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Research Article

MICROBIAL BIOMASS CARBON AND ORGANIC CARBON FRACTIONS IN PADDY SOIL AS INFLUENCED BY RICE STUBBLE MANAGEMENT

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Abstract- A laboratory experiment was conducted during November 2018 to April 2019 to evaluate the effects of rice stubble (RS) management practices on soil organic carbon fractions and microbial biomass carbon in a rice soil through a fifteen weeks incubation period under constant moisture regime. Untreated and glyphosate-yogurt treated rice stubble was either incorporated or left on the surface of soil-filled (15 cm depth on 5 cm sand at the bottom) poly vinyl chloride (PVC) pipe (25 cm long and 8.44 cm diameter), mounted on tray maintaining a constant water depth of 5 cm and incubated for 105 days. The evolution of CO_2, organic carbon, easily oxidizable carbon and microbial biomass carbon were monitored periodically during the experiment. Incorporation of untreated rice stubble influenced organic carbon content of soil at ninth and twelfth week of incubation. The easily oxidizable organic carbon in soil was affected neither by incorporation nor glyphosate-yogurt treatment of rice stubble. Soil microbial biomass carbon was influenced only at ninth week of incubation due to incorporation of rice stubble, with or without glyphosate-yogurt treatment.

Keywords- *Carbon mineralization, Soil microbial biomass carbon, Rice stubble, Paddy soil*
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Introduction

Rice is the major source supplying about 40 percent and 80 percent of the food energy and protein requirements, respectively for several South and South east countries of Asia and Pacific region. The huge quantity of rice stubble available after harvest of winter rice crop in the state of Assam mostly remains unutilized, and their efficient recycling may provide substantial amounts of nutrient to soil [1]. Composting of rice stubbles in situ through aerobic decomposition is cumbersome [2] and energy consuming process. Rice straw co-composting with poultry manure was shown to reduce carbon loss [3]. In situ decomposition of rice stubbles after harvest of winter rice could be accelerated by spraying glyphosate as a mixture with cellulose degrading microbe [4] or commercial yogurt culture [5, 6], which otherwise is slow and long process without increase in ambient temperature and rainfall. Increased grain yield of succeeding crop was reported when crop residues, treated with cellulose degrading bacterial culture, were incorporated into the soil [7]. Spraying of commercially available yogurt or CDM culture with glyphosate reduced dry weight and C:N ratio of rice stubble [5,8].

Crop residue input was a primary factor for stabilizing the soil carbon [9]. The rate of residue decomposition depends on the amount of the crop residue retained in the soil, the characteristics of the soil and the composition of the crop residues used [10]. The equilibrium level of soil organic carbon depends upon the equilibrium between the input in the soil as organic residues or other biomass and output from the soil by degradation, leaching and erosion [11]. A four months incubation study revealed that the carbon mineralized during the period increased with temperature, and the mineralizable carbon pool varied for different soils [12]. The decomposed carbon is either mineralized as CO_2 or assimilated by the soil microflora, consequently leading to humification and secondary carbon mineralization [13]. A strong positive correlation between the CO_2 evolution and the field water content of the soils was reported [14, 15]. Paddy soils had been reported to have a greater potential of carbon sequestration than the upland cultivated soils and to experience remarkable carbon enrichments with time [16, 17]. The soil organic carbon sequestration is influenced by microbial population, moisture and temperature fluctuation in the soil [18]. The rice soils undergoing alternate wetting and drying cycle are unique in terms of net carbon mineralization [19], and stubble addition than tillage system had greater effects on soil microbial properties [20-23]. Accordingly, a laboratory incubation study was carried out to evaluate the effect of rice stubble management practices on carbon mineralization and microbial properties in a rice soil.

