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Impact of Rice Husk Biochar on Nitrogen Mineralization and Methanotrophs Community Dynamics in Paddy Soil

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ABSTRACT

Investigations related to application of crop residues in benefit of soil fertility improvement, microbial diversity enhancement and agricultural productivity are scare. Therefore, impact of rice husk biochar (RHB) and microbial bio-formulation (CSR-BIO) on nitrogen (N) transformations and methanotrophs (CH_4 consuming bacteria) population and soil properties were examined in paddy field condition during the year 2016. In this experiment, three treatment plots (in triplicates) i.e. (a) control, (b) RHB, (c) CSR-BIO, and (d) RHB+CSR-BIO were established in completely randomized block design (RBD). Across treatments, the average N-mineralization (1.83±0.05 µg g^{-1} dry soil) and methanotrophs community (73.33 ± 9.50 × 10^5 MPN cells g^{-1} dry soil) was highest in RHB+CSR-BIO treated plots. ANOVA showed significant differences in soil properties (EC, pH, soil moisture, bulk density and WHC) due to treatments in faddy field condition. Results suggested that the application of RHB and CSR-BIO singly or in combination significantly enhance the rate of soil N-mineralization and number of CH_4 consuming bacteria as well as soil properties improvement in paddy field.

Key words: *CSR-BIO, Methanotrophs, Nitrogen mineralization, Rice husk biochar, Soil.*

INTRODUCTION

The indiscriminate growth in human population is a serious problem for future food accessibility due to poor agricultural productivity. Everyday increasing the demands of food supply but due to inadequate amount of food, most of the population has been moved to hunger crises. Mismanaged agricultural practices are responsible for the reduced productivity of agricultural yields across the world. The application of sustainable and eco-friendly soil improving materials in agriculture can be helpful strategies for increasing the production of agricultural crops. Rice husk biochar, 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.

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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) [1,2,3]. Most of the study has been conducted on the soil organic nitrogen dynamics[4,5]. However, application of rice husk biochar and its impact on N-mineralization in paddy field condition is still lacking.

Central Soil Research Bio (CSR-Bio), is a patented culture media for increasing the productivity of crops, is potential growth enhancer for sodic and normal soils based on integration of the dynamic microbial consortia[6]. 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[7]. During the paddy crop cycle more than 50% of N is absorbed by rice plants from the soil[8]. 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[9]. During the process of microbial mediated N-mineralization, the organic-N is transformed in to inorganic-N[10]. The pH and texture of the soil are important factors which affect the microbial community composition in paddy soil[11, 12, and 13]. 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 have been reported[14]. According to Prommer et al[14] 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[15] was 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[15]. The higher doses of biochar application in soil accelerated also the increment of ammonium contents in soil[16]. 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[14]. Methanotrophs, group of bacteria that utilize CH_4 as the sole carbon and energy source. So, methanotrophs can play an important role in removal of atmospheric CH_4 load[4]. 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 biochar application in combination of microbial bio-formulation-CSR-BIO (consortia of *Bacillus pumilus*, *Bacillus thurigensis,* and *Trichoderma harzianam*)[6] on soil physico-chemical properties, N-mineralization and methanotrophs abundance in paddy agriculture field conditions.

MATERIAL AND METHODS

EXPERIMENTAL SITE AND DESIGN

The present experiment was carried out at Agriculture Research Farm of Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh (India) along with the co-ordinates 26^0 46' 05.51" N Lat. and 80^0 55' 39.50" E Long, with average altitude of 100-355 msl. Experimental field was prepared after removing the plant debris and concretes from the surface during land preparation manually. Total 12 experimental plots (4 treatments × 3 replicates) each having dimension of 3×2 meter area were established in completely randomized block design (CRBD). Four treatments i.e. i) control ii) RHB, iii) CSR-BIO, and iv) RHB+CSR-BIO (each in triplicates) was applied with different doses in paddy field soil.

