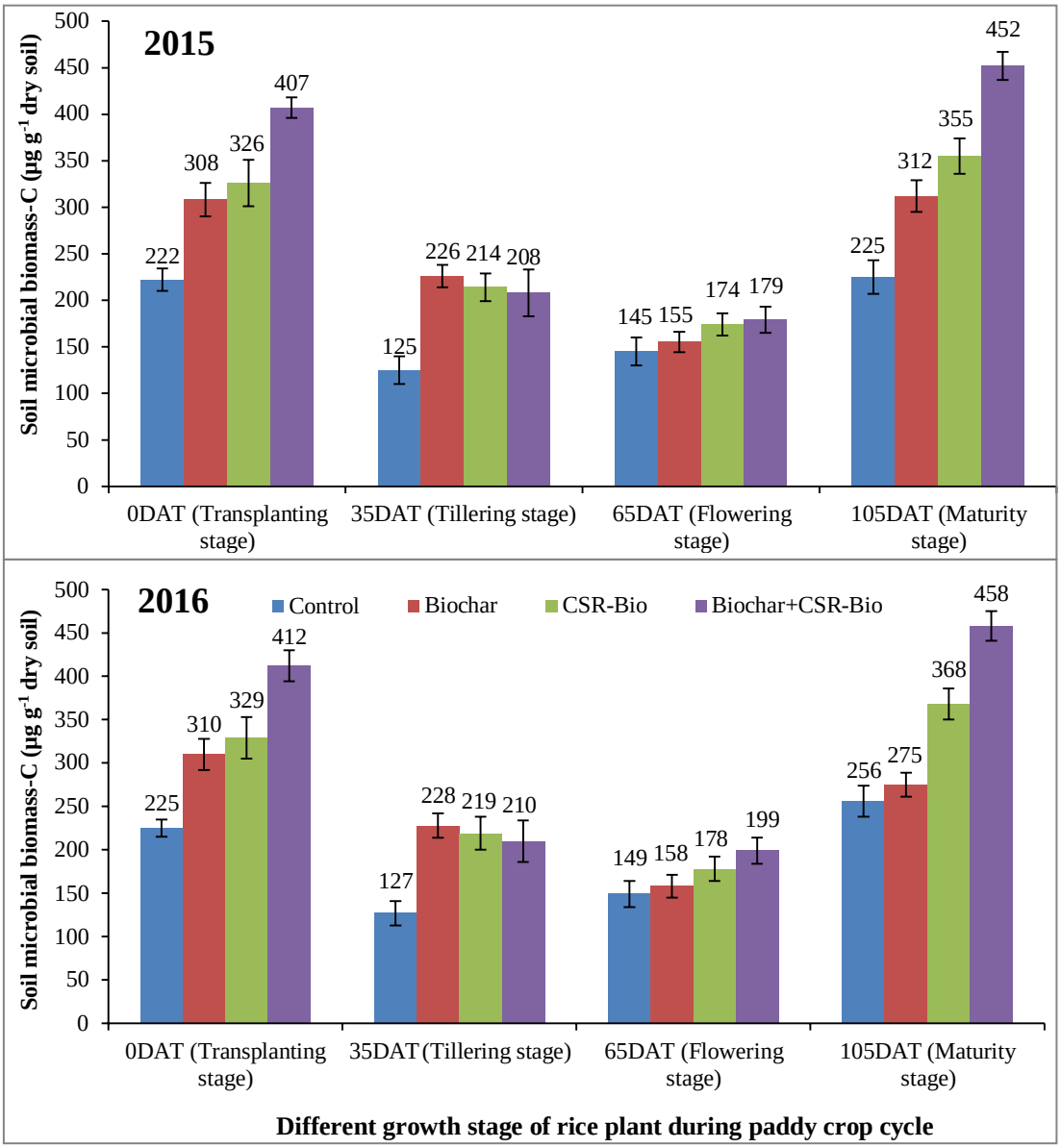

Figure 6.1. Variations insoil microbial biomass-C (µg g $^{-1}$dry soil) at different sampling dates after transplantation (DAT) during paddy crop cycle for the year 2015 and 2016. Vertical line on each bar represent ± 1SE. ANOVA for impact of sampling dates on soil microbial biomass-C across different treatments has been given in **Table 6.2**.

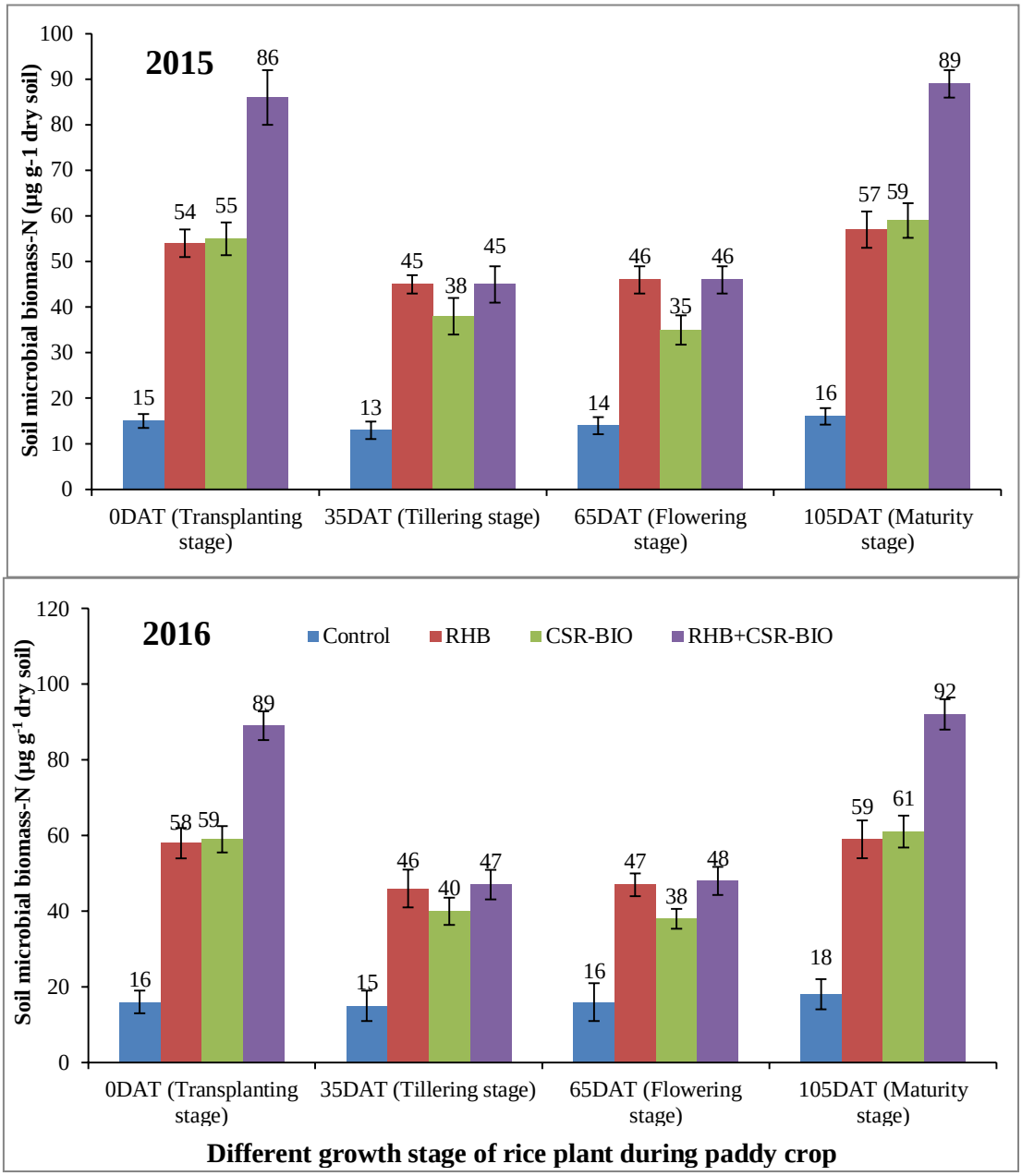

Figure 6.2. Variations insoil microbial biomass-N (µg g $^{-1}$dry soil) at different sampling dates after transplantation (DAT) during paddy crop cycle for the year 2015 and 2016. Vertical lines on each bar represent ± 1SE. ANOVA for impact of sampling dates on SMB-N across different treatments has been given in **Table 6.2**.

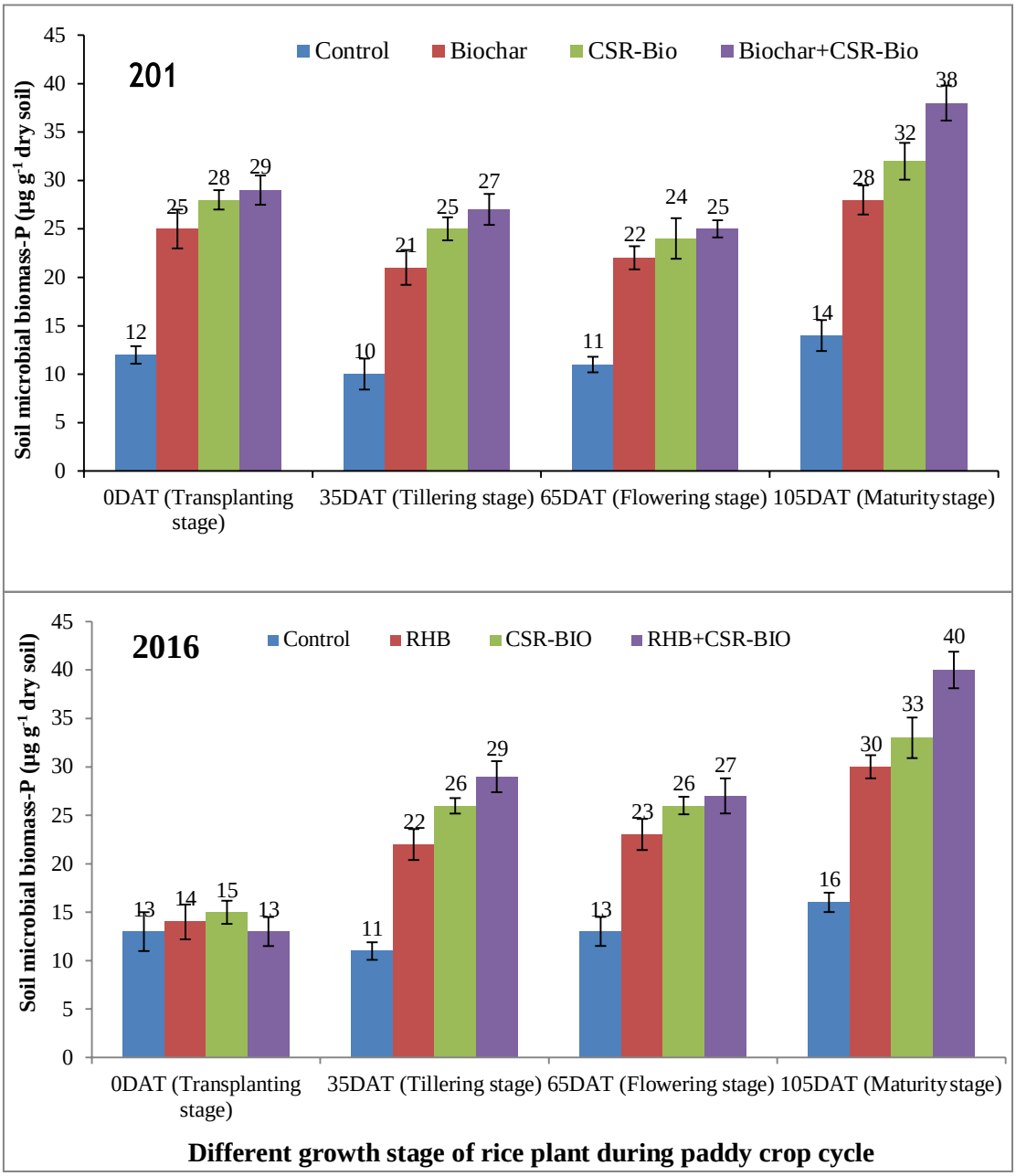

Figure 6.3. Variations insoil microbial biomass-P (µg g $^{-1}$dry soil) at different sampling dates after transplantation (DAT) during paddy crop cycle for the year 2015 and 2016. Vertical line on each bar represent ± 1SE. ANOVA for impact of sampling dates on soil microbial biomass-P across different treatments has been given in **Table 6.2**.

6.4 Conclusions

In conclusion, present study indicated that RHB and CSR-BIO added alone or in combinations to the soil contributes significantly to the enhancement of soil physico-chemical properties and therefore, microbial biomass in nutrient poor paddy agriculture soils of tropical regions. The results also revealed that RHB and CSR–BIO amendments notably enhanced the SMB–C, –N and –P. The RHB, having large surface area, pore size and nutrient elements provide favourable soil conditions for the growth and multiplication of microbial communities and consequently higher SMB levels, in nutrient deprived paddy agriculture soil. The RHB generation from paddy rice husk and its application with beneficial microbial inoculants may be a sustainable crop residues waste management option to enhance the nutrient status, microbial community of nutrient poor agriculture soils. It is suggested that, use of chemical fertilizers could be reduced to enhance the economic benefits and agriculture soil health because of RHB and CSR–BIO application. Available N immobilization and its release by SMB is an adaptation to provide nutrients to plants in nutrient limited ecosystems. The results of this investigation are based on short-term duration so, larger scale paddy cultivation experiments under field conditions, are required to verify the mechanisms involved in RHB-microbes interactions for soil fertility improvement and microbial community compositions.

7

Chapter-07

To Assess the Influence of CSR-BI0 and Biochar Amendment on Methanotrophs Abundance and Diversity

CHAPTER -7

TO ASSESS THE INFLUENCE OF CSR-BIO AND BIOCHAR AMENDMENTS ON METHANOTROPHS ABUNDANCE AND DIVERSITY.

7.1 Introduction

Methane (CH_4) is one of the most important greenhouse gasses, accounting for about 15% of the global warming (IPCC, 2007). More than that, it seems that the overall contribution of CH_4 to the global warming process has been underestimated, since some recent study just recently demonstrated that CH_4 can be readily formed *in-situ* in terrestrial plants. This newly identified source may have important implications for the global CH_4 budget and may call for a reconsideration of the role of natural CH_4 sources in the historical and the ongoing climate change. These new findings will only increase the importance of CH_4 sinks to reduce its atmospheric concentration. Besides reaction with hydroxyl (OH^-) radicals in the troposphere and stratosphere, CH_4 oxidation in soils removes about 30 Tg CH_4 yr^{-1}, accounting for about 5% of the total CH_4 sink. Thus, the most plausible way to increase the sink for CH_4 is increasing the performance of the soil CH_4 oxidizers. Therefore, knowledge about the ecology and responses of the CH_4 consuming bacterial diversity and abundance in relation to paddy agricultural practices are of particular interest.

Paddy agriculture is one of the most important sources of Global CH_4 emissions, contributing about 33-40 Tg $year^{-1}$ (Ciais *et al*., 2013).Moreover, as a greenhouse gas, CH_4 is 25-30 times more potential than CO_2 to capturing solar radiation and its effectiveness in the atmosphere is about 9 years (Hartmann *et al*., 2013; Tiwari *et al*., 2015). Additionally, CH_4 isproduced from several others sources such as biomass burning, biofuels landfills, waste degradation and livestock farming,

may contributing major share in global CH_4 budget. However, with intervention of some eco-friendly agricultural practises such as application of suitable amendments in soils can be minimized the CH_4 emission rate from agricultural fields. Biochar play important role to minimize the CH_4 emissions by influencing the microbial oxidation of CH_4 by methanotrophic bacteria, found in paddy soil (Singh, 2011). Methanotrophs are unique bacteria that utilize CH_4 as sole source of carbon and energy (Glaser *et al*., 2002; Fazli*et al*., 2013; Fazli *et al*., 2013; Abujabhah *et al*., 2016). Therefore, rice husk biochar (RHB) and microbial consortium (CSR-BIO) based agricultural cultivation can be a profitable and eco-friendly practice to achieve the higher paddy crop production and CH_4 mitigation by enhancing the abundance of methanotrophs in paddy agriculture soils.

Methanotrophs, a group of Gram-negative bacteria, utilize CH_4 as their sole source of carbon and energy, thus reducing the concentration of atmospheric CH_4 and contributing significantly as only known biological known CH_4 sinkin mitigation of global warming (Trotsenko and Murrell, 2008). The capability to consume CH_4 by methanotrophs for their carbon and energy requirements is because of unique methane monooxygenase (MMO) (Tiwari *et al*., 2015). In all known methanotrophs the MMO exits in two well known forms: i) the cytoplasmic soluble form (sMMO), and ii) the membrane-bound, particulate form (pMMO). All methanotrophs are equipped with either sMMO or pMMO while only few are reported to possess both the forms. Based on the literature available, two marker genes are fundamental in detecting the MMO: i) *mmoX* (a gene encoding a subunit of soluble methane monooxygenase), and ii) *pmoA* (a gene encoding a subunit of particulate methane monooxygenase) (Dong *et al*., 2015).

To date, several novel methanotrophic genera and species such as *Methylogaea oryzae* (Geymonat *et al*., 2011), *Methyloglobulus morosus* (Deutzmann *et al*., 2014), *Methylomonas lenta* (Hoefman *et al*., 2014b), *Methylocaldum marinum* (Takeuchi *et al*., 2014) *Methylomarinovum caldicuralii* (Hirayama *et al*., 2014). *Methyloparacoccus murrellii* (Hoefman *et al*., 2014a), *Methyloprofundus sedimenti* (Tavormina *et al*., 2015), *Methylocapsa palsarum* (Dedysh *et al*., 2015), etc. have been described by research scientist working in the area methanotrophic ecology for different natural ecosystems and laboratory throughout the world. The increase in the number of the novel methanotrophic strains could be attributed to the recent advances in molecular tools and techniques applied for isolation and identification from various unexplored agriculture and natural ecosystems.

In general, methane-oxidizing bacteria are placed in the group of *Proteobacteria* and classified into three phylogenetically distinct groups: Type I, Type II, and Type X. Type-I and Type-X methanotrophs belong to the Methylococcaceae family within the *Gammaproteobacteria*; Type-II methanotrophs belong to the family Methylocystaceae within the *Alphaproteobacteria*. All methanotrophs are capable to utilise CH_4 as the sole source of carbon and energy, though some methanotrophs are also capable of using other C1 compounds, such as methanol or methylamine (Bowman, 2006). After molecular analysis of methanotrophs such as sequencing of 16S rRNA and *pmoA* genes was investigated that Type I and Type II methanotrophs commonly found in paddy soil and as well as inside the paddy roots (Macalady *et al*., 2002; Shrestha *et al*., 2008; Gilbert *et al*., 1998; Eller and Frenzel, 2001; Kolb *et al*., 2003; Eller *et al*., 2005; Jia *et al*., 2007). All methanotrophs have the enzyme methane monoxygenase (MMO) to catalyze the oxidation of methane to methanol by serine and RuMp pathway, This enzyme exists

in two forms, the soluble form (sMMO) and the particulate form (pMMO) but pMMO enzyme is found predominantly in all group of methanotrophic bacteria (Bowman, 2006; Semrau *et al*., 2010).

A third class (Type-III) methanotrophs have also been reported as*Verrucomicrobia*, phylogenetically different from methanotrophsbelonging to Proteobacteria (Knief, 2015). Type-I methanotrophs are placed in *Gammaproteobacteria* and belong to the family Methylococcaceae and cover the following genera such as *Methylomonas, Methylobacter, Methylomicrobium, Methylosphaera, Methylococcus, Methylocaldum,* and *Methylothermus* (Stralis-Pavese *et al*., 2004; Bowman, 2006). Type-I methanotrophs have been reported predominantly throughout the environment and oxidizing CH_4 at atmospheric concentrations (<12 ppm; (Le Mer and Roger, 2001). Likewise Type-II methanotrophs also placed in the group of *alphaproteobacteria* within the family *Methylocystaceae* including the genera for instance *Methylosinus and Methylocystis* (Bowman, 2006). Type-II methanotrophs are oxidised higher concentration of CH_4 (Le Mer and Roger, 2001) than Type-I methanotrophs. They also can oxidise non CH_4 volatile organic compounds by soluble form of methane monooxygenase enzyme (sMMO). Scheutz *et al*., 2004; Huber-Humer *et al*., 2008). Methanotrophs can be studied using either cultivation-based techniques or cultivation-independent techniques that target specific genetic sequences. In this objective we studied methanotrophic abundance and diversity by cultivation and cultivation independent techniques using MPN and DGGE, respectively (Amann *et al*., 1995; Suzuki and Giovannoni, 1996). The cultivation independent approach using the polymerase chain reaction followed by denaturing gradient gel electrophoresis (DGGE) (targeting

pMMO gene of MOB) was used to assess methanotrophs abundance and diversity were present in paddy soil (Yargicoglu *et al*., 2017).

Methanotrophs can mitigate the effect of CH_4 emission and certain species also contribute as bio-fertilizer in paddy soil (Feng *et al*., 2012; Zhanga *et al*., 2012; Tiwari *et al*., 2015). Agriculture supports a major share of national income and export of agro based products raise the earning in several developing countries. India and china, both are major rice producing countries thought to be major contributors of global CH_4 budget. Synthetic fertilizers decrease the yield of crops including the soil fertility and microbial community as well as climate imbalance. However, CSR-BIO is an environmental supportive bio-formulations that can be added to the paddy agriculture crops in combination with biochar for greater paddy yields (Mukherjee and Lal, 2013; Damodaran *et al*., 2013; Gul*et al*., 2015). Biochar is known as soil conditioner to improve the plant growth directly with providing the suitable nutrients as well as eradicating the unwanted belongings indirectly, when compared to chemical fertilizers, insecticides and pesticides (Kuppusamy *et al*., 2016; Hussain *et al*., 2016). The application of biochar in soils is based on its properties such as: (i) agricultural value from enhanced soils nutrient maintenance, (ii) permanent carbon sequestration, and (iii) reduced GHG emissions, particularly CH_4 and N_2O (Lehmann *et al*., 2006). But, no information about the CH_4 consuming bacterial diversity and abundance under different biochar amended paddy fields is available, which may act as a strong sink for CH_4. It is also not known whether application of different biochar quantity and microbial formulation in combinations to paddy soils has similar of different CH_4 consuming bacterial population. Further, we don't know about their diversity in a given paddy agro-ecosystem treated with biochar and other amendments. It is expected that this research work would provide answer to some of

the questions raised here. The investigations on methanotrophs population dynamics from natural and upland paddy agro-ecosystems has indicated that dry tropical soils act as strong CH_4 sink.