Materials and Methods

Soil and climate

The present investigation was carried out during November 2018 to April 2019 at Assam Agricultural University (26°44'N, 94°10'E and 91 m above MSL), Jorhat (Assam), India. The daily temperature of Jorhat decreases from November to January and then increases from February to April with an average maximum temperature of 28°C in November to 23°C in January, and then 24°C in February to 28°C in April, and with an average minimum temperature of 16°C in November to 8°C in January, and thereafter 13°C in February to 19°C in April. Bulk surface (0-15 cm) soils were collected during November 2019 from field after harvest of winter rice crop. The collected soils were air dried and ground to pass through 2 mm sieve and the processed soil was used for the incubation experiment. Rice stubbles were sprayed in the field as per treatment and both the treated and untreated stubbles were collected one hour after spray for incubation study. The soil for the experiment had a sandy clay loam texture with 56.1 percent sand, 25.1 percent clay having bulk density and particle density of 1.39 and 2.36 Mg/m³, respectively. The soil had total porosity of 41.1 percent, maximum water holding capacity of 43.1 percent and field capacity moisture content of 21.6 percent (w/w). The pH of the soil was 4.6 with exchangeable acidity, total acidity and total potential acidity fractions as 0.55, 3.41 and 18.8 c mol (p^+)/kg, respectively. The lime requirement (to raise the pH to 6.4) of the soil in terms of $CaCO_3$ was 11.9 t/ha. The cation exchange capacity of the soil was 5.46 c mol (p^+)/kg soil, and exchangeable Al^{3+} content was 0.45 c mol (p^+)/kg soil. The other exchangeable cations contents were 0.17, 0.23, 1.12, 0.78 and 0.11 c mol (p^+)/kg soil for K+, NH_4+, Ca^{2+}, Mg^{2+} and Na+, respectively with a base saturation of 38.6 percent.

Experimental set up

The incubation was carried out using 25 cm long poly vinyl chloride (PVC) hollow pipe, the bottom of which was temporarily closed by fixing a woven stainless wire cloth (diameter ≤ 0.2 mm) with rubber and adhesive tape. Each PVC pipe (internal diameter 8.44 cm and wall thickness 0.28 cm) was filled with sand up to 5 cm from the bottom [Fig-1], followed by the processed soil to a thickness of 15 cm maintaining the dry bulk density of the soils, estimated earlier during collection of the samples. The soil-filled PVC pipes were mounted in a 5.5 cm high plastic tray and required mass of rice stubble was applied to each column as per the treatments and incubated for 105 days. A water level of 5 cm thickness was maintained in the plastic tray throughout the incubation period.

Treatments and experimental design

A mixture of glyphosate (2.05 g/L a.i.) and edible yogurt (5 g/L) in water was freshly prepared and used as spray solution [5, 6]. Glyphosate [N-(phosphonomethyl) glycine, C3H8NO5P] is a non-selective herbicide with a water solubility of 12 g/L at 25 0C. The edible yogurt was collected from the local market and used for the spray. The spray was done on 20-12-2019 using a manual operated knapsack sprayer fitted with hollow cone nozzle, with a spray volume of 550 litre per hectare. After the spray the stubble was kept for one hour in the field before collection for laboratory incubation. Both the treated and untreated rice stubbles were collected one hour after spray from the field, immediately chopped into small pieces (2.0 to 2.5 cm) and added to the soil columns as per treatments. Accurately weighed 4.0 gram of fresh biomass (with 60.4% moisture content, w/w) was added to respective soil column for treated and untreated rice stubbles. The mass of rice stubble to each soil column was calculated on the basis of surface area of the PVC pipe and average dry weight of stubbles in the field per unit area taking five random samples using a 1m x 1m quadrate. Five treatments were imposed to respective columns and comprised of T1 – without rice stubble (RS), T2 - RS untreated and retained on the surface, T3 - RS untreated and incorporated into soil, T4 - RS treated (glyphosate+yogurt) and retained on the surface and T5 - RS treated (glyphosate+yogurt) and incorporated into soil. Five sets of the columns in a completely randomized design with four replications were incubated up to 105 days of imposition of the treatments.