SOIL SAMPLING AND ANALYSIS

The soil samples, from each treatment plots, were collected in triplicates at 0, 35, 65 and 105 days after transplantation of paddy plants. The collected soil samples were transported to the laboratory for analysis of N-mineralization, methanotrophs abundance and soil physico-chemical properties affected by different treatments. 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[17]. 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; HANNA, USA) according to Pansu and Gautheyrou[18] and Nigussie et al [19]. Soil moisture was calculated by oven-drying the soil samples at 105 ^{o}C for 48 hours as suggested by Black[20]. 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[21]. Total N and P were measured according to Jackson method [22].

BIOCHAR PREPARATION

Rice husk procured from rice mill from the Lucknow area was used for the RHB production in the presence of partial supply of oxygen, called as pyrolysis (Figure 1). The pyrolysis was carried out in open environmental condition near to the experimental field site by drum method as described by Srinivasarao et al [23]. In brief a 50 kg capacity-drum was moderately filled with rice husk and heated for 30 minutes on 250 ^{0}C. Again after 40 minutes, the temperature was increased up to 300 ^{0}C for the production of precious quality of biochar[24]. After some time this the temperature was kept constant for few minutes and after 30 minutes the drum was left for cooling and dark-black colour RHB (Figure 1B) was harvest in container for the application in experimental field.

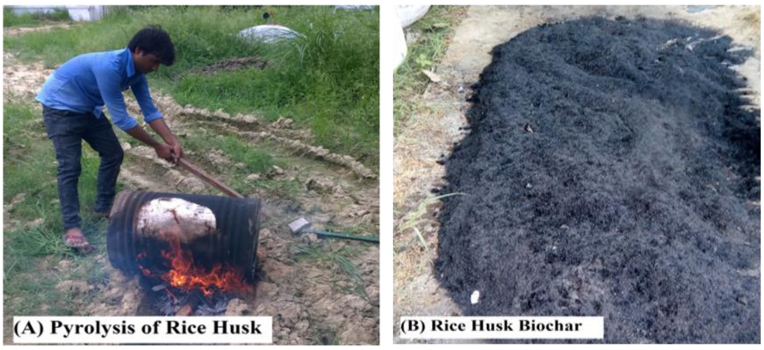

Fig. 1: Production of rice husk biochar by drum method as described by Srinivasarao *et al*. [23]

QUANTIFICATION OF SOIL N-MINERALIZATION AND METHANOTROPHS COMMUNITY SIZE

Estimation of inorganic nitrogen mineralization from organic nitrogen was done according to methods described by Hach[25] and APHA[26]. The number of methanotrophic bacteria (MB) was enumerated by the new Most Probable Number (MPN) technique as described by Saitoh et al[27] on mineral salt solid medium injecting the methane gas and air (1:1, v/v) and incubated at 30 ^{0}C in serum bottles for about 28 days. This modified MPN technique currently being used for the precise estimation of small population densities of methanotrophs for the paddy soils[28].

STATISTICAL ANALYSIS

The impact of various treatments on the soil parameters and methanotrophs community dynamics was assessed by mean, standard deviation and analysis of variance (ANOVA), using the Statistical software IBM SPSS computing version 20.

RESULTS AND DISCUSSION

PHYSICO-CHEMICAL PROPERTIES OF SOIL

The data on impact of rice husk biochar (RHB) and CSR-BIO amendment on physico-chemical properties of paddy field soil has been given in Table 1. Compared to treated plots, maximum electrical conductivity (EC) (5.5 ± 0.05µmho ms^{-1}) and pH (8.6 ± 0.25) was measured for untreated (control) plot. Among different treatments soil moisture (SM) and water holding capacity (WHC) were highest (25.60 ± 0.15 % and 77.3 ± 1.15%, respectively) in RHB + CSR-BIO treated plots and lowest (19.54 ± 0.10 % and 60.5 ± 1.00 %, respectively) in control plots. Compared to treated plots, bulk density (BD) was lowest in control plot (1.21 ± 0.03 gm cm^{-3}). ANOVA revealed that the soil physico-chemical properties 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) varied significantly due to application of various amendments (Table 1).