A number of studies have been conducted from agro-ecosystem to terrestrial and forest ecosystems regarding ecology of methanotrophs population dynamics. But to date, uses of RHB on community composition and population dynamics of methane consuming bacteria from paddy field soils are missing. Needless to say that no attempt has been made to understand the diversity and abundance of methanotrophs in the paddy soils treated with RHB and microbial bio-formulations such as CSR-BIO. Therefore, this study investigated the impact of RHB in combination of microbial bio-formulation-CSR-BIO (consortia of *Bacillus pumilus*, *Bacillusthuringensis*, and *Trichodermaharzianum*) on methanotrophs abundance in paddy agriculture field conditions.

7.2 Material and methods

The details of experimental design and field preparation, RHB + CSR-BIO treatment application, paddy nursery establishment and transplantation, have already been described in **Chapter 3**.

7.2.1 Methanotrophs abundance quantification

The soil samples were collected at 0, 35, 65 and 105 day after transplanting (DAT) from 0-15 cm soil depth in triplicates for MPN quantification of methanotrophs abundance. The soil samples were immediately transported to laboratory and stored in polyethylene bags at 4 ^{0}C for the analysis of methanotrophic bacterial abundance and community.

7.2.2 Quantification of methanotrophs abundance by MPN method

The number of methanotrophic bacteria was enumerated by the new Most Probable Number (MPN) technique according to Saitoh *et al.* (2002). This cultivation dependant experiment was carried out in50 mL serum bottle. Present modified MPN technique helps, not only in exact enumeration of small population densities of methanotrophs in paddy soil, but also it requires less equipments, labour, and is superior to conventional MPN methods (Roslev and King, 1994; Singh *et al*., 2010) however methanotrophic diversity was analysed by DGGE based molecular technique according to (Zheng *et al*., 2008).

The serum vials used for the isolation, quantification and purification of methanotrophs are shown in **Figure 7.1** and **7.2**. The culturable methanotrophic community structure was identified using modified most probable number (MPN) (Saitoh, 2002) method. A modified nitrate mineral salt (NMS) media (Whittenbury *et al*., 1970) used for methanotrophs cultivation contains: $MgSO_4.7H_2O$, 1 g; KNO_3, 1 g; $Na_2HPO_4.12H_2O$, 0.71 g; ferric ammonium EDTA, 5 gm, chelated iron solution 02 mL which contains ferric (III) ammonium citrate 0.01 g or ferric chloride 0.05 g, EDTA sodium salt 0.2 g, 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 ^oC for 15 minutes for sterilization. The CH_4 gas was injected with 1:1 ratio with air into serum vials and Petri plates as carbon source for the growth of methanotrophs.The serially diluted soil samples of different land use sites were spread on Petri plates containing NMS media for the enumeration of methanotrophs abundance and

purification of bacterial isolates. 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). The methanotrophic growth was observed in serum vials. Serum vials containing 30 mL freshly prepared NMS media were taken and inoculated with 0.1 mL of log phage synchronized methanotrophic culture of collected soil samples in separate vials and incubated at 30 ºC for 28 days.

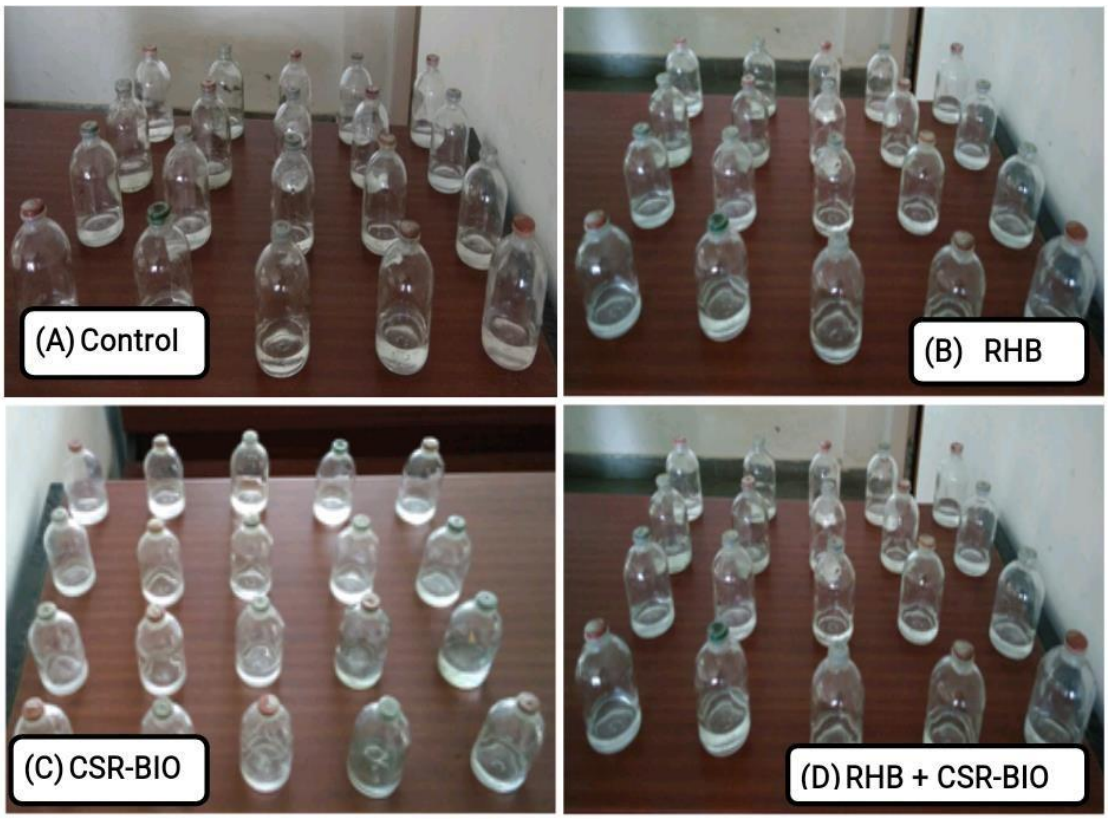

Figure 7.1. Culture of methanotrophs grown in serum vials containing NMS medium for the quantification of methanotrophs abundance in paddy soil for the year 2015.

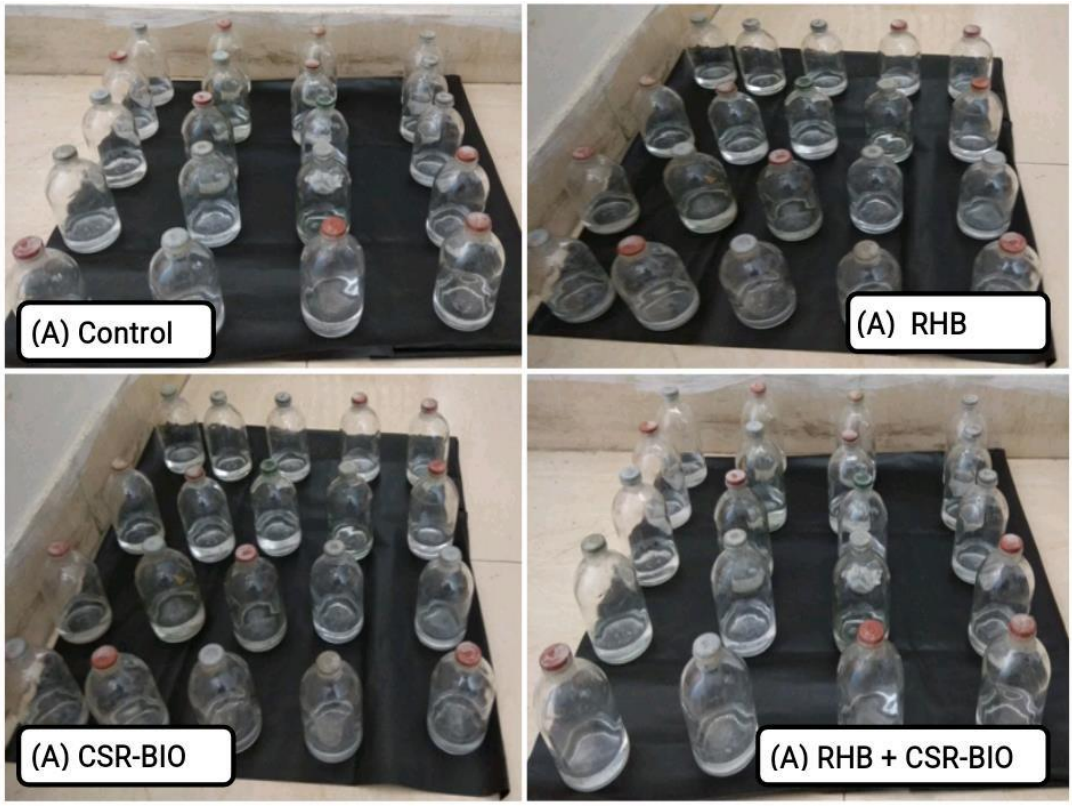

Figure 7.2. Culture of methanotrophs grown in serum vials containing NMS medium for the quantification of methanotrophs abundance in paddy soil for the year 2016.

7.2.3 Biochemical tests

The biochemical characterization of cultivated methanotrophs was carried out according to Smibert and Krieg (1981) and details are given in **Figure 7.6** and **Table 7.2.**

7.2.4 Identification of methanotrophs community by molecular methods

7.2.4.1 Genomic DNA extraction and purification from paddy soil

The DNA was extracted from fresh soil (0.25 g) with the help of MoBio power soil® DNA isolation kit **(**MO-BIO Laboratories, USA US 7,459,548 B2, Cat. No. 12888-50 and 12888-100) according to manufacturer's instructions using a bead-beating extraction protocol.

7.2.4.2 Amplification of the *pmoA* geneand PCR-DGGE analysis of methanotrophic community structure

The extracted DNA from paddy soils of different treatments was amplified by PCR–DGGE according to Dianou *et al*. (1999). The sequences of the universal forward and reverse primersin this experiment were A189F-GGTGACTGGGACTTCTGG and Mb661-CCGGMGCAACGTCYTTACC respectively, as used by Holmes*et al*.(1995). The GC clamp-A189f CCCCCCCCCCCCCCGCCCCCCGCCCCCCCCCC CGCCGCCGGNGACTGGGACTTCTGG (Henckel, 1999). The GC clamp attached to the 5′ end of A139F primer andamplification of methanotrophic bacterial DNA was carried out by PCR. During PCR the requirements were: 2.0 µL of each primer, 2.0 µL of deoxyribonucleoside triphosphate (dNTP), 0.7 µL of $MgCl_2$, 2.5 µL PCR buffer, 0.05 unit/µL of *Taq* polymerase, 3.75 µL Betain, 0.5 µL BSA and 50 ng of template DNA. All these materials were combined with super purified H_2O in a 0.5-mL tube. The PCR reaction was performed with the use of T-100 thermo cycler PCR and the initial denaturation was carried out for 5 minutes at 94 ℃, followed by 20 touchdown cycles (65 to 55℃).Further, 10 cycles were carried out at 55 ℃ for 1 minute, followed by 72 ℃ for 1 minute and a final extension of 72℃ for 5 minutes. Then after that the targeted DNA were separated and purified from products of PCR amplification. The purified DNA was loaded on 6% (vol/vol) acrylamide/bisacrylamide (37.5:1) gel, made with a denaturing gradient (urea: formamide-40: 60%) were run according to the procedures of Henckel *et al*. (1999) and Bourne *et al*. (2001) **Figure 7.3**. The potential DNA bands were excised from DGGE geland suspended in 100 mL of water overnight to elute the DNA. The bands were re-amplified and run again on the DGGE gel to check the quality and mobility of DNA for further analysis.

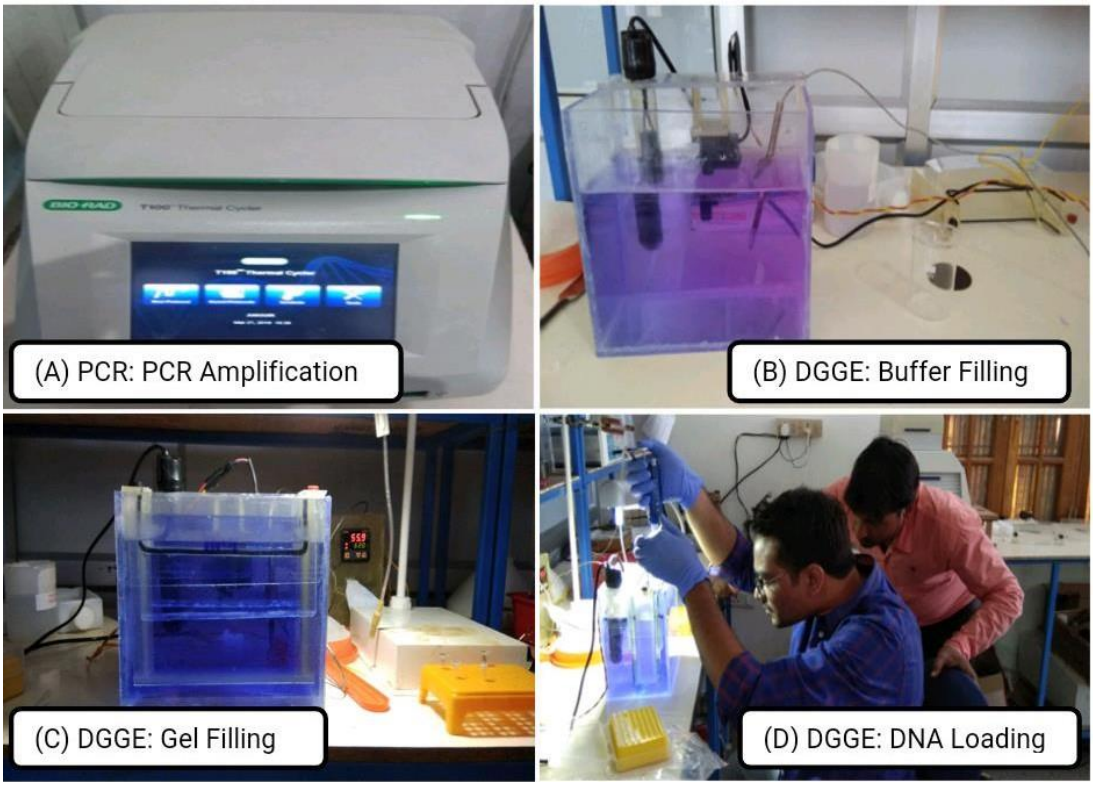

Figure 7.3. Equipments and experimental setup used for DGGE analyses of methanotrophs from paddy soils.

7.2.4.3 Sequencing of the *pmoA* genes and phylogenetic analysis

Theexcised DNA bands, sequencing was done by the sequencer Applied Biosystems® 3730/3730xl DNA Analyzer (Thermo Fisher Scientific, USA) according to manufacturer's guidelines. The obtained DNA sequences were manually checked edited and aligned using BioEdit version 7.2. The collected sequences werealigned with the help of using *pmoA* gene sequences from the National Center for Biotechnology Information (NCBI) database using the BLAST version 2.7.0 searching program and phylogeneticanalyses were conducted using MEGA 7 version 7.0.26.

7.3 Results and discussion

7.3.1 Methanotrophs abundance

During the two consecutive years (2015-2016) of paddy crop cycle,the average methanotrophs populations were counted highest in the soil of RHB + CSR-BIO treated plots for both the study years 2015 (54.75± 0.98 × 10^{-5} cells g^{-1} dry soil) and 2016 (57.25± 0.88(× 10^{-5} cells g^{-1} dry soil) (**Table 7.1**). Across different sampling dates the maximum methanotrophs abundance was noted at 65 DAT compared to control plotduring both the years (**Figure 7.4A**). At all the soil sampling days the RHB+CSR-BIO amended plot showed higher number of methanotrophs population compared to other treatments. The ANOVA showed that differences in methanotrophs population due to treatments were statistically significant (P=<0.001) (**Table 7.1**) for both the study years. The higher methanotrophic bacterial community in RHB+CSR-BIO treated soil could be due to the development favourable soil conditions because of higher organic contents and optimum soil moisture (**Table 4.1, Chapter-4**) that may support the growth and multiplication of methanotrophic bacteria (Wang *et al*., 1993; Quilliam *et al*., 2013; Tiwari *et al*., 2015; Singh and Gupta, 2016). The variations in soil physico-chemical properties (**Table 4.1, Chapter-4**) due to RHB and CSR-BIO amendments could be one of the major reasons for the variations in methanotrophic abundance across the treatments. While unfavourable soil in control plot may suppress the methanotrophs population growth. Beneficial microbial agents present in CSR-BIO formulation might create beneficial conditions to enrich the soil physico-chemical environment therefore; a higher number of methanotrophs might be build up in nutrient rich soil. The increase in methanotrophs abundance in RHB treated soil could be due to the improved soil physico-chemical properties as well as nutrient sources because of favourable soil conditions that supports the growth and

multiplication of methanotrophs. Further, the lager pore size of RHB could provide shelter to soil methanotrophs and protects them from soil microbes feeding (predator) such as protozoa, nematodes, etc. (Warnock *et al*., 2010). It is suggested that RHB may optimize the soil aeration and moisture conditions which favours the colonisation of methanotrophs (Zhong and Cai 2007; Henckel, 1999). It is expected that long term application of RHB in soil could promote activity, growth and community structure of methanotrophs (Bender and Conrad 1995; Henckel 1999). So, increasing methanotrophic abundance and diversity in paddy soils due to application of RHB can contribute toenhance the CH_4 consumption.

Table 7.1. Variations of methanotrophs abundance in rice husk biochar (RHB) and CSR-BIO treated paddy field. The values for the each parameter are average means of three replicates ± SE. Total number of soil samples analysed for each parameter and year was N=12 (3 replicates × 4 treatments).

Treatments	**Year**	**Methanotrophs number (× 10^5 cells g^{-1} dry soil)**
Control	2015	18.75± 0.89
	2016	20.00± 0.58
RHB	2015	46.00± 0.65
	2016	48.00± 0.78
CSR-BIO	2015	25.25± 0.19
	2016	26.75± 0.16
RHB + CSR-BIO	2015	54.75± 0.98
	2016	57.25± 0.88
One-way ANOVA	2015	F=56.62; N=12; P=<0.001
	2016	F=36.24; N=12; P=<0.001

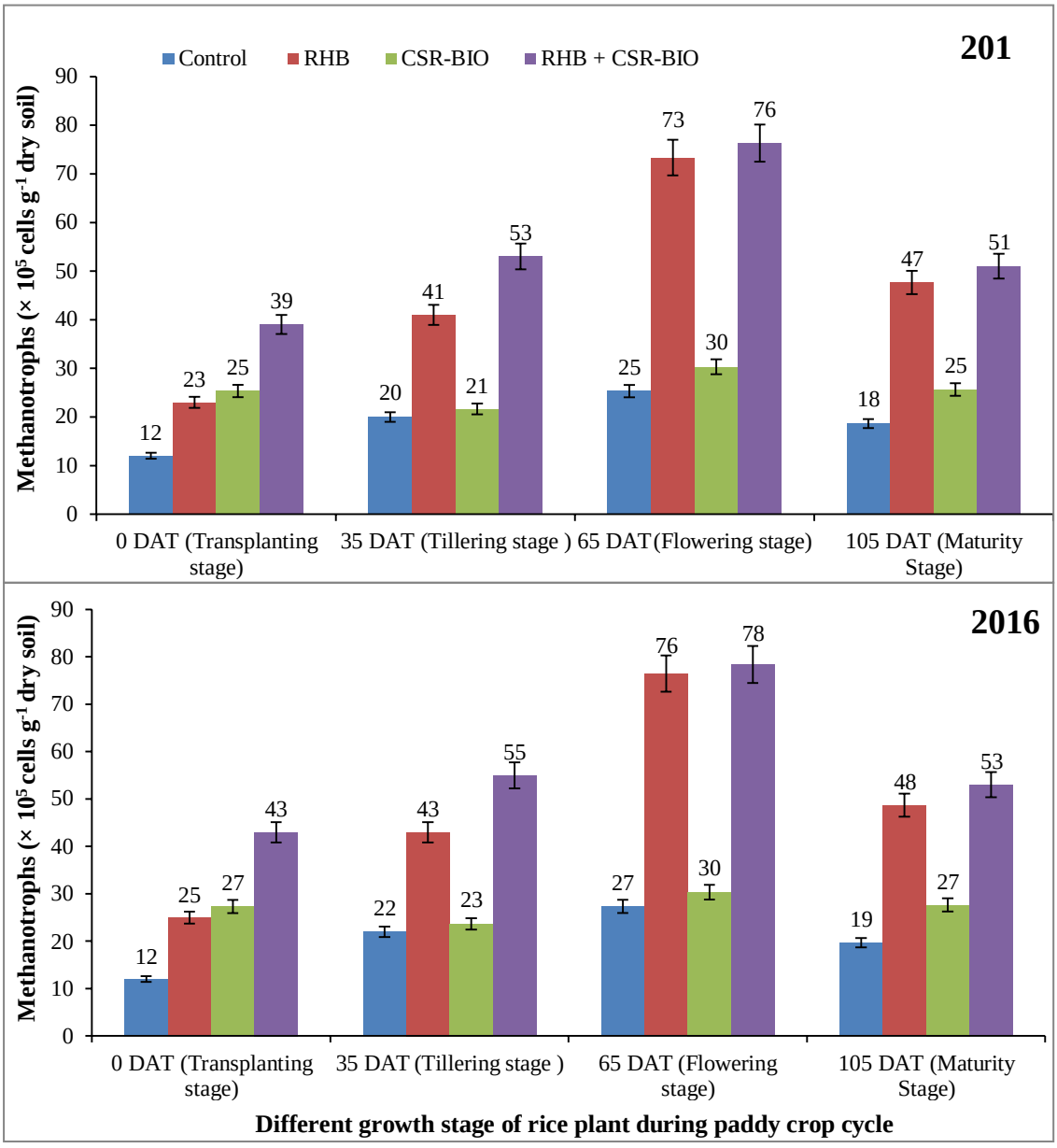

Figure 7.4A. Influence of RHB and CSR-BIO treatments on methanotrophs abundance at different sampling days during paddy cycle for the two years 2015 and 2016.Vertical lines on each bar represents means of three replicates ± SE. When the data were pooled across the treatments and sampling dates, one-way ANOVA indicated significant differences in methanotrophs abundance both the years 2015 (F=15.55; N=48; P=<0.001) and 2016 (F=23.15; N=48; P=<0.001).