Sampling and soil analysis

The CO_2 evolution from the soil column was collected weekly for the first 6 weeks, and thereafter every third day up to 105 days after treatments. The CO_2 evolution during incubation absorbed in 15 ml 0.1 M NaOH solution kept on the soil surface and covered with a 50 ml beaker until the sampling date, followed by titration of excess NaOH against 0.1 M HCl solution [24]. The volume of NaOH used for absorption of CO_2 was estimated, the volume of CO_2 evolved during the period was calculated and expressed as CO_2-C mg/kg soil. One of the several sets maintained for the experiment was dismantled periodically for analysis of soil properties at each of the 14th, 28th, 42th, 56th and 70th day of imposition of the treatments. The organic carbon content was determined by wet oxidation method [25]. Soils were centrifuged after adding 0.333 M $KMnO_4$ solution and easily oxidizable organic carbon was estimated colorimetrically [26]. The microbial biomass carbon (MBC) of the soil samples was determined using the chloroform fumigation-extraction method [27]. Fumigated and unfumigated samples were extracted respectively with 100 ml of 0.5 M K_2SO_4 for 1 hour. The MBC was expressed as the difference in carbon between the unfumigated and fumigated samples [28]. A one-way ANOVA was carried out to compare the means of the different treatments. When significant F-values were detected, the differences between individual means were tested using the least significant difference (LSD) test.

Results and Discussion

The soil moisture content was monitored periodically and the values varied from 27.2 to 31.6 percent (w/w) during the incubation period. The gravimetric moisture content of soils was not statistically significant among the treatments and across the sampling dates.

Carbon mineralization in soil at different stages of incubation

CO_2-C evolution from soil

The CO_2 evolution from soil increased up to 60 days of incubation due to addition of rice stubble, except for incorporation of RS with or without gluphosate-yogurt treatment [Fig-1]. In soil incorporated with gluphosate-yogurt treated RS the CO_2-C evolution reached peak at about 50 days after incubation, remained constant till 65 days after incubation and thereafter continued to decrease till the end of the incubation period. The CO_2 evolution from soil with incorporation of untreated RS reached maximum at about 56 days after incubation and decreased thereafter. In well-aerated soils, saccharides emanating from cellulose degradation are oxidized to carbon dioxide [29]. Variation in rate of CO_2 evolution was earlier reported by several workers [30-32]. Addition of straw significantly increased CO_2 emission from soil compared to soil without rice stubble. About four folds increase in CO_2 emission from soil with rice straw than that without it was reported under flooded condition [33]. However, the rate of straw addition was about 3.5 times more than that followed in the present work.

Faster decomposition of rice straw treated with microbe culture was earlier reported [34]. The efficiency of decomposition process in treated and incorporated rice stubble was due to their synergistic activity of the inoculated and native microbes leading to effective growth, biological processes and enzyme activities [35]. The compatible relationship among different strains of microbes forms a basis for stability and efficiency [36]. The organic residue degrading ability of microorganisms is dependent upon structure and production skills of the community [37] and the capability of such consortium to grow in organic residues of low nutrient concentration had been reported [38]. Incorporation of rice stubble treated with yogurt increased the microbial community diversity facilitating synergistic biological processes and enhanced decomposition compared to untreated incorporated or treated unincorporated stubbles. The reduction in acid detergent fibre, neutral detergent fibre and crude fibre was ascribed for effective decomposition of rice straw inoculated with cellulose degrading microbe [34].

Soil organic carbon, easily oxidizable C and microbial biomass C

The soil organic carbon content significantly increased after 63 days of incorporation of glyphosate-yogurt treated rice stubble compared to soil without rice stubble addition and without incorporation of untreated stubble [Table-1]. The highest organic carbon content was observed for incorporation of glyphosate-yogurt treated rice stubble and the lowest value was recorded in soil without rice straw at all the stages of the incubation. The organic carbon content of soil was not affected by the treatments up to 42 days after incubation. Significantly higher organic carbon content of soil was recorded in soil with incorporation of glyphosate-yogurt treated rice stubble over that with rice stubble removed and that with untreated rice stubble left on the surface at 63 to 105 days of incubation. The easily oxidizable organic carbon in soil for different treatments and stages of incubation is presented in [Table-2].