Due to unique physical properties (large surface area and porous internal structure) and nutrient composition, application of RHB has potential to alter the physico-chemical properties of paddy agriculture soil (Table 1). The addition of biochar has been reported to changes in soil physical and chemical properties such as BD, WHC, soil moisture, EC and pH[29], cation exchange capacity[30], accumulation[31] and soil particle spacing[32]. The changes in soil properties in this study could be due to features of RHB e.g., the surface charge, soil particle density and pore size allocation, which are dependent on the composition of feedstock and pyrolysis condition. Therefore, the soil which is directly influenced by the chemical and physical properties of biochar may ultimately affect plant-rhizospheric soil characteristics[33]. However, the relationship between biochar and the soil properties have not been intensely described yet. There is also a wide gap in our knowledge regarding interactions between the soil biota and biochar. Biochar can act as a soil refiner by improving the soil physical properties, but the exact mechanism of biochar in soil are not yet well define. This calls for standardized and key investigation related to soil-biochar dynamics to assess the potential consequences of well-known application of wonderful plant residues mediated soil fertility improvement product.

Table 1: Application of rice husk biochar (RHB) and CSR-BIO amendment on soil physico-chemical properties in paddy field. Values are means of three replicates ± SE

Treatments	**Parameters**				
	EC (µmho ms^{-1})	**pH**	**Soil moisture (%)**	**Bulk density (gm cm^{-3})**	**WHC (%)**
Control	5.5 ± 0.05	8.6 ± 0.25	19.54 ± 0.10	1.21 ± 0.03	67.3 ± 2.08
RHB	4.3 ± 0.05	6.9 ± 0.05	24.81 ± 0.04	1.13 ± 0.01	77.1 ±1.25
CSR- BIO	4.8 ± 0.05	7.3 ± 0.1	23.64 ± 0.34	1.19 ± 0.01	72.6 ± 0.57
RHB + CSR-BIO	4.4 ± 0.05	7.2 ± 0.1	25.60 ± 0.15	1.38 ± 0.44	77.3 ± 1.15
ANOVA	F=40.77, N=12, P=<0.001	F=68.03, N=12, P=<0.001	F=399.60, N=4, P=<0.001	F=5.41, N=4, P= 0.025	F=6.73, N= P=0.014

EFFECT OF BIOCHAR AND CSR-BIO ON N-MINERALIZATION AND METHANOTROPHS NUMBERS

The application of rice husk biochar treatment on nitrogen mineralization has been given in (Figure 1). Across different treatments and study days, the highest nitrogen mineralization rate 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[34]. 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.

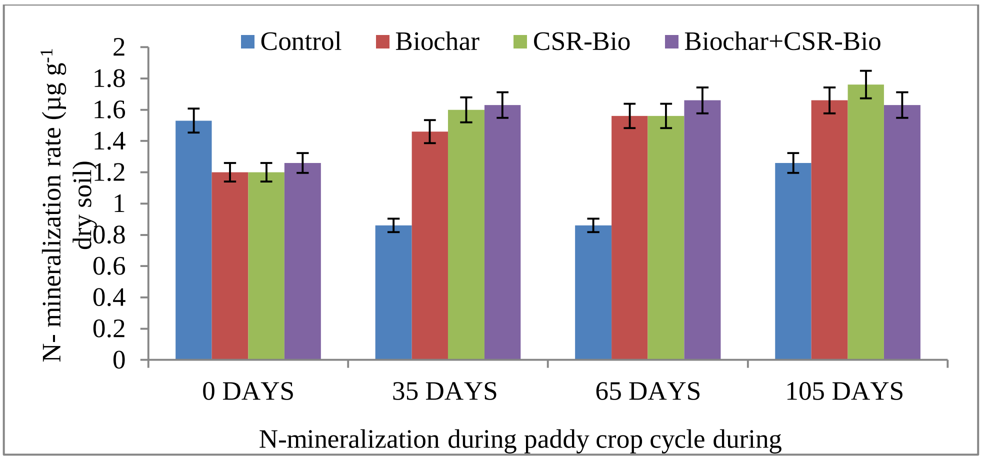

Fig. 2: Nitrogen mineralization (µg g^{-1}dry soil) at different days after rice husk biochar amendments in paddy agriculture. Values are means of three replicates ± SE