To find out the impact of different treatments in paddy soil, the correlation analysis was performed between methanotrophs number and some relevant soil properties such as soil moisture, WHC, soil pH, total-C, ammonium- and nitrate-N (**Figure 7.4B**). A positive significant correlation between methanotrophs number and soil moisture (N=12; R^2 = 0.6734; P=<0.001) showed that soil moisture is an important factor that regulates growth and multiplication of methanotrophs in dry tropical paddy soils. It has been also reported that methanotrophs are very sensitive microbes affected by soil moisture conditions (Singh *et al*., 2010a). This indicates that adequate soil moisture could be a necessary factor for the optimal functioning of methanotrophs in paddy soil. The information available about the impact of soil moisture on methanotrophs in RHB treated paddy soil indicate that soil moisture could be a very crucial parameter that may govern the community composition of these unique group of bacteria and their role in methane sink activity from upland paddy soil.

A negative correlation of methanotrophs number with ammonium-N (N=12; R^2 = -0.2162; P= < 0.001) and nitrate-N(N=12; R^2 = -0.2682; P = <0.001) across treatments indicating that the higher concentration of soil ammonium-N and nitrate-N significantly suppressed the number of methanotrophs in paddy soils (**Figure 7.4B**). Additionally, the negative relationship between methanotrophs abundance and inorganic-N contents in RHB treated paddy soil of present study can be explained with the results of Bender and Conrad, (1994), who confirms that ammonium-N, inhibits the methanotrophs population in paddy soil. Zheng *et al*. (2008)also showed that long term inorganic fertilizers applications negatively impacted on population dynamics and abundance soil methanotrophs in a Chinese paddy soil. Though, number of study with reference to effect of fertilizer application

on CH_4 sink activity in paddy soil have been conducted, but the impact on the methanotrophs abundance and diversity have not been investigated in detail. Therefore, precise and extensive research work related to effect of inorganic fertilizers application on methanotrophic community composition in paddy crop field conditions is required.

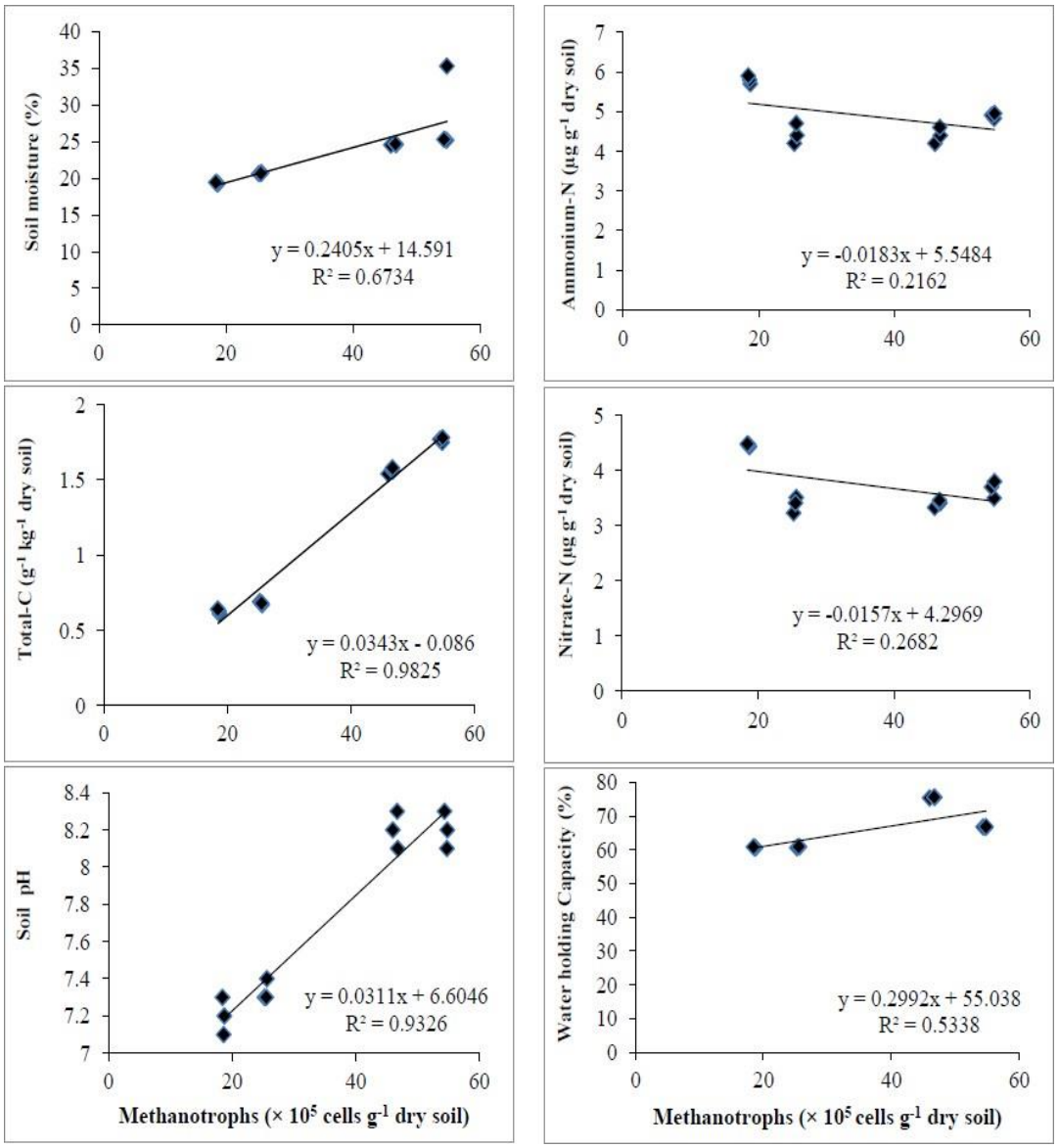

Figure 7.4B. Relationship between methanotrophs abundance and some relevant soil physico-chemical soil properties across different treatments. Total number of soil samples use for this correlation analysis was N=12 (4 treatments × 3 replicates).

An appropriate dilution of liquid culture samples of methanotrophs was spread on NMS agar plates and placed at 30 ^{0}C inside the incubator and 5:5 mL ratio of CH_4 and air were transferred weekly. After 3 week of incubationpinkish to yellowish colour methanotrophs colonies were appeared on Petri plates containing NMS agar medium (**Figure 7.5**).

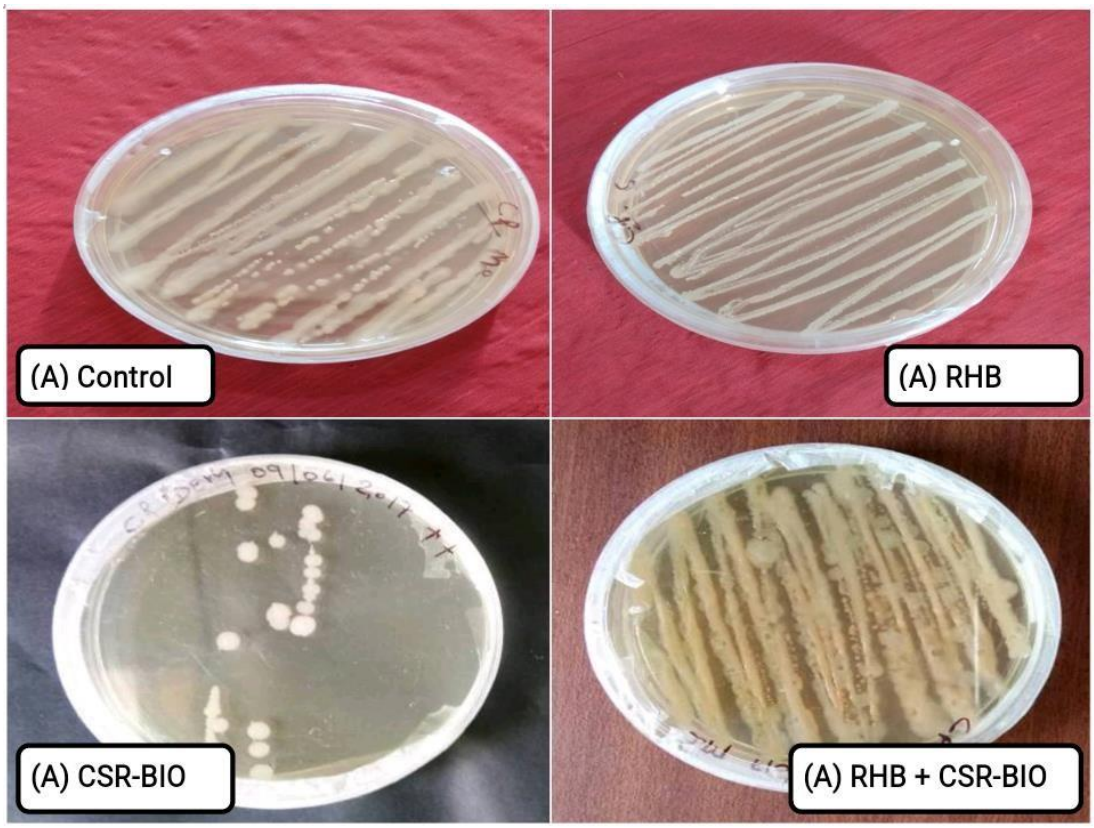

Figure 7.5. Methanotrophs gown on NMS agar medium from the paddy soils.

From these bacterial growth plates cells of methanotrophs was used for SEM and biochemical test. The biochemical kits **(A)** control **(B)** after addition of 0.5 mL of methanotrophs culture has been given in **Figure 7.6.** Results of biochemical test performed for methanotrophs isolated from soil samples are shown in **Table 7.2.** The results of biochemical test and SEM analysis of methanotrophs are in accordance with the results of Whittenbury *et al.* (1970). The bacterial cells were negative towards

Gram staining. The colony morphology of the methanotrophs growing on NMS agar plates was investigated visually. The strain exhibited a white, coccus and semi rod, entire colony with 3–4 mm in diameter after 15 days of incubation at 30 ^{0}C.

Figure 7.6. Biochemical kits **(A)** control **(B)** after addition of 0.5 mL of methanotrophs culture.

Table 7.2. Results of biochemical test performed for methanotrophs isolated from soil samples.

SNo.	Test	Results
1.	Methyl red	-
2.	Voges Proskaur's	-
3.	Ureas	+
4.	H_2S Production	+
5.	Citrate utilization	-
6.	Lysine utilization	+
7.	ONPG	-
8.	Lactose	+
9.	Arabinose	-
10.	Maltose	+
11.	Sorbitol	-
12.	Dulcitol	-
13.	Oxidase	+
14.	Gram staining	-

The isolated methanotrophs on NMS medium were subjected to phase contrast and SEM analysis (**Figure 7.7**). The bacterial cell shape was rod shaped and pinkish in colour.

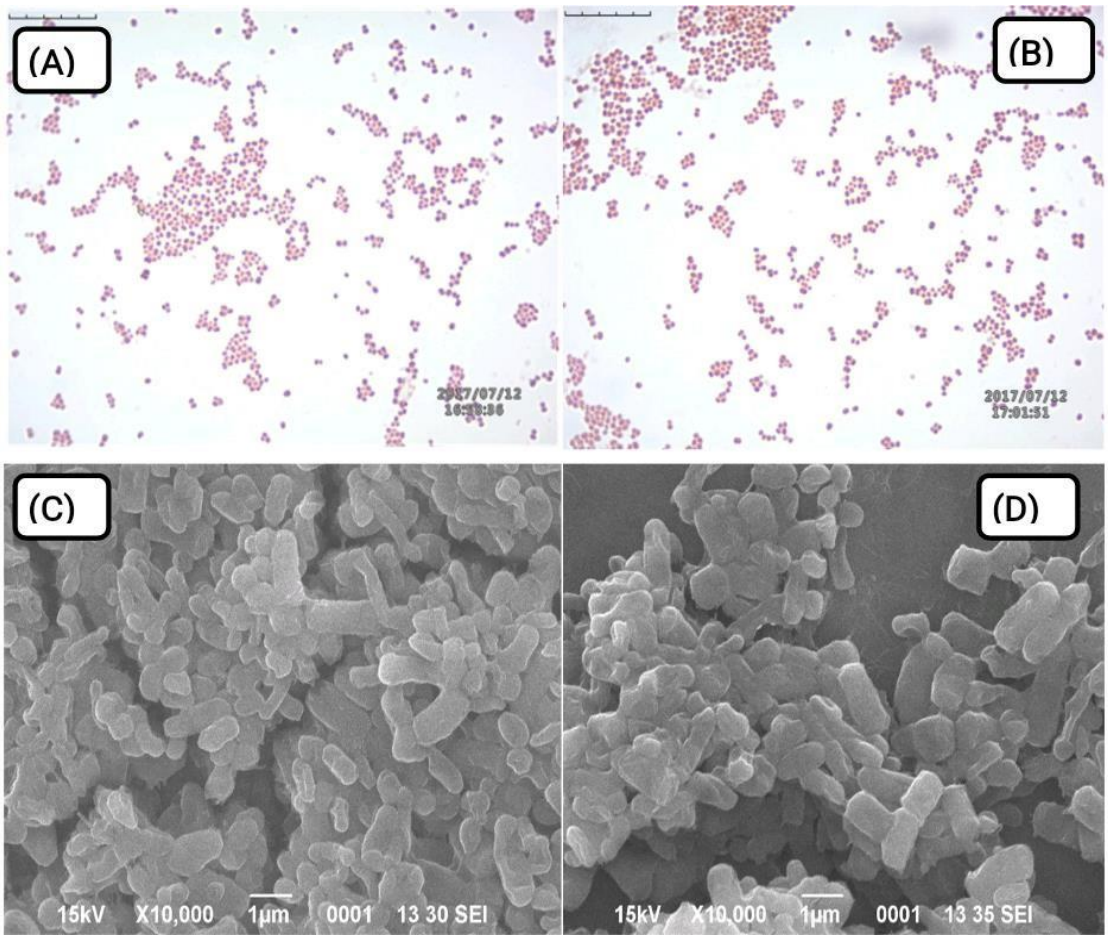

Figure 7.7. Images of isolated methanotrophs. (A) and (B) are images of phase contrast and (C) and (D) are taken by scanning electron microscope (SEM).

7.3.2 Methanotrophs community composition

The objective of present study was to characterize the methanotrophic communities present in the paddy soil treated with RHB and CSR-BIO. The extracted genomic DNA from paddy was subjected to molecular biology techniques such as DGGE and *pmoA* gene sequencing.

7.3.3 PCR-DGGE amplification of the *pmoA* gene

Methanotrophs use CH_4 as their sole source of carbon and energy with the help of broad spectrum enzyme methane monooxygenase (MMO) (Singh and Singh, 2017). Two common forms of MMO are: i) the sMMO (encoded by *mmoX* gene) and ii) pMMO (encoded by *pmoA* gene). Since, in most of the study conducted earlier only the pMMO containing methanotrophic community has been reported as dominant group in upland soils. Therefore, it was of interest to investigate whether the extracted genomic DNA from paddy upland soil contains *pmoA* gene or not. To answer this question, PCR-amplification of *pmoA* marker gene which is essential in detecting *pmoA* (a gene encoding a subunit of particulate methane monooxygenase) (Dong *et al*., 2015) was carried out using the specific primers. Amplification of *pmoA* genes was performed with universal primers A189F/mb661R using the standard recommended PCR protocols resulted in a product of about ~ 500 bp lengths (4 different bands) has been shown in Figure 7.7. The identified PCR-DGGE bands were further determined by sequence analyses as *pmoA* genes.

7.3.4 Sequence analysis of the DGGE bands across the treatments

During DGGE analysis of methanotrophic DNA total 4 bands (**Figure 7.8**). The band-1 represents the control soil, while the remainingthree 3 bands represents the RHB treated paddy soil.These DGGE bands were excised from the DGGE gel for PCR amplification and sequencing analyses. After sequencing analyses (aligning with the BLAST in NCBI database), the 4 sequences has been identified as *pmoA*genefragments which is categorised astype-I methanotrophs (*Gammaproterobacteria*) (**Figure 7.9** and **Table 7.3**). On the basis of distance metric analysis of phylogenetic tree,the CP1 band-5 (RHB soil) and CP2 band-1 (control soil) was much closed tothe *Methylobacter*and *Methylosarcina*sp., respectively.

However, CP1 band-1 and CP1 band-4 (RHB soil) was not classified in single bacterial species of methanotrophs due to the highest similarity from type I group of methanotrophs, therefore, both sequences were submitted as family uncultured Methylococcaceae bacterium, to the DNA Data Bank of Japan (DDBJ). **Figure 7.9.**

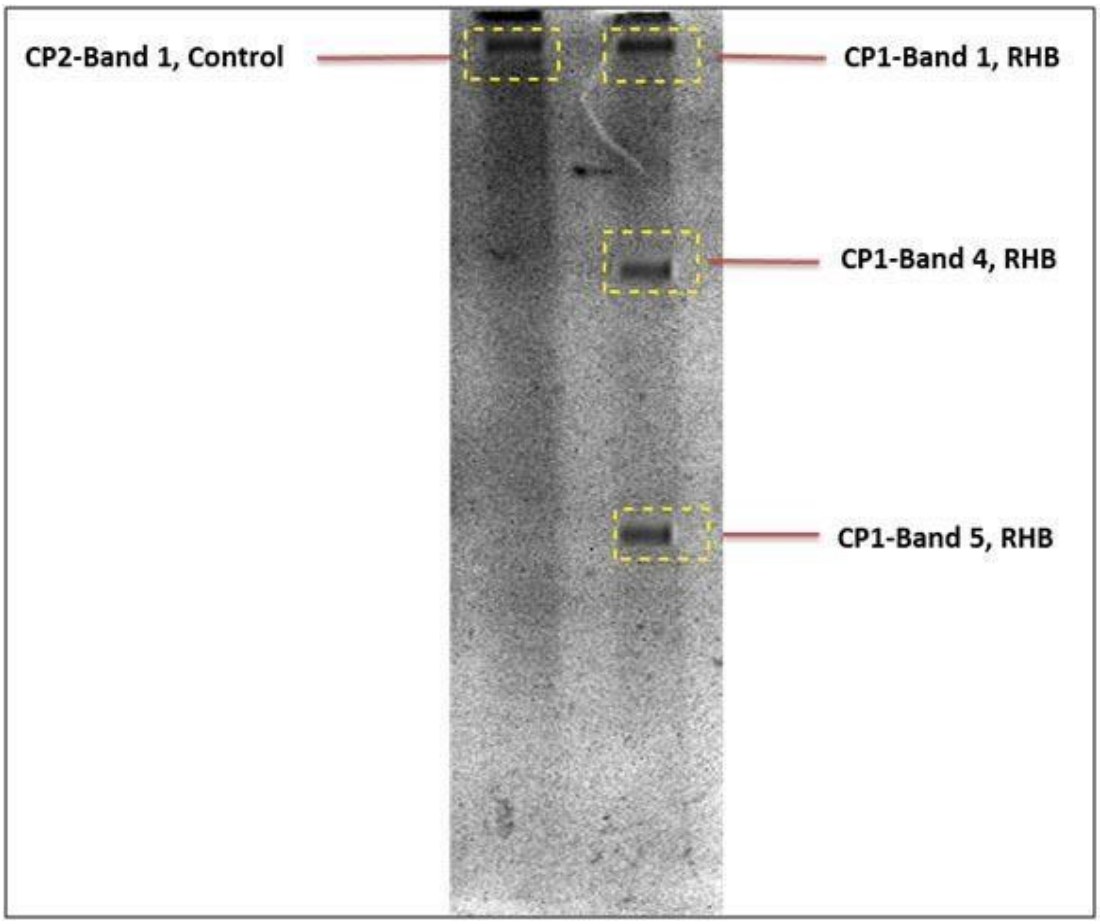

Figure 7.8. DGGE profile of *pmoA* gene fragments of paddy soil. Total 4 bands were excised from the selected area for DNA sequencing analysis.

7.3.5 Obtained nucleotide sequences from methanotrophs of paddy soil

Total 4sequences (3 sequences in RHB treated soil and 1 Sequence in control) were obtained after sequencing of *pmoA* gene of methanotrophs by DGGE analysis of paddy soil.