During the paddy crop cycle average methanotrophs populations were counted highest in the soil of biochar treated plots at 65 days (73.33 ± 9.50 × 10^5 MPN cells g^{-1} dry soil) compared to control (unamended) (25.33 ± 2.30 × 10^5 MPN cells g^{-1} dry soil) (Figure 2). At all the soil sampling days (0-105 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 (Figure 2). 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 contents (Table 1) that may support the growth and multiplication of bacteria[35,36,37,38]. The variations in soil physico-chemical properties due to biochar and CSR-BIO amendments could be one of the major reasons for the variations in methanotrophic community compositions across treatments. While unfavourable conditions and greater soil salinity[28, 39, 40, 41] at control plot may suppress the methanotrophs population growth. Microbial population, present in CSR-BIO formulation might create beneficial conditions to enrich the soil physic-chemical environment therefore; the number of methanotrophs might be positively influenced in nutrient rich soil.

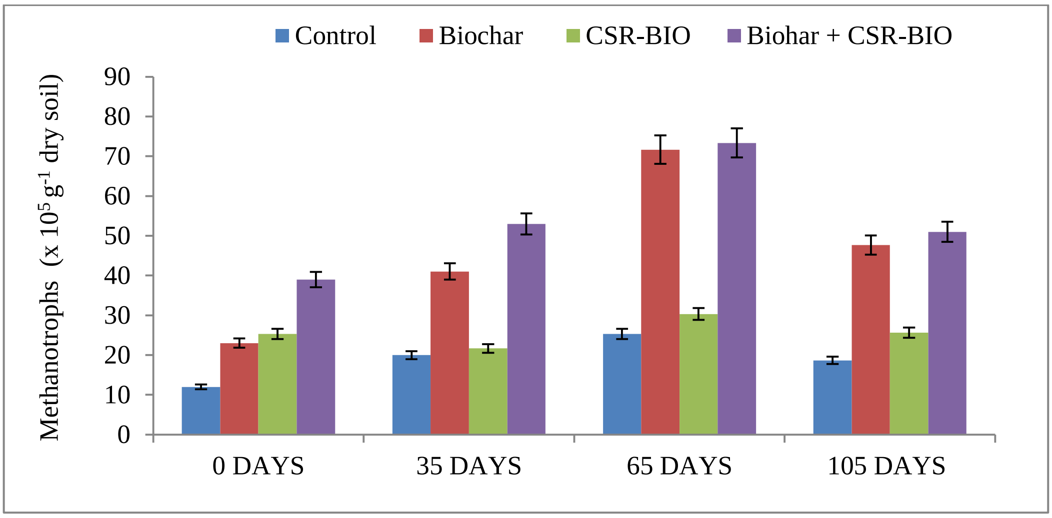

Fig. 3: Influence of biochar and CSR-BIO on methanotrophs abundance at different days during paddy cycle. Values are means of three replicates ± SE

CONCLUSION

This study demonstrates that RHB and CSR-BIO application improves the soil physico-chemical conditions that in turn enhance the rate of soil N-mineralization and number of methanotrophs community structure in paddy soil. The long term use of RHB may beneficial to enhance the number of methanotrophs in paddy field and therefore, removal of methane load from the atmosphere. 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 nitrogen transformation and beneficial microbial diversity including methane consuming bacteria of 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) and other crops residues may be used for sustainable agriculture and environment development.

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