CP1 Band 1 (RHB)

TTTTTCACCCATTCAGACGTACTCAGGAACGCCGGTTATTAGGCAATACTAACCT
ACGAAGGGAGCCAAGGGCATATGCATACCACTTTCTTCAATAGGAAAAAGGAAG
GGAGCCATATGAGGCCACTTACCGGGGTGAAAACCGAAACCAAAAACCAAGCGG
CCTCCAAACGCTTTTAACTAACATATTTTAGATACCAACAATACAAAATCCAATA
CGATAGCCCCGGGAATTAGTTGTGATGGGAATATGAAATTAACGGGGAAGGATG
TCCAGCCCCAAAAATTGAATTCCCGCTTGACCCCCTAGACCAAGCCCAACCCAAT
GAATCAAAAGGTTAGACCAAAGCCGCTGCTATAATGCCGCCGTTAAACGGTTAA
AACTGCCTCTGGGAAGGTAAGATCCCAACTCGATAAAAAGATTACCCGATAAAG
TTGTACT

CP1 Band 4 (RHB)

CACATTGGGTTACTGACTCCCCAATGGTAACAATTACTTTCCCAACTGAGTGAAA
AGCTATCTTCAGGCGAGTTTACACGTTTTCTTTTCGGGGAGTTCTTTTGCGTTCGG
GGTTGCTTTAGGGCGAATGGGCCAACAGTGACCTGAAGTTCAGGGGTCGGACTTA
CTTCCCCATTAAGTTCCCGTCCCCTCTGAACCTGGTCGCCGGCGCAACAGTTCGTT
GCGTTGTCATGGAGTTGTGAAAAGCGAATGTATTGACTGCTCTTTCCGGCGGTTG
GGGCTTCGGTCTGGTTTTTATCCCAGGCAACTGGTCAATCATTGCCCCAATTCATG
TTCCTGTCGATCAAAACGGCATGATGCTGGCTTTGGCTGACTTCAAGGTTACCAA
CTCCGTTTGAACTGGTACACCTGATGACCACAGAATGGTTGAAAAAGGTACATTG
AGAACTTTCG

CP1 Band 5 (RHB)

ACGTCTATGGGTAACTGTTACACCCTAGATTTTCAATCTCTTTCCCTGACGCTGTT
CAAGTAAGATTCTGGCTGGCTTACCGTCTGACTTTCGCGTGGTAACTTTTGTCTTA
GGTTTCCCCCCGGGTGGAGGGGTTAACCTTTACCTCAAACTTCGGGCATGGAGAG
TTTTCCCTGCCACCTTCTGGCTTCCTTACAAACCTGATCCAGGGCGCTTTAGTTCA
TTAAGTTCCATTCATGGAGTATAACAGCATGACGTTGACAGCGGTTGTTGGTGGT
ATGGCTTGGGGTCTACGGTTCTACCTGGCAACTGGTCAAATTCACATTCCATTAC
ACAATCCGGTTGAATGCAATGGCATGAGGTTTCCTCTGGGTGGGTTGCAAGGTTT
CCTCTACGTAAGAACTGGTACTCCTGATAACATGAGAAGGGTTAAAAAAGGTTCA
CTAAAAAG

CP2 Band 1 (Control)

CGACTGCTGTGGGAAACCGAATTGCCAATAGTTGGTATTACGTTCCCGGCCGCTC
CCCAAGTAGTTCTCTGGAGGGCTTACCGTTTACCTTTAGGCGGGGTTCTTTCTCTA
GTTGGGCTCCTCTTGGTCGAGTGGTTCATGGGTCAACAGATACTTCAACTTCGGG
GAAATAGTTCAAATTTCCCTAAACTTCGAGAGCCCATCAAACTCAAAGCCAGGTC
CTATCGTTATGGCAGTTATCCTGATGCCGTCTAACAGCATCAAGCGGACAGAGGT
TACGGGCGCATTGGGTTTTCTTTTTTGTTCTACCCAGGTAAATGGCCTGCTCCATT
TCCCTTGCCCACGCCTCTTGAAGACAACGGATTGGTAATGAATCTGGCTGCATTG
CAAGGTTCCCACTATGTTGGAACCGGTACTCCTGCGTGCATCCGTAGGATTAAAA
AAGGTACATTGATAACGTTCGGTAA

7.3.6 Methanotrophic bacterial diversity in paddy soil

The DGGE profile of the study soil samples showed variations in methanotrophic community composition in treated and control soils (**Figure 7.9**). It is clear from the occurrence of more diverse bands in RHB treated soil attributed toa greater methanotrophic community diversity compared to control soils. Since, all the methanotrophic genera in this study were identified as the type-I methanotrophs

(*Gammaproteobacteria*) it may be proposed that type-I methanotrophs are predominant in paddy soils. The CP1 band-1 and band-4 of RHB treated soil showedhighest similarity with the two methanotrophic genera of family Methylococcaceae. So, both these bands may belong to the methanotrophic genera such as *Methylomonas*, *Methyloglobulus* and *Methylobacter*. However, CP1 band-5 revealed highest closeness to the methanotrophic genera *Methylobacter*. Likewise in control soil CP 2 band 1 was closely related toonly single methanotrophic genera *Methylosarcina*. The *pmoA* gene sequences revealed that the strains were closely related to the above described methanotrophs genus with similarity ranged from 97-98%. In this study, the differences in banding patterns observed in the DGGE profile, suggesting that RHB treatments in paddy soil altered the methanotrophic community structure.Therefore, the long term application of RHB and other organic amendments in upland paddy soil could be a beneficial option to enhance the methanotrophic abundance and diversity in disturbed saline soils. This study revealed that the long term application of RHB in paddy fields could suppress the emission of CH_4 due to increasing the diversity and abundance of methanotrophs in RHB amended soil. But the study related to RHB amendments and methanotrophs in paddy fields, regarding CH_4 reduction and higher crop production is ambiguous so, there is the need of further investigations because paddy agriculture contribute major share in the production CH_4 due to anthropogenic sources.

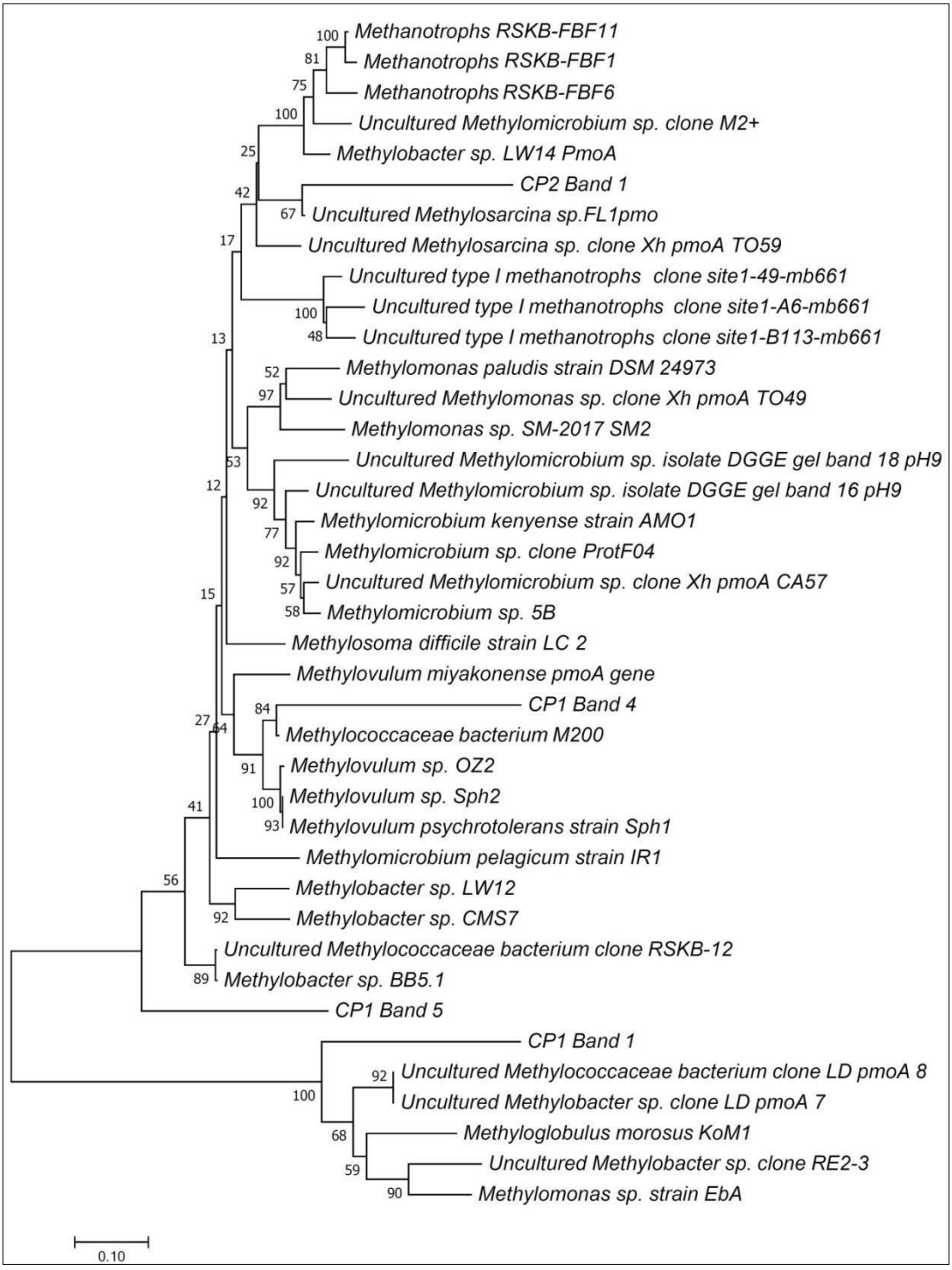

Figure 7.9. Phylogenetic relationship of identified methanotrophs community,based on *pmoA* genes amplified from excised bands from methanotrophic DGGE gel of paddy soils.

Table 7.3. On the basis of evolutionary distance matrix of phylogenetic relationship based on **Figure 7.9** the possible genera and family of methanotrophsidentified from paddy soils are mentioned below.

Treatments	Name and number of bands	Category/Type	Possible Methanotrophic genera/ family
Control	CP2- band 1	Type-I (*Gammaproteobacteria*)	*Methylosarcina* sp.
RHB	CP1-band 1	Type-I (*Gammaproteobacteria*)	Methylococcaceae
	CP1- band 4	Type-I (*Gammaproteobacteria*)	Methylococcaceae
	CP1- band 5	Type-I (*Gammaproteobacteria*)	*Methylobacter* sp.

7.3.7 Nucleotide sequences and accession numbers of *pmoA* gene

The obtained *pmoA* gene sequences of four bands have been deposited to the DNA Data Bank of Japan (DDBJ) under the following accession numbers.

Submitted bands sequences	Accession No
CP1-Band 1	LC386857
CP1-Band 4	LC386858
CP1-Band 5	LC386859
CP2-Band 1	LC386860

7.4 Conclusions

The present study showed a higher methanotrophic bacterial abundance and community in RHB treated soil compared to control plots of saline paddy soils. The variations in soil physico-chemical properties due to RHB amendments has been found as major reasons for the variations in methanotrophic abundance across the treatments. The unfavourable soil conditions in control plot suppressed the number of methanotrophs. The improved soil physico-chemical properties as well as nutrient sources in RHB treated soil supported the growth and multiplication of methanotrophs. The lager pore size of RHB provide shelter to soil methanotrophs and

protects them from soil microbes feeding (predator) such as protozoa, nematodes, etc. and therefore, a higher methanotrophs population build up.

Our study demonstrated that the abundance and methanotrophic microbial community are apparently affected RHB treatments in paddy soils and hence that bacterial abundance and diversity has to be taken into account in the global biodiversity debate. Alteration in soil physico-chemical characteristics due RHB treatments are crucial factors that may provoke shifts in methanotrophs abundance and community compositions in paddy up land soils. Type-I methanotrophs has been found as the dominant bacterial group in the paddy soil of present study. However, to confirm this, extensive ecological experiments have to be carried out with pure cultures of methanotrophs isolated from upland paddy soils differing in methanotrophs abundance and diversity. The plant residues based biochar and fertilizer addition studies have to be carried out in paddy soil habitats with dominance of methanotrophs types (type-I, type-II, etc.) and of, where methane consumption has to be linked with diversity by using advanced tools and techniques. Finally it can be said that biodiversity conservation and land use policy makers should also consider methanotrophic abundance and diversity, which is apparently important for global green house gases particularly methane reduction in soils.

8

Chapter-08
Summary

CHAPTER- 8

SUMMARY

India being an agriculture-dominant country produces more than 500 million tons of crop residues wastes annually. The huge crop residues of rice, wheat, cotton, maize, millet, sugarcane, jute, rapeseed-mustard, groundnut and other crops are typically burnt on agriculture farm sites across different states of the India. A large portion of unused crop residues are burnt every year in the fields primarily to clear the left-over straw and stubbles after the crop harvest. The problem is more severe in the irrigated paddy agriculture, particularly in the mechanized rice-wheat system of the north-west region of the country. Non availability of technically trained peoples, high cost of residue removal from the field and increasing use of combines in harvesting the crops, are main reasons behind burning of huge amount of crop residues at the fields. Burning of crop residues not only causes environmental pollution, but is also responsible for the loss of agriculturally important soil microbial community and abundance (Singh *et al.*, 2017). Since, microbes are the crucial biological agents Therefore, appropriate management strategies of crop residues to agricultural use may assumed as a viable and sustainable option for enhancing the key beneficial microbial community in soils to agriculture and environment (Singh *et al.*, 2011; Singh and Pandey, 2013; Singh, 2015). It is need of the hour to develop recent research efforts in conservation of agriculture-based crop management technologies which may be beneficial to enhance the beneficial soil microbial diversity and biomass (Singh and Gupta, 2018), agriculture soil fertility and crop productivity.

Sustainable degraded agriculture land management approaches such as organic farming, novel microbial inoculation with suitable bio-inoculant carriers have been

considered as key tools for combating the loss of soil fertility and crop productivity. The inoculation of microbial bio-formulations, developed from agriculturally important microbes, in combination with suitable organic amendments has been demonstrated to speed up the restoration of degraded land soil fertility within short period of time (Singh *et al*., 2016). Evidently, the direct inoculation of beneficial microbial consortia in combination with suitable supporting soil amendments/carrier material can be a viable and new efficient tool to contribute significantly in enhancement of microbial density and biomass, which can help considerably to agro-ecosystem sustainability and crop productivity. Therefore, in this experiment, the CSR-BIO, a commercial bio-formulation consortia prepared from agriculturally beneficial microbes (Damodaran et al., 2013), was used with cow dung manure (as carrier material) and RHB as soil conditioner. We hypothesized that the addition of RHB with CSR-BIO mixture will have positive effects on soil physico-chemical properties, methanotrophs abundance, SMB levels and paddy productivity. It may also be further assumed that the application of RHB in combination with novel microbial bio-formulation mixture (CSR-BIO developed by Damodaran *et al*., 2013, IISSR, Lucknow) would increase synergistically the soil available inorganic-N nutrients to paddy plants in nutrient deprived soils. However, the experimental evidences and answers for the above raised arguments and questions in field conditions are still to be investigated. Therefore, this study focused on impact of RHB application in combination of microbial bio-formulation-CSR-BIO (consortia of *Bacillus pumilus*, *Bacillus thurigensis* and *Trichoderma harzianam*) on soil physico-chemical properties, paddy crop yields, SMB, and methanotrophs diversity and abundance in paddy field.

Since, application of RHB in combination with suitable commercialized microbial bio-formulation (CSR-BIO) as supporting amendment, from agriculture paddy filed conditions are lacking therefore, to find out the answers of above raised questions, the present doctoral research work has been carried out with the following objectives:

1. To assess the impact of CSR-BIO and Biochar application on soil physico-chemical properties of saline paddy fields.
2. To find out the influence of CSR-BIO and Biochar treatments on paddy yields.
3. To study the microbial biomass-C, -N and P variations as affected by CSR-BIO and biochar amendments.
4. To assess the influence of CSR-BIO and Biochar amendments on methanotrophs abundance and diversity.

Experimental design, treatments and paddy cultivation

A field experiment for two consecutive years (2015-2016) with paddy cultivation was carried out at Agriculture Research Farm of Babasaheb Bhimrao Ambedkar University located in Lucknow, Uttar Pradesh (India). Total 12 experimental plots each having dimension of 3×2 meter was established in completely randomized block design (RBD). Four treatments i.e. rice husk biochar (RHB), CSR-BIO (a commercial microbial bio-formulation), and RHB+CSR-BIO, including one control plot (without any treatment) was also established in triplicate. Except control plot, RHB and CSR-BIO were applied at a rate of 10 t ha^{-1} as described, respectively by Zhang *et al*. (2010) and Damodaran *et al*. (2013). For this study, paddy (*Oryza sativa*) was selected as experimental crop. For the rice variety namely HUR 9-10 Hindu University Rice-9-10 was obtained from Department of Geneticsand Plant Breeding, Institute of Agriculture Sciences, Banaras HinduUniversity (South campus),

Mirzapur, Uttar Pradesh. The nursery of rice cultivar was prepared on 20 June during both the years 2015 and 2016. After 25 days of nursery growth, the paddy seedling was transfer to the experimental plots. Three hills (each with 2 seedlings) were transplanted on 25 July in both the years. Frequent irrigation (a water level of 6-12 cm) avoiding waterlogged condition, was provided throughout the crop cycle. At 105 day after transplantation (DAT), the matured paddy crop was harvested for the determination of selected paddy agronomic variables. The paddy agronomic variables such as panicle length (cm), tiller numbers (plant^{-1}), rice grain yield (t ha^{-1}) and paddy straw yield (t ha^{-1}) was determined according to Mahamud *et al*. (2013) and Amanullah and Inamullah (2016).

1. Impact of RHB and CSR-BIO and application on soil physico-chemical properties in paddy soils

The results showed that compared to treated plots, maximum electrical conductivity (EC) and pH, respectively was noted for untreated (control) plot in both the years. Across different treatments gravimetric soil moisture (GSM) and water holding capacity (WHC) were highest in RHB + CSR-BIO treated plots and lowest in control plots for each year. Compared to treated plots, bulk density (BD) was lowest in control plot. During both the years, among different treated plots the values of total-N, total-C and total-P were lowest in control plot than the treated soils. ANOVA revealed that studied soil physico-chemical characteristics such as EC, pH, SM, WHC, BD, total-N, C and -P varied significantly due to treatments. Across different treatments, inorganic-N (ammonium- and nitrate-N) levels and N-mineralization were minimum in control plots and maximum in RHB + CSR-BIO treated plots in both the years. Across different sampling dates, ammonium-N and nitrate-N levels were noted minimum on 35 DAT (tillering stage) and maximum on 105 DAT (maturity stage) in

both the years. Across different sampling dates, N-mineralization was noted minimum on 0 DAT and maximum on 105 DAT (maturity stage) in both the years. ANOVA indicated significant difference in ammonium-N, nitrate-N and N-mineralization due to sampling dates for 2015 ($P = < 0.001$) and 2016 ($P = < 0.001$).

This study demonstrates that RHB and CSR-BIO application improves the soil physico-chemical conditions that in turn enhance the availability of inorganic-N (ammonium- and nitrate-N) and rate of soil N-mineralization in paddy soil. The long term use of RHB may beneficial to enhance the soil nutrient status of nutrient deprived and saline soils. Thus, the plant residues after plant harvest such rice husk can be converted into RHB and in combination with CSR-BIO or other PGPR bio-formulations/amendments could be important strategies for enhancing the rate of soil N transformation and beneficial available N nutrients in poor paddy agriculture soil. This experiment was carried out only with the application of RHB and CSR-BIO, but other suitable organic amendments, green manures, farm yard manure (FYM) derived from crops residues may be used for restoration of soil fertility of disturbed soils.

2. Influence of RHB and CSR-BIO treatments on paddy yields

The results suggest that variation in paddy agronomic variables (variables such as panicle length, tiller number, rice grain and paddy straw yield) due to RHB and CSR-BIO treatments for two consecutive years (2015 and 2016) are statistically significant. All the selected paddy agronomic parameters were found greater in treated plots (maximum in RHB + CSR-BIO treatment) compared to untreated (control) plot. It was interesting to note that among the various selected paddy agronomic variables the influence of RHB treatment was more effective for rice grain yield.

All the selected paddy agronomic parameters (panicle length, tiller number, rice grain and paddy straw yield) were found greater in treated plots (maximum in

RHB + CSR-BIO treatment) compared to untreated (control) plot. ANOVA showed significant difference (P = < 0.001) in paddy agronomic variables due to treatments. The percent increase in panicle length, tiller number, rice grain yield and paddy straw yields in RHB + CSR-BIO treated plot was comparatively than other treatments for both the years. Further, it was interesting to note that among the various selected paddy agronomic variables the influence of RHB treatment was more efficient for rice grain yield. The high levels of soil organic carbon accumulation in RHB amended plots in present study can enhance N efficiency and increase paddy productivity in nutrient poor paddy soils of tropical regions. Though, the effect of biochar on the selected paddy agronomic variables (tiller numbers and panicle length) is difficult to elucidate because there is insufficient information based on the present experiment or previous studies. However, the positive effects of RHB treatments on paddy yields in present study may be attributed to the nutrients directly available to the paddy plants by the RHB because of having sufficient trace elements (**Table 3.2 Chapter 3**). Further, the increase in paddy yield witnessed is basically due to increase in nutrient mobilization to the paddy crop plants from the rhizosphere soil which has been enabled by the inoculated microbial consortia present in CSR-BIO to harness the available nutrients from the soil strata in field condition.

When the data were pooled across different treatments and sampling dates, the paddy agronomic variables showed negative relationship with inorganic-N (ammonium- and nitrate-N) contents and positive with SMB-C, -N and -Pas shown in the table given below. The results showed that SMB levels in the paddy soil were drastically reduced on 35 (tillering stage) and 65 (flowering stage) days after transplantation (DAT) (**Figures 6.1, 6.2 and 6.3, Chapter 6**). It is assumed that the paddy plant during these days (an active crop growth period) may possibly require a

greater amount of soil available nutrients to support the vegetative growth of crop plants. This could be the reason for a significant reduction in SMB quantity during active paddy growth periods (tillering and flowering stage). During active paddy growth period, a strong demand of soil nutrients by the crop plants may compete for available soil nutrients with microbial community and consequently led to a reduced SMB levels. The higher levels of SMB-C, -N and -P in this study on 105 DAT (crop maturity stage) could be due to a reduced demand of available soil nutrients by the inactive paddy crop growth conditions. Furthermore, the situation of higher SMB levels at the paddy crop maturity sampling date (105 DAT) could have also arisen because of greater microbial nutrient N-immobilization due to reduced available-N nutrients demand by matured paddy plants. These situations make easy availability of nutrients to micro-flora and consequently a higher SMB build-up. In view of the above arguments, when the pooled data across different sampling days were considered for correlation analysis, a positive relationship between SMB and paddy plant growth parameters might be expected as given in the **Table 8.1**.

Table 8.1. Linear regression parameters, correlation coefficient and significance levels for the relationships of paddy agronomic variables (*Y*) and soil variables (*X*) in rice husk biochar (RHB) and CSR-BIO treated agriculture soil.

***Y*-variables**	***X*-variables (µg g^{-1}dry soil)**	**a**	**b**	**N**	**R^2**
Panicle length (cm)	Ammonium-N	-0.1454	8.208	12	-0.1454x + 0.8407**
	Nitrate-N	-0.1063	6.2462	12	0.7653**
	SMB-C	13.615	12.989	12	0.6544*
	SMB-N	5.5753	83.341	12	0.7502**
	SMB-P	1.5278	15.031	12	0.9547**
Tiller number ($plant^{-1}$)	Ammonium-N	-0.1137	7.4575	12	0.8381**
	Nitrate-N	-0.0823	5.6752	12	0.7464**
	SMB-C	11.092	46.531	12	0.7077**
	SMB-N	4.5389	58.888	12	0.8102**
	SMB-P	1.2042	7.3682	12	0.9665**
Rice grain yield (t ha^{-1})	Ammonium-N	-0.3997	6.335	12	0.551*
	Nitrate-N	-0.2177	4.5697	12	0.2782^{NS}
	SMB-C	52.819	99.208	12	0.8539**
	SMB-N	20.592	33.135	12	0.8873**
	SMB-P	4.3081	4.2099	12	0.6582*
Paddy straw yield (t ha^{-1})	Ammonium-N	-0.2309	6.5405	12	0.3648^{NS}
	Nitrate-N	-0.1004	4.4786	12	0.1174^{NS}
	SMB-C	35.667	30.733	12	0.7728**
	SMB-N	13.656	57.838	12	0.7746**
	SMB-P	2.5355	1.6155	12	0.4526 *

* P = < 0.05; ** P < 0.001; NS= Not significant; SMB= soil microbial biomass

The paddy plant growth parameters (number of tillers and panicle length) and yields (rice grain and paddy straw) enhancement, following to RHB and CSR–BIO application, could be attributed to the synergistic effects of combined

amendments on soil nutrients availability to paddy crop plants in nutrient poor soils. The RHB generation from paddy rice husk and its application with beneficial microbial inoculants may be a sustainable crop residues waste management option to enhance the nutrient status and paddy productivity of nutrient poor agriculture soils.

3. Impact of RHB and CSR-BIO treatments on soil microbial biomass (SMB)-C, -N and -P in paddy field

Across the treatments highest quantity of SMB-C, -N and -P was observed in RHB + CSR BIO treated plots compared to other treatments. Data showed that variations in SMB-C, -N and -P due to treatments were significant ($P = < 0.001$). Across different sampling dates, the SMB-C, -N and -P was recorded minimum on 65 DAT (tillering stage) and maximum on 105 DAT (maturity stage). ANOVA indicated significant differences ($P = < 0.001$) in SMB-C, -N and -P quantity due to treatments and sampling dates in both the years 2015 and 2016.

The result suggests that RHB and CSR-BIO added alone or in combinations to the soil contributes significantly to the enhancement of soil physico-chemical properties and therefore, microbial biomass in nutrient poor paddy agriculture soils of tropical regions. The results also revealed that RHB amendments notably enhanced the SMB. The RHB, having large surface area, pore size and nutrient elements provide favourable soil conditions for the growth and multiplication of microbial communities and consequently higher SMB levels, in nutrient deprived paddy agriculture soil. The RHB generation from paddy rice husk and its application with beneficial microbial inoculants may be a sustainable crop residues waste management option to enhance the nutrient status, microbial community of nutrient poor agriculture soils. It is suggested that, use of chemical fertilizers could be reduced to enhance the economic benefits and agriculture soil health because of RHB and CSR–

BIO application. Available N immobilization and its release by SMB is an adaptation to provide nutrients to plants in nutrient limited ecosystems. The results of this investigation are based on short-term duration so, larger scale paddy cultivation experiments under field conditions, are required to verify the mechanisms involved in RHB-microbes interactions for soil fertility improvement and microbial community compositions.

4. Influence of CSR-BIO and RHB amendments on methanotrophs abundance and diversity

During the two consecutive years (2015-2016) of paddy crop cycle, the average methanotrophs populations were counted highest in the soil of RHB + CSR-BIO treated plots for both the study years 2015 ($54.75 \pm 0.98 \times 10^{-5}$ cells g^{-1} dry soil) and 2016 ($57.25 \pm 0.88 \times 10^{-5}$ cells g^{-1} dry soil) (**Table 7.1**). Across different sampling dates the maximum methanotrophs abundance was noted at 65 DAT compared to control plot during both the years (**Figure 7.4A**). At all the soil sampling days the RHB+CSR-BIO amended plot showed higher number of methanotrophs population compared to other treatments. The ANOVA showed that differences in methanotrophs population due to treatments were statistically significant (P=<0.001) (**Table 7.1**) for both the study years. The higher methanotrophic bacterial community in RHB+CSR-BIO treated soil could be due to the development favourable soil conditions because of higher organic contents and optimum soil moisture (**Table 4.1, Chapter-4**) that may support the growth and multiplication of methanotrophic bacteria (Wang *et al*., 1993; Quilliam *et al*., 2013; Tiwari *et al*., 2015; Singh and Gupta, 2016). The variations in soil physico-chemical properties (**Table 4.1, Chapter-4**) due to RHB and CSR-BIO amendments could be one of the major reasons for the variations in methanotrophic abundance across the treatments. While unfavourable soil in control

plot may suppress the methanotrophs population growth. Beneficial microbial agents present in CSR-BIO formulation might create beneficial conditions to enrich the soil physico-chemical environment therefore; a higher number of methanotrophs might be build up in nutrient rich soil. The increase in methanotrophs abundance in RHB treated soil could be due to the improved soil physico-chemical properties as well as nutrient sources because of favourable soil conditions that supports the growth and multiplication of methanotrophs. Further, the lager pore size of RHB could provide shelter to soil methanotrophs and protects them from soil microbes feeding (predator) such as protozoa, nematodes, etc (Warnock *et al*., 2010). It is suggested that RHB may optimize the soil aeration and moisture conditions which favours the colonisation of methanotrophs (Zhong and Cai 2007; Henckel, 1999). It is expected that long term application of RHB in soil could promote activity, growth and community structure of methanotrophs (Bender and Conrad 1995; Henckel, 1999). So, increasing methanotrophic abundance and diversity in paddy soils due to application of RHB can contribute to enhance the CH_4 consumption.

To find out the impact of different treatments in paddy soil, the correlation analysis was performed between methanotrophs number and some relevant soil properties such as soil moisture, WHC, soil pH, total-C, ammonium- and nitrate-N (**Figure 7.4B**). A positive significant correlation between methanotrophs number and soil moisture (N=12; R^2 = 0.6734; P=<0.001) showed that soil moisture is an important factor that regulates growth and multiplication of methanotrophs in dry tropical paddy soils. It has been also reported that methanotrophs are very sensitive microbes affected by soil moisture conditions (Singh *et al*., 2010a). This indicates that adequate soil moisture could be a necessary factor for the optimal functioning of methanotrophs in paddy soil. The information available about the impact of soil

moisture on methanotrophs in RHB treated paddy soil indicate that soil moisture could be a very crucial parameter that may govern the community composition of these unique group of bacteria and their role in methane sink activity from upland paddy soil.

A negative correlation of methanotrophs number with ammonium-N (N=12; R^2 = -0.2162; P= < 0.001) and nitrate-N (N=12; R^2 = -0.2682; P = <0.001) across treatments indicating that the higher concentration of soil ammonium-N and nitrate-N significantly suppressed the number of methanotroph in paddy soils (**Figure 7.4B**). Additionally, the negative relationship between methanotroph abundance and inorganic-N contents in RHB treated paddy soil of present study can be explained with the results of Bender and Conrad (1994), who confirms that ammonium-N, inhibits the methanotrophs population in paddy soil. Zheng *et al*. (2008) also showed that long term inorganic fertilizers applications negatively impacted on population dynamics and abundance soil methanotrophs in a Chinese paddy soil. The DGGE profile of the study soil samples showed variations in methanotrophic community composition in treated and control soils (**Figure 7.9**). It is clear from the occurrence of more diverse bands in RHB treated soil attributed to a greater methanotrophic community diversity compared to control soils. Since, all the methanotrophic genera in this study were identified as the type-I methanotrophs (*Gammaproteobacteria*) it may be proposed that type-I methanotrophs are predominant in paddy soils. The CP1 band-1 and band-4 of RHB treated soil showedhighest similarity with the two methanotrophic genera of family Methylococcaceae. So, both these bands may belong to the methanotrophic genera such as *Methylomonas*, *Methyloglobulus* and *Methylobacter*. However, CP1 band-5 revealed highest closeness to the methanotrophic genera *Methylobacter*. Likewise in control soil CP 2 band 1 was

closely related to only single methanotrophic genera *Methylosarcina*. In this study, the differences in banding patterns observed in the DGGE profile, suggesting that RHB treatments in paddy soil altered the methanotrophic community structure. Therefore, the long term application of RHB and other organic amendments in upland paddy soil could be a beneficial option to enhance the methanotrophic abundance and diversity in disturbed saline soils. This study revealed that the long term application of RHB in paddy fields could suppress the emission of CH_4 due to increasing the diversity and abundance of methanotrophs in amended soil. But the study relating RHB and methanotrophs in paddy fields, regarding CH_4 reduction and higher crop production is ambiguous so there is the need to further study because paddy agriculture is contribute major share in the production CH_4 of total anthropogenic sources.

The present study showed a higher methanotrophic bacterial abundance and community in RHB treated soil compared to control plots of saline paddy field. The variations in soil physico-chemical properties due to RHB amendments has been found as major reasons for the variations in methanotrophic abundance across the treatments. The unfavourable soil conditions in control plot suppressed the number of methanotrophs. The improved soil physico-chemical properties as well as nutrient sources in RHB treated soil supported the growth and multiplication of methanotrophs. The lager pore size of RHB provide shelter to soil methanotrophs and protects them from soil microbes feeding (predator) such as protozoa, nematodes, etc. and therefore, a higher methanotrophs population build up.

Our study demonstrated that the abundance and methanotrophic microbial community are apparently affected RHB treatments in paddy soils and hence that bacterial abundance and diversity has to be taken into account in the global

biodiversity debate. Alteration in soil physico-chemical characteristics due RHB treatments are crucial factors that may provoke shifts in methanotrophs abundance and community compositions in paddy up land soils. Type-I methanotrophs has been found as the dominant bacterial group in the paddy soil of present study. However, to confirm this, extensive ecological experiments have to be carried out with pure cultures of methanotrophs isolated from upland paddy soils differing in methanotrophs abundance and diversity. The plant residues based biochar and fertilizer addition studies have to be carried out in paddy soil habitats with dominance of methanotrophs types (type-I, type-II, etc.) and of, where methane consumption has to be linked with diversity by using advanced tools and techniques. Finally it can be said that biodiversity conservation and land use policy makers should also consider methanotrophic abundance and diversity, which is apparently important for global green house gases particularly methane reduction in soils.

BIBLIOGRAPHY

Abdullah, H., and Wu, H.W. 2009. Biochar as a fuel: Properties and grind ability of biochars produced from the pyrolysis of mallee wood under slow-heating conditions. *Eng. Fuels* 23, 4174-4181.

Agegnehua, G., Srivastavab, A.K., and Michael, I.B. 2017. The role of biochar and biochar-compost in improving soil quality and crop performance: a review. *Appl. Soil Ecol.* 119, 156-170.

Amann, R.I., Ludwig, W., and Schleifer, K.H. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol. Rev*. 59, 143-169.

Amanullah, and Inamullah. 2016. Dry Matter Partitioning and Harvest Index Differ in Rice Genotypes with Variable Rates of Phosphorus and Zinc Nutrition. *Rice Sci.* 23, 78-87.

Anonymous. 2000. Nutrient management. In rice production, best management practices, 2000 (accessed:https://www.slide share.net/SatriaSakata/rice-production-best-management-practices).

Asai, H., Samson, B.K., Stephan, H.M., Songyikhangsuthor, K., Homma, K., Kiyono, Y., Inoue, Y., Shiraiwa, T., and Horie, T. 2009. Biochar amendment techniques for upland rice production in Northern Laos 1. Soil physical properties, leaf SPAD and grain yield. *Field Crop Res.* 111, 81-84.

Atkinson, C.J., Fitzgerald, J.D., and Hipps, N.A. 2010. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. *Plant Soil* 337, 1-18.

Ball, P.N., MacKenzie, M.D., DeLuca, T.H., and Holben, W.E. 2010. Wildfire and charcoal enhance nitrification and ammonium-oxidizing bacteria abundance in dry montane forest soils. *J. Environ. Qual*. 39, 1243-1253.

Bender, M., and Conrad, R. 1995. Effect of CH_4 concentrations and soil conditions on the induction of CH_4 oxidation activity. *Soil Biol. Biochem.* 27, 1517-1527.

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

Berglund, O., and Berglund, K. 2011. Influence of water table level and soil properties on emissions of Greenhouse gases from cultivated peat soil. *Soil Biol. Biochem.43*, 923-931.

Binod, P., Sindhu, R., Singhania, R.R., Vikram, S., Devi, L., Nagalakshmi, S., Kurien, N., Sukumaran, R.K., and Pandey, A. 2010. Bioethanol production from paddy straw: An overview. *Bioresour. Technol*. 101, 4767-4774.

Black, C.A. 1965. Methods of soil analysis: Part 1- Physical and Mineralogical Properties. In: *American Society of Agronomy*, Madison, Wisconsin, USA.

Bodrossy, L., Stralis-Pavese, N., Murrell, J.C., Radajewski, S., Weilharter, A., and Sessitsch, A. 2003. Development and validation of a diagnostic microbial microarray for methanotrophs. *Environ. Microbiol*.5, 566-582.

Borne, M., Dorr, H.,and I. Levin. 1990. Methane consumption in aerated soils of the temperate zone. *Tellus* 42, 2-8.

Bosse, U., and Frenzel, P. 1997. Activity and distribution of methane-oxidizing bacteria in flooded rice soil microcosms and in rice plants (*Oryza sativa*). *Appl. Environ. Microbiol.* 63, 1199-1207.

Bourne, D.G., Mcdonald, I.R., and Murrell, J.C. 2001. Comparison of *pmoA* PCR Primer Sets as tools for investigating methanotroph diversity in three Danish soils. *Appl Environ. Microbiol.*67, 3802-3809.

Bowman, J. 2006. The methanotrophs-the families Methylococcaceae and Methylocystaceae, pp. 266-289. The Prokaryotes, Springer, New York.

Braida, W.J., Pignatello, J.J., Lu, Y., Ravikovitch, P.I., Neimark, A.V., and Xing, B. 2003. Sorption hysteresis of benzene in charcoal particles. *Environ. Sci. Technol.* 37, 409-417.

Bregliani, M.M., Ros, G.H., Temminghoff, E.J., and Van, R.W.H. 2010. Nitrogen mineralization in soils related to initial extractable organic nitrogen: effect of temperature and time. *Commun. Soil Plant Anal.*41, 1383-1398.

Brentrup, F., and Palliere, C. 2008. Energy efficiency and greenhouse gas emissions in European nitrogen fertilizer production and use, pp. 1-25. *Proceedings Int. Fertiliser Soc. Conf.* Cambridge, UK.

Brookes, P.C., Landman, A., Pruden, G., and Jenkinson, D.S. 1985. Chloroform fumigation and release of soil N: A rapid direct extraction method to measure microbial biomass N in soil. *Soil Biol. Biochem.* 17, 837-842.

Bruun, E.W., Ambus, P., Egsgaard, H., and Hauggaard-Nielsen, H. 2012. Effects of slow and fast pyrolysis biochar on soil C and N turnover dynamics. *Soil Biol. Biochem.* 46, 73-79.

Bruun, E.W., Hauggaard-Nielsen, H., Ibrahim, N., Egsgaard, H., Ambus, P., Jensen, P.A., and Johansen, K.D. 2011. Influence of fast pyrolysis temperature on biochar labile fraction and short-term carbon loss in a loamy soil. *Biomass Bioenergy*35, 1182-1189.

Cai, Z.C., Xing, G.X., Yan, X.Y., Xu, H., Tsuruta, H., Yagi, K., and Minami, K. 1997. Methane and nitrous oxide emissions from rice paddy fields as affected by nitrogen fertilizers and water management. *Plant Soil* 196, 7-14.

Carriera, M., Hardieb, A.G., Urasa, U., Gorgensa, J., and Knoetze, J. 2012. Production of char from vacuum pyrolysis of South-African sugar cane bagasse and its characterization as activated carbon and biochar. *J. Anal. Appl. Pyrolysis* 96, 24-32.

Cassman, K.G., Dobermann, A.R., Walters, D.T., and Yang, H. 2003. Meeting cereal demand while protecting natural resources and improving environmental quality. *Annu. Rev. Environ. Res.*28, 315-358.

Castaldi, S., Riondino, M., Baronti, S., Esposito, F.R., Marzaioli, R., Rutigliano, F.A., Vaccari, F.P., and Miglietta F. 2011. Impact of biochar application to a Mediterranean wheat crop on soil microbial activity and greenhouse gas fluxes. *Chemosphere* 85, 1464-471.

Castro, M.S., Steudler, P.A., Melillo, J.M., Aber, J.D., and Bowden, R.D. 1995. Factors controlling atmospheric methane consumption by temperate forest soils. *Global Biogeochem. Cycles* 9, 1-10.

Chan, K.Y., and Xu, Z. 2009. Biochar: nutrient properties and their enhancement. *Sci. Technol.*1, 67-84.

Chen, Y., Shinogi, Y., and Taira, M. 2010. Influence of biochar use on sugarcane growth, soil parameters, and groundwater quality. *Soil Res.* 48, 526-530.

Chintala, R., Mollinedo, J., Schumacher, T.E., Malo, D.D. and Julson, J.L. 2013. Effect of biochars on chemical properties of acidic soil. *Arch. Agron. Soil Sci.*60, 393-404.

Chintala, R., Owen, R., Kumar, S., Schumacher, T.E., and Malo, D. 2014. Biochar impacts on denitrification under different soil water contents. *World Congress Soil Sci.* 6, 157-157.

Clough, T.J., Bertram, J.E., Ray, J.L., Condron, L.M., O'Callaghan, M., Sherlock, R.R., and Wells, N.2010. Un-weathered wood biochar impact on nitrous oxide emissions from a bovine-urine-amended pasture soil. *Soil Sci. Soc. Am. J.* 74, 852-860.

Conrad, R. 1989. Control of methane production in terrestrial ecosystems. In:Exchange of Trace Gases between Terrestrial Ecosystems and the Atmosphere.Andrea, M.O., and Schimel, D.S. (eds.), Dahlem Konferenzen, Wiley, New York. pp. 39-58.

Conrad, R. 1999. Soil microorganisms oxidizing atmospheric trace gasses (CH_4, CO, H_2, NO). *Indian J. Microbiol.* 39, 193-203.

Cooperman, Y. 2016. Biochar and carbon sequestration. Solution centre for nutrient management perspectives on nutrient management in California agriculture.

Dalal, R.C., and Allen, D.E. 2008. Greenhouse gas fluxes from natural ecosystems. *Aust. J. Bot.*56, 369-407.

Damodaran, T., Rai, R.B., Mishra, V.K., Sharma, D.K., Ram, R.A., Rai, S., Kumar, H. 2011. Integrated farming system and livelihood security-an approach. CSSRI, Karnal, India.pp. 1-108.

Damodaran, T., Rai, R.B., Sharma, D.K., Mishra, V.K., Jha, S.K. 2013b. Rhizosphere engineering-an approach for sustainable vegetable production in sodic soils. National Symposium on Abiotic and Biotic Stress Management in Vegetable Crops,

NorthAmerica,<http://conference.isvs.org.in/Index.php/conf_Doc/ncab/paper/view. pp 382.

Damodaran, T., Mishra, V.K., Sharma, D.K., Jha, S.K., Verma, C.L., Rai, R.B., Kannan, R., Nayak, A.K., and Dhama, K. 2013c. Management of sub-soil sodicity for sustainable banana production in sodic soil-an approach. *Int. J. Curr. Res.* 5, 1930-1934.

Damodaran, T., Rai, R.B., Jha, S.K., Dhama, K., Mishra, V.K., Sharma, D. K., Singh, A. K., and Himanshu, D., 2013a. Impact of CSR-Bio- An eco-friendly bio-growth enhancer on increasing the profitability of horticultural crops to small and marginal land holders. *Int. J. Curr.Res.*5, 2682-2685.

Dannenberg, S., and Conrad, R. 1999. Effect of rice plant on methane production and rhizospheric metabolism in paddy soil. *Biogeochemistry*45, 53-71.

Davidson, E.A. 2009. The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860. *Nat. Geosci.* 2, 659-662.

De Souza Silva, C.M.M., and Fay, E.F. 2012. Effect of salinity on soil microorganisms, soil health and land use management. In:Soil Health and Land Use Management.Maria, D., and Soriano C.H. (eds.), In Tech. pp. 177-198.

Deenik, J.L., McClellan, T., Uehara, G., Antal., M.J., and Campbell, S. 2010. Charcoal volatile matter content influences plant growth and soil nitrogen transformations. *Soil Sci. Soc. Am. J.*74, 1259-1270.

DeLuca, T.H., MacKenzie, M.D., Gundale, M.J., and Holben, W.E. 2006. Wildfire-produced charcoal directly influences nitrogen cycling in Ponderosa pine forests. *Soil Sci. Soc. Am. J.*70, 448-453.

Demirbas, A. 2001. Biomass resource facilities and biomass conversion processing for fuels and chemicals. *Energy Convers Manage*. 42, 1357-1378.

Demisie, W., and Zhang, M. 2015. Effect of biochar application on microbial biomass and enzymatic activities in red soil. *Afr. J. Agric. Res.* 10, 755-766.

Dempster, D.N., Gleeson, D.B., Solaiman, Z.M., Jones, D.L., and Murphy, D.V. 2012. Decreased soil microbial biomass and nitrogen mineralization with eucalyptus biochar addition to a coarse textured soil, *Plant Soil*354, 311-324. Dessureault-Rompre, J., Zebarth, B.J., Georgallas, A., Burton, D.L., Grant, C.A., and Drury, C.F. 2010. Temperature dependence of soil nitrogen mineralization rate: Comparison of mathematical models, reference temperatures and origin of the soils. *Geoderma*157, 97-108.

Dev, M.S., and Rao, C.N. 2004. Food processing in Andhra Pradesh opportunities and challenges. Centre for Economic and Social Studies, Hyderabad.

Dianou, D., and Adachi, K. 1999. Characterization of methanotrophic bacteria isolated from a subtropical paddy field. *FEMS Microbiol. Lett.* 173, 163-173

Dolfing, J. 1988. Acetogenesis. In: Anaerobic Microbiology.Zehnder, A.B. (ed.).Wiley, New York. pp. 417-468.

Dong, D., Yang, M., Wang, C., Wang, H., Li, Y., Luo, J., and Wu, W. 2013. Responses of methane emissions and rice yield to applications of biochar and straw in a paddy field. *J. Soils Sediments* 13, 1450-1460.

Dong, D., Feng, Q., McGrouther, K., Yang, M., Wang, H., and Wu, W. 2015. Effects of biochar amendment on rice growth and nitrogen retention in a waterlogged paddy field. *J. Soils Sediments* 15, 153-162.

Downie, A., Crosky, A., and Munroe, P. 2009. Physical properties of biochar. In: Biochar for Environmental Management: Science and Technology. Lehmann, J., and Joseph S. (eds.).Earthscan, London. pp. 13-32.

Dubey, S.K. 2011. Methane emission and rice agriculture. *Curr. Sci.* 81, 345-346.

Eller, G., and Frenzel, P. 2001. Changes in activity and community structure of methane-oxidizing bacteria over the growthperiod of rice. *Appl. Environ. Microbiol*. 67, 2395-2403.

Eller, G., Kruger, M., and Frenzel, P. 2005. Comparing field and microcosm experiments: a case study on methano- and methylo-trophic bacteria in paddy soil. *FEMS Microbiol. Ecol.*51, 279-291.

EPA, 2010. Methane and nitrous oxide emission from natural sources. U.S. Environmental Protection Agency, Washington, DC, USA.

Erisman, J.W.W., Bleeker, A., Galloway, J., and Sutton, M.S. 2007. Reduced nitrogen in ecology and the environment. *Environ. Pollut*. 150, 140-149.

Eykelbosh, A.J., Johnson, M.S., Queiroz, E.S.D., Dalmagro, H.J., Couto, E.G. 2014. Biochar from sugarcane filter cake reduces soil CO_2 emissions relative to raw residue and improves water retention and nutrient availability in a highly-weathered tropical soil. *PLoS ONE* 9, 98523.

Fageria, N.K., Slaton, N.A., and Baligar V.C. 2003. Nutrient management for improving lowland rice productivity and sustainability. *Adv. Agron*. 88, 63-152.

FAO. 2004. Fertilizer use by crop in Malaysia: land and plant nutrition management service, Land and Water Development Division, FAO, Rome.

Feng, Y., Xu, Y., Yu, Y., Xie, Z., and Lin, X. 2012. Mechanisms of biochar decreasing methane emission from Chinese paddy soils. *Soil Biol. Biochem*. 46, 80-88.

Fowles, M. 2007. Black carbon sequestration as an alternative to bioenergy. *Biomass Bioenergy* 31, 426-432.

Frenzel, P., Therbrath, B., and Conrad, R. 1990. Oxidation of methane in the toxic surface layer of deep lake sediment (Lake Constance). *FEMS Microbiol. Ecol.*73, 149-158.

Gai, X., Wang, H., Liu, J., Zhai, L., Liu, S., Ren, T., and Liu, H. 2014. Effects of feedstock and pyrolysistemperature on biochar adsorption of ammonium and nitrate. *PLoS ONE* 9, 1-19.

Gaunt, J.L., and Johannes, L. 2008. Energy balance and emissions associated with biochar sequestration and pyrolysis bioenergy production. *Environ. Sci. Technol.* 42, 11-4152.

Gilbert, B., Asmus, B., Hartmann, A., and Frenzel, P. 1998. *In-situ*localization of two methanotrophic strains in the rhizosphereof rice plants. *FEMS Microbiol. Ecol*. 25, 117-128.

Gioseffi, E., de Neergaard, A., and Schjoerring, J.K. 2012. Interactions between uptake of amino acids and inorganic nitrogen in wheat plants. *Biogeoscience* 9, 1509-1518.

Giri, D.D., Kumar, A., Sahu, P.K., Mishra, P.K., Pandey, K.D. 2014. Temperature dependent decline in soil methane oxidizing bacterial population in tropical dry deciduous forest ecosystems. *Int. J. Sci. Technol. Res.* 3, 2277-8616.

Giria, D., Shuklaa, P.N., Kashyap, S., Singha, P., and Kashyap, A.K. 1996. Revised IPCC guidelines for national greenhouse gas inventories: Reference Manual Deen.

Glaser, B., Balashov, E., Haumaier, L., Guggenberger, G., and Zech, W. 2000. Black carbon in density fractions of anthropogenic soils of the Brazilian Amazon region. *Org. Geochem.* 31, 669-678.

Glaser, B., Haumaier, L., Guggenberger, G., and Zech, W. 2001. The'Terra Preta'phenomenon: a model for sustainable agriculture in the humid tropics. *Naturwissenschaften*, 88, 37-41.

Glick, B.R., Cheng, Z., Czarny, J., and Duan, J., 2007. Promotion of plant growth by ACC deaminase-producing soil bacteria. *Eur. J. Plant Pathol*. 119, 329-339.

Golloway, J., Raghuram, N., and Abrol, Y.P., 2008. A perspective on reactive nitrogen in a global, Asian and Indian context. *Curr. Sci*. 94, 1375-1381.

Goodwin, S., and Zeikus, J.G. 1987. Ecophysiological adaptations of anaerobic bacteria to low pH: analysis of anaerobic digestion in acid bog sediments. *Appl. Environ. Microbiol.* 53, 57-64.

Graef, C., Hestnes, A.G., Svenning, M.M., and Frenzel, P. 2011. The active methanotrophic community in a wetland from the high arctic. *Environ. Microbiol. Rep.* 3:466-472.

Granatstein, D., Kruger, C.E., Collins, H., Galinato, S., Garcia-Perez, M., and Yoder, J. 2009. Use of biochar from the pyrolysis of waste organic material as a soil amendment. Final project report, centre for sustaining agriculture and natural resources, Washington State University, Wenatchee.

Groffman, P.M., Eagan, P., Sullivan, W., and Lemunyon, J.L. 1996. Grass species and soil type effects on microbial biomass and activity. *Plant Soil*183, 61-67.

Grossman, J.M., O 'Neill, B.E., Tsai, S.M., Liang, B., Neves, E., Lehmann, J., and Thies, J.W. 2010. Amazonian anthrosols support similar microbial communities that differ distinctly from those extant in adjacent, unmodified soils of the same mineralogy. *Microbe. Ecol*. 60, 192-205.

Gul, S., Whalen, J.K., Thomas, B.W., Sachdeva, V., Deng, H. 2015. Physico-chemical properties and microbial responses in biochar-amended soils mechanisms and future directions. *Agric. Ecosyst. Environ.* 206, 46-59.

Guo, J., and Chen, B. 2014. Insights on the molecular mechanism for the recalcitrance of biochars: Interactive effects of carbon and silicon components. *Environ.Sci. Technol*. 48, 9103-9112.

Guo, L.B., and Gifford, R.M. 2002. Soil carbon stocks and land use change: a meta-analysis. *Global Change Biol.* 8, 345-360.

Gupta, S.K., Gupta, R.C., Chhabra, S.K., Eskiocak, S., Gupta, A.B., and Gupta, R. 2008. Health issues related to N pollution in water and air. *Curr. Sci.* 94, 1469-1477.

Gupta, R. 2010. The economic causes of crop residue burning in Western Indo-Gangetic plains, pp. 1-26. Paper presented at conference held at Indian Statistical Institute, Delhi centre.

Han, B., Chen, Y., Abell, Y., Jiang, H., Bodrossy, L., Zhao, J., Murrel, J.C., and Xing, X. 2009. Diversity and activity of methanotrophs in alkaline soil from a Chinese coalmine. *FEMS Microbiol. Ecol.* 70,196-207.

Handa, B.K. 1986. Pollution of ground water by nitrate in India BHU-JAL News Quart. *J. CGWB.* 1, 16-19.

Hanson, R.S., and Hanson, T.E. 1996. Methanotrophic bacteria. *Microb. Rev.* 60, 439-471.

Hassan, S.S.M., Awwad, N.S., Aboterika, A.H.A. 2008. Removal of mercury (II) from wastewater using camel bone charcoal. *J. Hazard. Mater.* 154, 992-997.

Hayashi, K. 2013. The role of biochar and prospects for its use in rice production in Southeast Asia. Proceedings of the National Workshop on Biochar for Future Food Security: Learning from Experiences and Identifying Research Priorities, Bogor, West Java, Indonesia.pp. 1-4.

Hazelton, P.A., Murphy, B.W. 2007. Interpreting soil test results: what do all the numbers mean?CSIRO Publishing: Melbourne.

Henckel, T., Friedrich, M., and Conrad, R., 1999. Molecular analyses of the methane-oxidizing microbial community in rice field soil by targeting the genes of the 16s rRNA, particulate methane monooxygenase, and methanol dehydrogenase.*Appl. Environ. Microbiol*. 69, 1980-1990

Hmid, A., Mondelli, D., Fiore, S., Fanizzi, F.P., Ziad, A.C., and Dumontet, S. 2014. Production and characterization of biochar from three-phase olive mill waste through slow pyrolysis. *Biomass Bioenergy* 71, 330-339.

Hofstrand, D. 2009. Biochar-A multitude of benefits AgMRC, Renewable Energy Newsletter.

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

Houghton, J.T., Filho, L.G.M., Bruce, J., Lee, H., Callander, B.A., Haites, E., Harris, N., Maskell, K. 1995. Radiative forcing of climate change. Cambridge University Press Cambridge.pp. 231.

Hua, L., Wu, W., Liu, Y., McBride, M.B., and Chen, Y. 2009. Reduction of nitrogen loss and Cu and Zn mobility during sludge composting with bamboo charcoal amendment. *Environ. Sci. Pollut. Res.* 16, 1-9.

Huber-Humer, M., Gebert, J., and Hilger, H. 2008. Biotic systems to mitigate landfill methane emissions.*Waste Manage. Res*. 26, 33-46.

Hutsch, B.W. 2001. Methane oxidation, nitrification, and counts of methanotrophic bacteria in soils from a long-term fertilization experiment. *J Plant Nutri.*164, 21-28.

IARI, 2012. Crop residues management with conservation agriculture: Potential, constraints and policy needs, 32. Indian Agricultural Research Institute, New Delhi.

Intergovernmental Panel on Climate Change (IPCC), 2007. The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. In: Climate Change 2007: Cambridge University Press, 48 Climate Change and Environmental Sustainability. Cambridge, United Kingdom and New York, NY, USA.pp. 433-497.

Intergovernmental Panel on Climate Change (IPCC), 2000. Emissions Scenarios, Special Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, UK.

Intergovernmental Panel on Climate Change (IPCC), 2007. Agriculture. In: Climate change 2007: mitigation, contribution of working group III to the fourth assessment report of the Intergovernmental Panel on Climate Change.Metz, B., and Bosch, P.R. (eds.). Cambridge University Press, Cambridge, UK/New York., pp. 498-540.

Ishii, T., and Kadoya, K., 1994. Effects of charcoal as a soil conditioner on citrus growth and vesicular-arbuscular mycorrhizal development. *J. Jpn. Soci Hortic. Sci.* 63, 529-535.

Izaurralde, R.C., Rosenberg, N.J., and Lal, R. 2001. Mitigation of climate change by soil carbon sequestration: issues of science, monitoring, and degraded lands. *Adv. Agron.,* 70, 1-75.

Jaafar, N.M., 2014. Biochar as a habitat for arbuscular mycorrhizal fungi. In: mycorrhizal fungi: Use in sustainable agriculture and land restoration, solaiman, pp. 297-311. Z.M., Abbott, L.K., and Varma, A. (eds.), Springer, Berlin, Heidelberg.

Jackson, M.L., 1958. Soil chemical analysis, Prentice Hall, New Jersey, USA.

Jang, I., Lee, S., Hong, J.H., and Kang, H. 2006. Methane oxidation rates in forest soils and their controlling variables: A review and a case study in Korea. *Ecol. Res.* 21, 849-854.

Jia, Z.J., Sugano, A., Asari, N., Tun, C.C., Ishihara, R., Asakawa, S., and Kimura, M. 2007. Molecular characterization of methane-oxidizing bacteria associated with rice straw decomposition in a rice field. *Soil Sci. Plant Nutr*. 53, 729-739.

Jien, S.H., and Wang, C.S. 2013. Effects of biochar on soil properties and erosion potential in a highly weathered soil. *Catena* 110, 225-233.

Jindo, K., Suto, K., Matsumoto, K., García, C., Sonoki, T., Sanchez-Monedero, M.A. 2012. Chemical and biochemical characterisation of biochar-blended composts prepared from poultry manure. *Bioresour. Technol.* 110, 396-404.

Jindo, K., Mizumoto, H., Sawada, Y., Sanchez-Monodero M.A., and Sonoki, T. 2014. Physical and chemical characterization of biochars derived from different agricultural residues. *Biogeoscience*11, 6613-6621.

Johannes, L. 2007. Bio-energy in the black. *Front. Ecol. Environ.* 5, 381-387.

Jones, D., Murphy, D., Khalid, M., Ahmad, W., Edwards-Jones, G., and DeLuca, T. 2011. Short-term biochar-induced increase in soil CO_2 release is both biotically and abiotically mediated. *Soil Biol. Biochem*. 43, 1723-1731.

Joseph, S., Peacocke, C., Lehmann, J., and Munroe, P. 2009. Developing biochar classification and test methods. In:Biochar for Environmental Management: Science and TechnologyLehmann, J., and Joseph, S. (eds.),Earthscan, London. pp. 107-126.

Jouiada, M., Al-Nofeli, N., Khalifa, N., Benyettouc, F., and Yousef, L.F. 2015. Characteristics of slow pyrolysis biochars produced from rhodes grass and fronds of edible date palm. *J. Anal. Appl. Pyrolysis* 111, 183-190.

Kamanula, J., Sileshi, G.W., Bolmain, S.R., Sola, P., Mvumi, B.M., Nyirenda, S.P., and Stevenson, P.C. 2011. Farmers insect pest management practices and pesticidal plant use in the protection of stored maize and beans in Southern Afr. *Int. J. Pest Manage*. 57, 41-49.

Kamara, A., Kamara, H.S., and Kamara, M.S. 2015. Effect of rice straw biochar on soil quality and the early growth and biomass yield of two rice varieties. *Agric. Sci.* 6, 798-806.

Karhu, K., Mattila, T., Bergstrom, I., and Regina, K. 2011. Biochar addition to agricultural soil increased CH_4 uptake and water holding capacity-results from a short-term pilot field study. *Agric. Ecos. Environ.* 140, 309-313.

Keppler, F., Hamilton, J.T.G., Brab, M., and Rockmann, T. 2006. Methane emissions from terrestrial plants under aerobic conditions. *Nature* 439, 187-191.

Khalil, M.A.K., and Rasmussen, R.A. 1994. Global emissions of methane during the last several centuries. *Chemosphere* 29, 833-842.

Khalil, M.A.K., and Shearer, M.J. 2006. Decreasing emissions of methane from rice agriculture. *Int. Congr. Ser.* 1293, 33-41.

Khan, S.T., and Mubeen, U. 2012. Wheat straw: a pragmatic overview. *Curr. Res. J. Biol. Sci.* 4, 673-675.

Khodadad, C.L., Zimmerman, A.R., Green, S.J., Uthandi, S. and Foster, J.S. 2011. Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments. *Soil Biol. Biochem.*43, 385-392.

Kim, J.S., Sparovek, S., Longo, R.M., De Melo, W.J., and Crowley, D. 2007. Bacterial diversity of terra preta and pristine forest soil from the Western Amazon. *Soil Biol. Biochem.* 39, 684-690.

Kim, S.S., Agblevor, F.A., and Lim, J. 2009. Fast pyrolysis of chicken litter and turkey litter in a fluidized bed reactor. *J. Ind. Eng. Chem.* 15, 247-252.

Kim, J., Yoo, G., Kim, D., Ding, W., and Kang, H. 2017. Combined application of biochar and slow-release fertilizer reduces methane emission but enhances rice yield by different mechanisms. *Appl. Soil Ecol.* 118, 57-62.

King, G.M. 1997. Responses of atmospheric methane consumption by soils to global climate change. *Global Chang Biol.* 3, 351-362.

Knief, C. 2015. Diversity and habitat preferences of cultivated and uncultivated aerobic methanotrophic bacteria evaluated based on pmoA as molecular marker. *Front. Microbiol.* 6, 13-46.

Knoblauch, C., Maarifat, A.A., Pfeiffer, E-M., and Haefele, S.M. 2011, Degradability of black carbon and its impact on trace gas fluxes and carbon turnover in paddy soils. *Soil Biol. Biochem.* 43, 1768-1778.

Knoblauch, C., Marifaat, A.A., Haefele, M.S. 2008. Biochar in rice-based system: impact on carbon mineralization and trace gas emissions. (accessed: http://www.biocharinternational.org/2008/conference/posters).

Kogel-Knabner, I., Amelung, W., Cao, Z., Fiedler, S., Frenzel, P., Jahn, R., Kalbitz, K., Kölbl, A., and Schloter, M. 2010. Biogeochemistry of paddy soils. *Geoderma*157, 1-14.

Kolb, S., Knief, C., Stubner, S., Conrad, R. 2003. Quantitative detection of methanotrophs in soil by novel pmoA-targeted real-time PCR assays. *Appl. Environ. Microbiol.* 69, 2423-2429.

Kollah, B., Dubey, G., Parasai, P., Saha, J.K., Gangil, S. and Mohanty, S.R. 2015. Interactive effect of biochar size and organic amendments on methane consumption in a tropical vertisol. *Soil Use Manege.* 31, 52-59.

Krause, S., Ke, C.L., Frenzel, P. 2010. Succession of methanotrophs in oxygen–methane counter-gradients of flooded rice paddies. *Int. Soc. Microb. Ecol. J.* 4, 1603-1607.

Krishnakumar, S., Rajalakshmi, A.G., Balaganesh, B., Manikandan, P., Vinoth, C. and Rajendran, V. 2014. Impact of biochar on soil health. *Int. J. Adv. Res.* 2, 933-950.

Kumar, M., Bauddh, K., Sainger, M., Sainger, P.A, Singh, J.S., and Singh, R.P., 2012. Increase in growth, productivity and nutritional status of rice (*Oryza sativa* L. cv. Basmati) and enrichment in soil fertility applied with an organic matrix entrapped urea. *J. Crop Sci. Biotech.* 15, 137-144.

Kuppusamy, S., Thavamani, P., Megharaj, M., Venkateswarlu, K., and Naidub, R. 2016. Agronomic and remedial benefits and risks of applying biochar to soil: Current knowledge and future research directions. *Environ. Int.*87, 1-12.

Kyaw, K.Z., 2016. Ph.D. Thesis, University, Luneburg, Germany.

Lai, L., Ismail, M.R., Muharam, F.M., Yusof, M.M., Ismail, R., and Jaafa, N.M. 2017. Effects of rice straw biochar and nitrogen fertilizer on rice growth and yield. *Asian J. Crop Sci.* 9, 159-166.

Lai, W.Y., Lai, C.M., Ke, G.R., Chung, R.S., Chen, C.T., Cheng, C.H., Pai, C.W., Chen, S.Y., Chen, C.C. 2013. The effect of woodchip biochar application on crop yield, carbon sequestration and greenhouse gas emission from soils planted with rice or leaf beet. *J. Taiwan Insti. Chem. Engin.* 44, 1039-1044.

Laird, D.A. 2008. The Charcoal Vision: A Win–Win–Win scenario for simultaneously producing bioenergy, permanently sequestering carbon, while improving soil and water quality.*Agron. J.* 100, 178-181.

Laird, D.A., Fleming, P., Davis, D.D., Horton, R., Wang, B., Karlen, D.L. 2010. Impact of biochar amendments on the quality of a typical Midwestern agricultural soil. *Geoderma* 158, 443-449.

Lashari, M.S., Liu, Y., Li, L., Pan, W., Fu, J., Pan, G., Zheng, J., Zheng, J., Zhang, X., Yu, X. 2013. Effects of amendment of biochar-manure compost in conjunction with pyroligneous solution on soil quality and wheat yield of a salt-stressed cropland from Central China Great Plain.*Field Crop Res*. 144, 113-118.

Le Mer, J., and Roger, P. 2001. Production, oxidation, emission and consumption of methane by soils: A review. *Eur. J. Soil Biol.* 37, 25-50.

Lehmann, J. 2007. Bio-energy in the black. *Front. Ecol. Environ.* 5, 381-387.

Lehmann, J., Gaunt, J., Rondon, M. 2006. Bio-char sequestration in terrestrial ecosystems-a review. *Mitig. Adapt. Strateg. Global Change* 11, 403-427.

Lehmann, J., Joseph, S. 2009. Biochar for environmental management: an introduction. In: Biochar for Environmental Management, Science and Technology. Lehmann, J., and Joseph, S. (eds.). Earthscan, UK and U. pp. 1-12.

Lehmann, J., Rillig, M.C, Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D. 2011. Biochar effects on soil biota a review. *Soil Biol. Biochem.* 43, 1812-1836.

Levy, H. Moxim W.J., Kasibhatla, P.S., and Logan, J.A. 1991. The global impact of biomass burning on tropospheric reactive nitrogen.In: Global Biomass Burning: Atmospheric, Climatic and Biosphere Implications. Levine, J. (ed.).pp. 363-369. MIT Press Cambridge, Mass.

Li, Y.L., and Wang, X.X. 2013. Root-induced changes in radial oxygen loss, rhizosphere oxygen profile, and nitrification of two rice cultivars in Chinese red soil regions. *Plant Soil* 36, 115-126.

Liang, B., Lehmann, J., and Sohi, S.P. 2010, Black carbon affects the cycling of non-black carbon in soil. *Org. Geochem*. 41, 206-213.

Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'Neill, B., Skjemstad J.O., Thies, J., Luizao F.J., Petersen. J., and Neves, E.G. 2006: Black carbon increases cation exchange capacity in soils. *Soil Sci. Soc. Am. J.* 70, 1719-1730.

Linquist, B., Van, Groenigen, K.J., Adviento-Borbe, M.A., Pittelkow, C., and Van, K.C. 2012. An agronomic assessment of greenhouse gas emissions from major cereal crops. *Global Change Biol*. 18, 194-209.

Liu, C.W., Wu, C.Y. 2004. Evaluation of methane emissions from Taiwanese paddies. *Sci. Total Environ.* 333, 195-207.

Liu, S., Li, Y., Wu, J., Huang, D., Su, Y., and Wei, W. 2010. Spatial variability of soil microbial biomass carbon, nitrogen and phosphorus in a hilly red soil landscape in subtropical China. *Soil Sci. Plant. Nutr.* 56, 693-704.

Liu, Y.X., Yang, M., Wu, Y.M., Wang, H.L., Chen, Y.X., and Wu, W.X. 2011. Reducing CH_4 and CO_2 emissions from waterlogged paddy soil with biochar. *J Soils Sediments* 11, 930-939.

Liu, X., Zhiming, Q.I., Quan, W., Zhiwen, M.A., and Lanhai, L.I. 2017. Effects of biochar addition on CO_2 and CH_4 emissions from a cultivated sandy loam soil during freeze-thaw cycles.*Plant Soil Environ.* 63, 243-249.

Lone, A.H., Najar, G.R., Ganie, M.A., Sofi, J.A., and Ali, T. 2015. Biochar for sustainable soil health: A review of prospects and concerns. *Pedosphere* 25, 639-653.

Lovley, D.R., and Klug, R.J. 1983. Methanogenesis from methanol and methylamines and acetogenesis from hydrogen and carbon dioxide in sediments of an eutrophic lake. *Appl. Environ. Microbiol.* 45, 1310-1315.

Lu, S.G., Sun, F.F., and Zong YT (2014) Effect of rice husk biochar and coal fly ash on some physical properties of expansive clayey soil (Vertisol). *Catena* 114, 37-44.

Ludmila, C., Julia, A., Rudoyk, R., Toms, M., and Lidstrom, E. 1998. G Transfer enzymes and coenzymes were linking methylotrophic bacteria and methanogenic Archaea. *J. Sci.* 281, 99-101.

Luke, C., Bodrossy, L., Lupotto, E., and Frenzel, P. 2011. Methanotrophic bacteria associated to rice roots: the cultivar effect assessed by T-RFLP and microarray analysis. *Environ. Microbiol*. *Reports*3, 518-525.

Luo, S., Wang S., Tian L., Li, S., Li Z., Shen, Y., and Tian, C. 2017. Long-term biochar application influences soil microbial community and its potential roles in semiarid farmland. *Appl. Soil Ecol.*117, 10-15.

Luo, Y., Durenkamp, M., De, Nobili, M., Lin, Q., and Brookes, P.C. 2011. Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. *Soil Biol. Biochem.* 43, 2304-2314.

Macalady, J.L., McMillan, A.M.S., Dickens, A.F., Tyler, S.C., and Scow, K.M. 2002. Population dynamics of type I and II methanotrophic bacteria in rice soils. *Environ. Microbiol* 4:148-157.

Mahamud. J.A, Haque, M.M., and Hasanuzzaman, M. 2013. Growth dry matter production and yield performance of transplanted Aman rice varieties influenced by seedling densities per hill. *Int. J. Sustain. Agric.* 5, 16-24.

Mahinpey, N., Murugan, P., Mani, T., and Raina, R. 2009. Analysis of bio-oil, biogas, and biochar from pressurized pyrolysisof wheat straw using a tubular reactor. *Energy Fuels*23, 2736-2742.

Major, J., Rondon, M., Molina, D., Riha, S.J., and Lehmann, J. 2010. Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. *Plant Soil* 333, 117-128.

Major, J., Steiner, C., DiTommaso, A., Falcao, NPS, and Lenmann, J. 2005. Weed composition and cover after three years of soil fertility management in the central Brazilian Amazon: compost, fertilizer, manure and charcoal applications. *Weed Biol. Manage.* 5, 69-76.

Manyong, V.M., Douthwaite, B., Coulibaly O., and Keatinge, J.D.H. 2001. Participatory impact assessment at the International Institute of Tropical Agriculture: functions and mechanisms. In: The future of impact assessment in the CGIAR: needs, constraints and options. Proceedings of a workshop organized by the Standing Panel on Impact Assessment of the Technical Advisory Committee, 3–5 May 2000. FAO, Rome, Italy. pp. 69-74.

Marris, E. 2006. Putting the carbon back: Black is the new green. *Nature*442, 624-626.

Masulili, A., Utomo, W.H., and Syechfani, M.S. 2010. The characteristics of rice husk biochar and its influence on the properties of acid sulphate soils and rice growth in West Kalimantan, Indonesia. *J. Agric. Sci*.2, 39-47.

McHenry, M.P. 2009. Agricultural bio-char production, renewable energy generation and farm carbon sequestration in Western Australia: Certainty, uncertainty and risk. *Agric. Ecosyst. Environ*. 129, 1-7.

McLaughlin, S., and Walsh, M. 1998. Evaluating environmental consequences of producing herbaceous crops for bioenergy. *Biomass Bioenergy* 14, 317-324.

Mekuria, W., and Noble, A. 2013. The role of biochar in ameliorating disturbed soils and sequestering soil carbon in tropical agricultural production systems. *Appl. Environ. Soil Sci.* 2013, 1-10.

Mekuria, W., Langan, S., Noble, A., and Johnston., R. 2017. Soil restoration after seven years of exclosure management in northwestern Ethiopia. *Land Degrad. Dev.* 28, 1287-1297.

Meyer, S., Glaser, B., and Quicker, P. 2011. Technical, economical and climate related aspects of biochar production technologies: a literature review. *Environ. Sci. Technol.*45, 9473-9483.

Meyer, S., Bright, R.M., Fischer, D., Schulz, H., and Glaser, B. 2012. Albedo impact on the suitability of biochar systems to mitigate global warming. *Environ. Sci. Technol.* 46, 12726-12734.

Milla, O.V., Rivera, E.B., Huang, W.J., Chien, C.C., and Wang, Y.M. 2013. Agronomic properties and characterization of rice husk and wood biochars and their effect on the growth of water spinach in a field test. *J. Soil. Sci. Plant. Nutr.* 13, 251-266.

MNRE, 2009. Ministry of New and Renewable Energy Resources, Govt. of India, New Delhi (accessed:www.mnre.gov.in/biomassrsources).

Mohammed, I.Y., Abakr, Y.A, Kazi, F.K., Yusuf, S., Alshareef, I., and Chi, S.A. 2015. Pyrolysis of napier grass in a fixed bed reactor: effect of operating conditions on product fields and characteristic. *Bio. Res.* 10, 6457-6478.

Mohanty, S.R., Bodelier, P.L.E., and Conrad, R. 2007. Effect of temperature on composition of the methanotrophic community in rice field and forest soil. *FEMS Microbiol. Ecol.* 62, 24-31.

Assumpção, C.R.M.. 20017. M.Sc. Thesis University of Helsinki Department of Forest Sciences.

Mukherjee, A., and Lal, R. 2013. Biochar impacts on soil physical properties and greenhousegas emissions. *Agronomy*3, 313-339.

Mukherjee, A., Zimmerman, A.R. and Harris, W. 2011. Surface chemistry variations among a series of laboratory-produced biochars. *Geoderma* 163, 247-255.

Murali, S., Shrivastava, R., and Saxena, M. 2010. Greenhouse gas emissions from open field burning of agricultural residues in India. *J. Environ. Sci. Eng*. 52, 277-284.

Namgay, T., and Singh, B. 2010. Influence of biochar application to soil on the availability of As, Cd, Cu, Pb and Zn to maize (*Zea mays* L.). *Aust. J. Soil Res.*48, 638-647.

Näsholm, T., Kielland, K. and Ganeteg, U. 2009. Uptake of organic nitrogen by plants. *New Phytol.* 182, 31-48.

Nelissen, V., Ruysschaert, G., Manka'Abusi, D., D'Hose, T., De Beuf, K., Al-Barri, B., Cornelis, W., and Boeckx, P. 2015. Impact of a woody biochar on properties of sandy loam soil and spring barley during a two-year field experiment. *Eur. J. Agron.* 62, 65-78.

Neue, H.U., Wassmann, R., Lantin, R.S., Alberto, M.C.R., Aduna, J.B., Javellana, A.M. 1996. Factors affecting methane emission from rice fields. *Atmos. Environ.* 30, 1751-1754.

Neue, N.U. 1993. Methane emission from rice fields. *Bioscience* 43, 466-474.

Nigussie, A., Kissi, E., Misganaw, M., and Ambaw, G. 2012. Effect of biochar application on soil properties and nutrient uptake of lettuces (*Lactuca sativa)* grown in chromium polluted soils American-Eurasian. *J. Agric. Environ. Sci.*12, 369-376.

O'Neill, B., Grossman, J., Tsai, M., Gomes, J., Lehmann, J., Peterson, J., Neves, E., and Thies, J. 2009. Bacterial community composition in Brazilian Anthrosols and adjacent soils characterized using culturing and molecular identification. *Microb. Ecol.* 58, 23-35.

Offermann, F., and Nieberg, H. 2000. Economic performance of organic farms in Europe. organic farming in Europe, economics and policy, Universität Hohenheim.

Oremland, R.S. 1988. Biogeochemistry of methanogenic bacteria. In:Anaerobic Microbiology.Zehnder, A.J.B. (ed.). Wiley, New York. pp. 641-705.

Pan, G.X., Zhou, P., Li, Z.P., Smith, P., Li, L.Q., Qiu, D.S., Zhang, X.H., Xu, X.B, Shen, S.Y., Chen, X.M. 2009. Combined inorganic/organic fertilization enhances N efficiency and increases rice productivity through organic carbon accumulation in a rice paddy from the Tai Lake region, China. *Agric. Ecosyst. Environ.* 131, 274-280.

Pandey, V.C., Singh, J.S., Singh, D.P., and Singh, R.P. 2014. Methanotrophs: promising bacteria for environmental remediation. *Int. J. Environ. Sci. Technol.*11, 241-250.

Pansu, M., and Gautheyrou, J., 2006. Handbook of soil analysis mineralogical, organic and inorganic method. Springer-verlag Heidelber. pp. 551-578.

Park, J.H., Choppala, G.K., Bolan, N.S., Chung, J.W., and Chuasavathi, T. 2011. Biochar reduces the bioavailability and phytotoxicity of heavy metals. *Plant Soil*348, 439-451.

Parr, J.F., 2006. Effect of fire on phytolith coloration. *Geoarchaeology* 21, 171-185.

Paungfoo-Lonhienne, C., Visser, J., Lonhienne, T.G.A., Schmidt, S. 2012. Past, present and future of organic nutrients. *Plant Soil* 359, 1-18.

Peake, L.R., Reid, B.J., and Tang, X. 2014. Quantifying the influence of biochar on the physical and hydrological properties of dissimilar soils. *Geoderma* 235, 182-190.

Peter, W. 2007. Biochar and bioenergy production for climate change mitigation. *New Zealand Sci. Rev.* 64, 5-10.

Peters, V., and Conrad, R. 1996. Sequential reduction processes and initiation of CH_4 production upon flooding of oxic upland soils. *Soil Biol. Biochem.* 28, 371-382.

Phillips, R., Whalen, S.C., and Schlesinger, W.H. 2001. Influence of atmospheric CO_2 enrichment on methane consumption in a temperate forest soil. *Global Chang Biol.* 7, 557-563.

Pietikäinen, J., Kiikkilä, O., and Fritze, H. 2000. Charcoal as a habitat for microbes and its effects on the microbial community of the underlying humus. *Oikos* 89, 231-242.

Piper, C.S. 1944. Soil and plant analysis. Inter Science Publication, Inc, Adelaide, Australia.

Pratiwi, E.P.A. and Shinogi, Y. 2016. Rice husk biochar application to paddy soil and its effects on soil physical properties, plant growth, and methane emission. *Paddy Water Environ.* 14, 521-532.

Prommer, J., Wanek, W., Hofhansl, F., Trojan, D., Offre, P., Urich, T., Schleper, C., Sassmann, S., Kitzler, B., Soja, G., Hood-Nowotny, R.C. 2014. Biochar decelerates soil organic nitrogen cycling but stimulates soil nitrification in a temperate arable field trial. *PLoS ONE* 9, 1-16.

Punia, M., Prasad, N.V., and Yogesh, Y. 2008. Identifying biomass burned patches of agriculture residue using satellite remote sensing data. *Curr. Sci.* 94, 1185-1190.

Quilliam, R.S., Glanville, H.C., Wade, S.C., and Jones, D.L. 2013. Life in the 'charosphere'-Does biochar in agricultural soil provide a significant habitat for microorganisms? *Soil Biol. Biochem.* 65, 287-293.

Quin, P., Joseph, S., Husson, O., Donne, S., Mitchell, D., Munroe, P., Phelan, D., Cowie, A., Van, Zwieten, L. 2015. Lowering N_2O emissions from soils using eucalypt biochar: the importance of redox reactions. *Sci. Rep.* 5, 16773.

Rawat, S.K., Singh, R.P., 2010. Seasonal variation of nitrate level in ground and surface water of Lucknow and its remediation using certain aquatic macrophytes. *Int. J. Lakes Rivers* 3, 25-36.

Reddy, K.R., Asce, F., Yargicoglu, E.N., Asce, S.M., Yue, D., Yaghoubi, P, 2014. Enhanced microbial methane oxidation in landfill cover soil amended with biochar. *J. Geotech. Geoenviron. Eng.* 11, 1090-0241.

Renner, R. 2007. Rethinking biochar. *Environ. Sci. Technol.* 41, 5932-5933.

Rillig, M.C., Wagner, M., Salem, M., Antunes, P.M., George, C., Ramke, H.G., Titirici, M.M. and Antonietti, M. 2010. Material derived from hydrothermal carbonization: effects on plant growth and arbuscular mycorrhiza. *App. Soil Ecol.* 45, 238-242.

Roberts, K.G., Gloy, B.A., Joseph, S., Scott, N.R., and Lehmann, J. 2010, Life cycle assessment of biochar systems: Estimating the energetic, economic, and climate change potential. *Environ. Sci. Technol*. 44, 827-833.

Robertson, G.P., and Groffman, P.M. 2007. Nitrogen transformations. In: Soil Microbiology, Ecology, and Biochemistry. Elsevier.Paul, E.A. (ed.). pp. 532.

Rondon, M.A., Molina, D., Hurtado, M., Ramirez, J., Lehmann, J., Major, J., and Amezquita, E. 2006. Enhancing the productivity of crops and grasses while reducing greenhouse gas emissions through biochar amendments to unfertile tropical soils. In: Proceedings of the 18th World Congress of Soil Science, Philadelphia, PA, USA. pp. 138-168.

Rondon, M.A., Lehmann, J., Ramírez, J., Hurtado, M. 2007. Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with bio-char additions. *Biol. Fertil. Soils*43, 699-708.

Rondon, M., Ramirez, J.A., and Lehmann, J. 2005. Charcoal additions reduce net emissions of greenhouse gases to the atmosphere. In: Proceedings of the 3 rd USDA Symposium on Greenhouse Gases and Carbon Sequestration, Baltimore, USA.pp. 208.

Roslev, P., and King, G.M. 1994. Survival and recovery of methanotrophic bacteria starved under oxic and anoxic conditions. *Appl. Environ. Microbiol*. 60, 2602-2608.

Rousk, J., Baath, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., and Fierer, N. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. *The ISME* 4, 134-151.

Rousk, J., Dempster, D.M., and Jones, D.L. 2013. Transient biochar effects on decomposer microbial growth rates: evidence from two agricultural case-studies. *Eur. J. Soil Sci.*64, 770-776.

Rousk, J., Dempster, D.M., and Jones, D.L. 2013. Transient biochar effects on decomposer microbial growth rates: evidence from two agricultural case-studies. *Eur. J. Soil. Sci.*64, 770-776.

Rutherford, D.W., Wershaw, R.L., and Cox, L.G. 2004. Changes in composition and porosity occurring during the thermal degradation of wood and wood components, U.S. Departmentof the Interior, U.S. Geological Survey: Denver, CO, USA.pp. 88.

Sadaka, S., Sharara, M.A., Ashworth, A., Keyser, P., Allen, F., and Wright, A. 2014. Characterization of biochar from switchgrass carbonization. *Energy* 7, 548-567.

Saitoh, S., Iwasaki, K., and Yagi, O. 2002. Development of a new most-probable-number method for enumerating methanotrophs, using 48-well microtitre plates. *Microbes Environ.* 17, 191-196.

Samonte, S.O.P.B., Wilson, L.T., Medley, J.C., Pinson, S.R.M., McClung, A.M., and Lales, J.S. 2006. Nitrogen utilization efficiency: Relationships with grain yield, grain protein, and yield-related traits in rice. *Agron. J.* 98, 168-176.

Sass, R.L., Fischer, J.F.M., and Huang, Y. 2000. A process-based model for methane emission from irrigated rice fields: experimental basis and assumption. *Nutri Cycl. Agroecosyst.*58, 249-258.

Scheer, C., Grace, P.R., Rowlings, D.W., Kimber, S., Van, Z.L. 2011. Effect of biochar amendment on the soil-atmosphere exchange of greenhouse gases from an intensive subtropical pasture in northern New South Wales, Australia. *Plant Soil* 345, 47-58.

Scheutz, C., Kjeldsen,Peter., Visscher J.E.B.A.D., Gebert, J., Hilger, H.A., Marion-Huber, Humer., Spokas, Kurt. 2009. Microbial methane oxidation processes and technologies for mitigation of landfill gas emissions. *Waste Manage. Res*. 27, 409-455.

Schimel, J.P., and Bennett, J. 2004. Nitrogen mineralization: Challenges of a changing paradigm. *Ecol. Soc. Am.* 85, 591-602.

Schlesinger, H.W. 1997. Biogeochemistry: An analysis of global change. Academic Press, New York.

Schulten, H-R. and Schnitzer, M., 1997. The chemistry of soil organic nitrogen: a review. *Biol. Fertil. Soils*26, 1-15.

Semrau, J. D., Dispirito, A. A., and Yoon, S. 2010. Methanotrophs and copper. *Fems Microbiol. Rev.* 34, 496-531.

Shackley, S., Carter, S., Knowles, T., Middelink, E., Haefele, S., Sohi, S., Cross, A., and Haszeldine, S. 2012. Sustainable gasification-biochar systems? A case-study of rice-husk gasification in Cambodia, Part I: Context, chemical properties, environmental and health and safety issues. *Energy Policy* 42, 49-58.

Shima, S.L. 1998. Mechanism of biological methane formation: Structure and function of methyl-coenzyme M reductase. *Prote Nucleic Acid Enzym* 43, 1461-1467.

Shrestha, M.S., Abraham, W-R, Shrestha, P.M., Noll, M., and Conrad, R. 2008. Activity and composition of methanotrophic bacterial communities in planted rice soil studied by flux measurements, analyses of *pmoA* gene and stable isotope probing of phospholipids fatty acid. *Environ. Microbiol*. 10, 400-412.

Siljanen, H.M.P., Saari, A., Krause, S., Lensu, A., Abell, G.C.J., Bodrossy, L., Bodelier, P.L.E., and Martikainen, P.J. 2011. Hydrology is reflected in the functioning and community composition of methanotrophs in the littoral wetland of a boreal lake. *FEMS Microbiol. Ecol.* 75, 430-445.

Singh, C., Tiwari, S., and Singh, J.S. 2017b. Impact of rice husk biochar on nitrogen mineralization and methanotrophs community dynamics in paddy soil. *Int. J. Pure Appl. Biosci.* 5, 428-435.

Singh, C., Tiwari, S., Boudh, S., and Singh, J.S. 2017a. Biochar application in management of paddy crop production and methane mitigation In: Agro-

Environmental sustainability: managing environmental pollution.Singh, J.S.,and Seneviratne, G. (eds.).Springer.pp. 123-146.

Singh, J.S. 2013a. Anticipated effects of climate change on methanotrophic methane oxidation. *Clim. Change Environ. Sustain.*1, 20-24.

Singh, J.S. 2015. Microbes: the chief ecological engineers in reinstating equilibrium in degraded ecosystems. *Agric. Ecosyst. Environ.* 203, 80-82.

Singh, J.S. Abhilash, P.C., and Gupta, V.K. 2016b. Agriculturally important microbes in sustainable food production, *Trend Biotechnol*. 34, 773-775.

Singh, J.S., and Gupta, V.K. 2018. Soil microbial biomass: A key soil driver in management of ecosystem functioning. *Sci. Total Environ*. 634, 497-500.

Singh, J.S., and Gupta, V.K., 2016c. Degraded land restoration in reinstating CH_4 sink. *Front. Microbiol.*7, 1-5.

Singh, J.S., and Pandey, V.C. 2013. Fly ash application in nutrient poor agriculture soils: impact on methanotrophs population dynamics and paddy yields. *Ecotoxicol. Environ. Saf.*89, 43-51.

Singh, J.S., and Strong, P.J. 2016a. Biologically derived fertilizer: a multifaceted bio-tool in methane mitigation. *Ecotoxicol. Environ. Saf.*124, 67-276.

Singh, J.S., Kashyap, A.K., and Singh, D.P. 2010. Microbial biomass C, N and P in disturbed dry tropical forest soils, India.*Pedosphere* 20, 780-788.

Singh, J.S., Kumar, A., Rai, A.N., and Singh, D.P. 2016b. Cyanobacteria: a precious bio-resource in agriculture, ecosystem, and environmental sustainability. *Front. Microbiol.* 7, 1-19.

Singh, J.S., Pandey, V.C., and Singh, D.P. 2011. Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. *Agric. Ecosyst. Environ.* 140, 339-353.

Singh, J.S., Singh, D.P., and Kashyap, A.K. 2009. Comparative account of the microbial biomass-N and N-mineralization of soils under natural forest, grassland and crop field from dry tropical region India. *Plant Soil Environ.*55, 223-230.

Singla, A., Dubey, S.K., Singh, A., and Inubushi, K. 2014. Effect of biogas digested slurry-based biochar on methane flux and methanogenic Archaea diversity in paddy soil.*Agric.Ecosyst. Environ*. 197, 278-287.

Smibert, R.M., and Krieg, N.R. 1981. General characterization. In: Manual of Methods for General Bacteriology. Gerhardt, P., Murray, R.G.E., Costillow, R.N., Nester, E.W., Wood, W.A., Krieg, N.R. and Phillips, G.B. (eds.). American Society for Microbiology, Washington, DC.pp. 409-443.

Smith, J.L., Collins, H.P., Bailey, V.L., 2010. The effect of young biochar on soil respiration. *Soil Biol. Biochem.* 42, 2345-2347.

Smith, P., D. Martino, Z. Cai, D. Gwary, H. Janzen, P. Kumar, B. McCarl, S. Ogle, F. O'Mara, C. Rice, B. Scholes, O. Sirotenko, 2007: Agriculture. In Climate Change 2007: In:Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Metz, B., Davidson, O.R., Bosch, P.R., Dave, R., Meyer, L.A. (eds.). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Soest, V.P.J. 2006. Review: Paddy straw, the role of silica and treatments to improve quality. *Animal Feed Sci. Technol.* 130, 137-171.

Sohi, S., Krull, E., Lopez-Capel, E., Bol, R. 2010. A review of biochar and its use and function in soil. *Adv. Agron.* 105, 47-82.

Sohi, S., Lopez-Capel, E., Krull, E., Bol, R. 2009. Biochar, climate change and soil: a review to guide future research. CSIRO Land and Water Science Report.

Solomon, S., Qin, D., Manning, M., Alley, R.B., Berntsen, T., Bindoff, N.L., Chen, Z., Chidthaisong, A., Gregory, J.M., Hegerl, G.C., Heimann, M., Hewitson, B., Hoskins, B.J., Joos, F., Jouzel, J., Kattsov, V., Lohmann, U., Matsuno, T., Molina, M., Nicholls, N., Overpeck, J., Raga, G., Ramaswamy, V., Ren, J., Rusticucci, M., Somerville, R., Stocker, T.F., Whetton, P., Wood, R.A., and Wratt, D. 2007a. Technical summary. In: Solomon, S.D., Qin, M,. Manning, Z., Chen, M., Marquis, K.B., Averyt, M., Tignor, H.L. (eds.), Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Solomon, S., Qin, M., Manning, Z., Chen, M., Marquis, K.B., Averyt, M.T., and Miller, H.L. 2007b. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom and New York, USA.

Songa,W., and Guo, M. 2012. Quality variations of poultry litter biochar generated at different pyrolysis temperatures. *J. Anal. Appl. Pyrol.* 94, 138-145.

Sparling, G.P., Whale, K.N., and Ramsa, A.J. 1985. Identifying the contribution from the soil microbial biomass to the extractable P levels of fresh and air dried soils. *Aust. J. Soil Res.* 23, 613-621.

Spokas, K.A., Reicosky, D.C. 2009. Impacts of sixteen different biochars on soil greenhouse gas production. *Annual Environ. Sci.*3, 179-193.

Srinivasarao, C., Gopinath, K.A., Venkatesh, G., Dubey, A.K., Harsha, W., Purakayastha, T.J., Pathak, H., Jha, P., Lakaria, B.L., Rajkhowa, D.J., Sandip, M., Jeyaraman, S., Venkateswarlu, B., and Sikka, A.K. 2013a. Use of biochar forsoil health enhancement andgreenhouse gas mitigation in India, NICRA Bulletin.

Srinivasarao, C., Vankateswarlu, B., Lal, R., Singh, A.K., Sumanta Kundu, Vittal, K.P.R., Balaguruvaiah, G., Vijaya Shankar Babu, M., Ravindra Chary, G., Prasadbabu, M.B.B., and Yellamanda Reddy, T. 2012. Soil carbon sequestration and agronomic productivity of an Alfisol for a groundnut-based system in a semiarid environment in southern India. *Eur. J. Agron.* 43, 40-48.

Srinivasarao, C.H., Venkateswarlu, B., Lal, R., Singh, A.K. and Sumanta Kundu. 2013b. Sustainable management of soils of dryland ecosystems for enhancing agronomic productivity and sequestering carbon. *Adv. Agron.* 121, 253-329.

Srivastava, S.C., and Singh, J.S. 1988. Carbon and phosphorus in the soil biomass of some tropical soils of India. *Soil Biol. Biochem.*20, 743-747.

Steiner, C., Das, K.C., Garcia, M., Forster, B., Zech, W. 2008. Charcoal and smoke extract stimulate the soil microbial community in a highly weathered xanthic Ferralsol. *Pedobiologia* 51, 359-366.

Steiner, C., Teixeira, W.G., Lehmann, J., Nehls, T., de Macêdo, J.L.V., Blum, W.E.H., Zech, W. 2007. Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered central Amazonian upland soil. *Plant Soil* 291, 275-290.

Stralis-Pavese, N., Sessitsch, A., Weilharter, A., Reichenauer, T., Riesing, J., Csontos, J. Murrell, J.C., and Bodrossy, L. 2004. Optimization of diagnostic

microarray for application in analysing landfill methanotroph communities under different plant covers. *Environ. Microbiol*. 6, 347-363.

Sugumaran, P., and Sheshadri, S. 2009. Evaluation of selected biomass for charcoal production. *J. Sci. Indus. Res.* 68, 719-723.

Suzuki, M. T., and Giovannoni, S. J. 1996. Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. *Appl. Environ. Microb*. 62, 625-630.

Svensson, B.H., and Sundh, I. 1992. Factors affecting methane production in peat soils. *Suo* 43, 183-190.

Tamai, N., Takenaka, C., and Ishizuka, S. 2007. Water soluble Al inhibits methane oxidation at atmospheric concentration levels in Japanese forest soil. *Soil Biol. Biochem.* 39, 1730-1736.

Theeba, M., Bachmann, R.T., Illani, Z.I., Husni, M.H.A., and Samsuri, A.W. 2012. Characterization of local mill rice husk charcoal and its effect on compost properties. *Malasian J. Soil Sci.*16, 89-102.

Timmusk, S., Behers, L., Muthoni, J., Muraya, A., and Aronsson, A.C. 2017. Perspectives and challenges of microbial application for crop improvement. *Front. Plant Sci.* 8, 1-10.

Tiwari, S.,Singh, J.S., and Singh, D.P. 2015. Methanotrophs and CH_4 sink: Effect of human activity and ecological perturbations. *Clim. Change Environ. Sustain.* 3, 35-50

Topp, E., and Pattey, E. 1997. Soils as sources and sinks for atmoshperic methane. *Can. J. Soil Sci.* 77, 167-178.

Van Zwieten, L.., Kimber, S., Morris, S., Chan, K.Y., Downie, A., Rust. J., Joseph, S., Cowie, A. 2010a. Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. *Plant Soil* 327, 235-246.

Van Zwieten, L., Singh, B., Joseph, S., Kimber, S., Cowie, A., and Yin Chan, K. 2009. Biochar and emissions of non-CO_2 greenhouse gases from soil. In: Biochar for Environmental Management.Lehmann, J., Joseph, S. (eds.). Earthscan: London Sterling, VA, USA.

Vance, E.D., Brookes, P.C., and Jenkinson, D.S. 1987. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.*19, 703-707.

Vantieghem, S. 2016. Carbon dioxide sequestration by means of biochar: analytical pyrolysis as a stability proxy method, faculty of bioscience engineering university of gent. Master's dissertation submitted in partial fulfilment of the requirements for the degree of Master in bioscience engineering: Chemistry and Bioprocess Technology.

Varela, M.O., Rivera, E.B., Huang, W.J., Chien, C., and Wang, Y.M. 2013. Agronomic properties and characterization of rice husk and wood biochars and their effect on the growth of water spinach in a field test. *J. Soil Sci. Plant Nutri.* 13, 251-266.

Velumurugan, A., Dadhwal, V.K., and Abrol, Y.P. 2008. Regional nitrogen cycle: An Indian perspective. *Curr. Sci.* 94, 1455-1468.

Venkataraman, C., Habib, G., Kadamba, D., Shrivastava, M., Leon, J., Crouzille, B., Boucher, O., and Streets, D. 2006. Emissions from open biomass burning in India: Integrating the inventory approach with high-resolution Moderate Resolution Imaging Spectroradiometer (MODIS) active-fire and land cover data. *Global Biogeochem. Cycles* 20, GB2013.

Verheijen, F., Jeffery, S., Bastos, A.C., vander-Velde, M., Diafas, I. 2009. Biochar application to soil- A critical scientific review of effects on soil properties, process and functions. EUR 24099 EN, office for the official publications of the European communities, Luxembourg.pp. 149.

Vimal, S.R., Singh. J.S., Arora, N.K., and Singh, S. 2017. Soil-plant-microbe interactions in stressed agriculture management: a review. *Pedosphere* 27, 177-192.

Walkley, A. 1947. A critical examination of a rapid method for determining organic carbon in soils-Effects of variations in digestion conditions and of inorganic soil constituents. *Soil Sci.*63, 251-264.

Wang, H., Lin, K., Hou, Z., Richardson, B., and Gan, J. 2010. Sorption of the herbicide terbuthylazine in two New Zealand forest soils amended with biosolids and biochars. *J. Soils Sediments* 10, 283-289.

Wang, Z.P., Delaune, R.D., Masscheleyn, P.B., Patrick, J.W.H. 1993. Soil redox and pH effects on methane production in a flooded rice soils. *Soil Sci. Soc. Am. J.* 57, 382-385

Wang, J.Y., Pan, X.J., Liu, Y.L., Zhang, X.L., and Xiong, Z.Q. 2012. Effects of biochar amendment in two soils on greenhouse gas emissions and crop production, *Plant Soil* 360, 287-298.

Ward, B., 2012. Fundamentals of Geobiology.In: Fundamentals of Geobiology. Knoll A.H., Canfield, D.E., and Konhauser, K.O. (eds). Chichester, UK: John Wiley and Sons, Ltd.pp. 36-48.

Warnock, D.D., Lehmann, J., Kuyper, T.W., Rillig, M.C. 2007. Mycorrhizal responses to biochar in soil concepts and mechanisms. *Plant Soil* 300, 9-20.

Warnock, D.D., Mummey, D.L., Major, B.J., Lehmann, J., and Rillig, M.C. 2010. Influences of non-herbaceous biochar on arbuscular mycorrhizal fungal abundances in roots and soils: results from growth-chamber and field experiments. *Appl. Soil Ecol.* 46, 450-456.

Wassmann, R., Lantin, R.S., Neu, H.D., Buendia, L.V., Corton, T.M., and Lu, Y. 2000. Characterization of methane emissions from rice fields in Asia. III Mitigation options and future research needs. *Nutr. Cycle Agroecosyst.* 58, 23-36.

Weligama, C., Sale, P.W.G., Conyers, M.K., Liu, D.L., and Tang, C. 2010. Nitrate leaching stimulates subsurface root growth of wheat and increase rhizosphere alkalization in highly acidic soil. *Plant Soil* 328, 119-132.

Whittenbury, R., Phillips, K.C., and Wilkinson, J.F. 1970. Enrichment, isolation and some properties of methane-utilizing bacteria. *J. Gen. Microbiol.* 61, 205-218.

World Meteorological Organization, 2014. WMO greenhouse gas bulletin-the state of greenhouse gases in the atmosphere based on global observations through 2013. Geneva.

Wu, F., Jia, Z., Wang, S., Chang, S.X., Startsev, A. 2013. Contrasting effects of wheat straw and its biochar on greenhouse gas emissions and enzyme activities in a chernozemic soil. *Biol. Fertil. Soils* 49, 555-565.

Wu, M., Feng, Q., Sun, X., Wang, H., Gielen, G., Wu, W. 2015. Rice (*Oryza sativa L*) plantation affects the stability of biochar in paddy soil. *Sci. Rep.*5, 1000.

Wuebbles, D.J., and Hayhoe, K. 2002. Atmospheric methane and global change. *Earth Sci. Rev.*57, 177-210.

Xiang, J., Liu, D., Ding, W., Yuan, J., and Lin, Y. 2015. Effects of biochar on nitrous oxide and nitric oxide emissions from paddy field during the wheat growth season. *J.Clean. Prod.*104, 52-58.

Xiong, Z.Q., Xing, G.X., and Zhu, Z.L. 2007. Nitrous oxide and methane emissions as affected by water soil and nitrogen. *Pedosphere* 17, 146-155.

Xu, G., Lv, Y., Sun, J., Shao, H., and Wei, L. 2012. Recent advances in biochar applications in agricultural soils: benefits and environmental implications. *Clean- Soil Air Water* 40, 1093-1098.

Xu, X., Zhao, Y., Sima, J., Zhao, L., Masek, O., and Cao, X. 2017. Indispensable role of biochar-inherent mineral constituents in its environmental applications: A review. *Bioresour. Technol.*241, 887-899.

Yadav, R.K., Yadav M.R., Kumar, R., Parihar, C.M., Yadav, N., Bajiya, R., Ram, H., Meena, R.K., Yadav, D.K., and Yadav, B. 2017. Role of biochar in mitigation of climate change through carbon sequestration. *Int. J. Curr. Microbiol. Appl.Sci.*6, 859-866.

Yagi, K., and Minami, K. 1990. Effects of organic matter application on methane emission from some Japanese paddy fields. *Soil Biol. Biochem.* 36, 599-610.

Yagi, K., Tsuruta, H., and Minami, K. 1997. Possible options for mitigating methane emission from rice cultivation. *Nutr. Cycle Agroecosyst*. 49, 213-220.

Yamamoto, N., and Lopez, G. 1985. Bacterial abundance in relation to surface area and organic content of marine sediments. *J. Exp. Mar. Bio. Ecol.* 90, 209-220.

Yamato, M., Okimori, Y., Wibowo, I.F., Anshori, S., and Ogawa, M. 2006. Effects of the application of charred bark of *Acacia mangium* on the yield of maize,

cowpea and peanut and soil chemical properties in south Sumatra, Indonesia. *Soil Sci. Plant Nutr.* 52, 489-495.

Yan, X., Shi, S., Du, L., and Xing, G. 2000. Pathways of N_2O emission from rice paddy soil. *Soil Biochem.*32, 437-440.

Yanai, Y., Toyota, K., and Okazaki, M. 2007. Effects of charcoal addition on N2O emissions from soil resulting from rewetting air-dried soil in short-term laboratory experiments. *Soil Sci. Plant Nutr.* 53, 181-188.

Yang, Y., Ma, S., Zhao, Y., Jing, M., Xu, Y., and Chen, J. 2015. A field experiment on enhancement of crop yield by rice straw and corn stalk-derived biochar in Northern China. *Sustain.* 7, 13713-13725.

Yao, H., and Conrad, R. 2001. Thermodynamics of propionate degradation in anoxic paddy soil from different rice-growing regions. *Soil Biol. Biochem*. 33, 359-364.

Yao, H., and Conrad, R. 1999. Thermodynamics of methane production in different rice paddy soils from China, the Philippines, and Italy. *Soil Biol. Biochem.* 31, 463-473.

Yargicoglu, E.N., Sadasivam, B.Y., Reddy, K.R., and Spokas, K. 2015. Physical and chemical characterization of waste wood derived biochars. *Waste Manage.*36, 256-268.

Yargicoglu, E.Y., and Reddy, K.R. 2017. Effects of biochar and wood pellets amendments added to landfill cover soil on microbial methane oxidation: A laboratory column study. *J. Environ. Manage.* 193, 19-31.

Yavitt, J.B., and Lang, G.E. 1990. Methane production in contrasting wetland sites: response to organic-chemical components of peat and to sulphate reduction.*Geomicrobiol. J.* 8, 27-46.

Yoo, G., and Kang, H. 2012. Effects of biochar addition on greenhouse gas emissions and microbial responses in a short-term laboratory experiment. *J. Environ. Qual.* 41, 1193-1202.

Zehnder AJB & Stumm W (1988) Geochemistry and biogeochemistry of anaerobic habitats.*In*: Anaerobic Microbiology.Zehnder, A.J.B. (ed.).Wiley, New York.pp. 1-38.

Zhang, A., Cui, L., Pan, G., Li, L., Hussain, Q., Zhang, X., Zheng, J., and Crowley, D. 2010a. Effect of biochar amendment on yield and methane and nitrous oxide emissions from a rice paddy from Tai Lake plain, China. *Agric. Ecosyst.Environ.* 139, 469-475.

Zhang, A., Liu, Yuming., Pan, G., Hussain, Q., Li, L., Zheng, J., and Zhang, X. 2012b, Effect of biochar amendment on maize yield and greenhouse gas emissions from a soil organic carbon poor calcareous loamy soil from Central China plain. *Plant Soil* 351, 263-275.

Zhang, H., Lin, K., Wang, H., and Gan, J. 2010b. Effect of Pinus radiate derived biochars on soil sorption and desorption of phenanthrene. *Environ. Pollut.* 158, 2821-2825.

Zhang H.Z., Chen, C.R., Gray, E.M., and Boyd, S.E. 2017. Effect of feedstock and pyrolysis temperature on properties of biochar governing end use efficacy Hanzhi. *Biomass Bioenergy* 105, 136-146.

Zhang, A., Bian, R., Pan, G., Cui, L., Hussain, Q., Li, L., Zheng, J., Zheng, J., Zhang, X., Han, X., and Yu, X. 2012a. Effects of biochar amendment on soil quality, crop yield and greenhouse gas emission in a Chinese rice paddy: A field study of 2 consecutive rice growing cycles. *Field Crops Res.* 127, 153-160.

Zhang, A., Cui, L., Pan, G., Li, L., Hussain, Q., Zhang, X., Zheng, J. and Crowley, D. 2010b. Effect of biochar amendment on yield and methane and nitrous oxide emissions from a rice paddy from Tai Lake plain, China. *Agric. Ecosyst. Environ.*139-469-475.

Zhang, Q., Dijkstra, F.A., Liu, Xr., Wang, Y., Huang, J., and Ning, L. 2014. Effects of biochar on soil microbial biomass after four years of consecutive application in the North China plain. *PLoSONE* 9, e102062.

Zhanga, A., Biana, R., Pana, G., Cuia, L., Hussaina, Q., Li, L., Jinwei, Z., Zhenga, J., Zhanga, X., Xiaojun, H., and Yua, X. 2010a. Effects of biochar amendment on soil quality, crop yield and greenhouse gas emission in a Chinese rice paddy: A field study of 2 consecutive rice growing cycles. *Field Crop Res.*127, 153-160.

Zhong, W.H., and Cai, Z.C. 2007. Long-term effects of inorganic fertilizers on microbial biomass and community functional diversity in a rice soil derived from quaternary red clay. *Appl. Soil. Ecol.* 36, 84-91.

Zhou, W., Lv, T.F., Chen, Y., Westby, A.P., and Ren, W.J. 2014. Soil Physicochemical and Biological Properties of Paddy-Upland Rotation: A Review. *Sci. World J.* 2014, 1-8.

Zhou, R.D., Coles, N., and Wu, J. 2015. Carbon mineralization following additions of fresh and aged biochar to an infertile soil.*Catena* 125, 183-189.

Zhu, Z. 1985. Advances in investigations on soil nitrogen supply and fate of fertilizer nitrogen in Soils of China. *Soils*17, 2-9.

Zimmerman, A.R. 2010. Abiotic and microbial oxidation of laboratory-produced black carbon (biochar). *Environ. Sci. Technol.* 44, 1295-1301.

Zimmerman, A.R., Gao, B., and Ahn, M. 2011. Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. *Soil Biol. Biochem.* 43, 1169-1179.

Zou, J., Huang, Y., Zheng, X., and Wang, Y. 2007. Quantifying direct N2O emissions in paddy fields during rice growing season in mainland China: dependence on water regime. *Atmos. Environ*. 41, 8030-8042.

Zwieten, V., Singh, L.B., Joseph, S., Kimber, S., Cowie, A., and Yin C.K. 2009. Biochar and emissions of non-CO_2 greenhouse gases from soil. In:Biochar for Environmental Management. Lehmann, J., and Joseph, S., (eds.). Earthscan: London Sterling, VA, USA.

Zwieten, V.L., Kimber, S., Morris, S., Chan, Y.K., Downie, A., Rust, J., Joseph, S., and Cowie, A. 2010. Effect of biochar from slow pyrolysisi of papermill waste on agronomic performance and soil fertility. *Plant Soil* 327, 235-246.