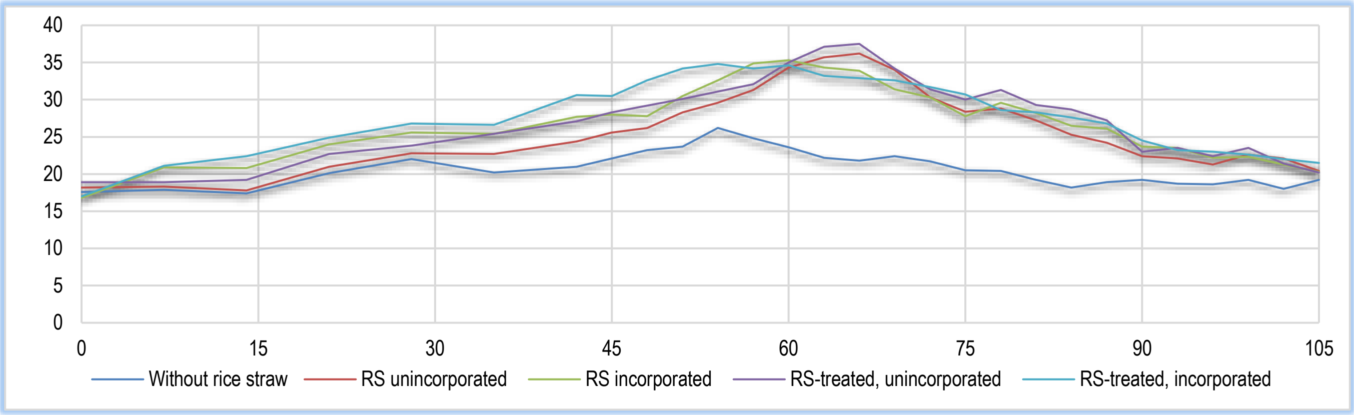

Fig-1 Influence of management on CO_2-C (mg/kg soil) evolution from soil at different stages of incubation

Table-1 *Soil organic carbon content at different days of the incubation*

Treatments	Days after incubation (g/kg soil)				
	21	42	63	84	105
Without rice straw (RS)	6.30	6.60	6.94	7.13	7.05
RS unincorporated	6.68	6.56	6.71	7.35	8.03
RS incorporated	6.60	6.98	7.46	8.38	8.70
RS treated, unincorporated	7.05	7.05	7.39	7.80	8.18
RS-treated, incorporated	7.13	7.24	7.69	8.25	8.93
$LSD_{P=0.05}$	NS	NS	0.70	0.88	0.87
CV %	6.4	5.4	6.1	7.6	6.8

The highest value was recorded for incorporation of untreated rice stubble at 105 days and the lowest in soil without rice stubble at 42 days after incubation. The easily oxidizable organic carbon in soil was unaffected by the treatments at 21 days after incubation. Incorporation of glyphosate-yogurt treated rice stubble resulted in significantly higher easily oxidizable organic carbon in soil compared to leaving untreated rice stubble on the surface at 63, 84 and 105 days of incubation. At 105 days of incubation, incorporation of untreated rice stubble and unincorporated glyphosate-yogurt treated rice stubble showed statistically higher easily oxidizable organic carbon in soil over leaving untreated rice stubble on the surface.

Table-2 *Easily oxidizable organic carbon in soil at different days of the incubation*

Treatments	Days after treatment (mg/kg soil)				
	21	42	63	84	105
Without rice straw (RS)	260.0	233.3	246.7	264.2	253.3
RS unincorporated	253.3	266.7	273.3	296.7	299.2
RS incorporated	273.3	290.0	293.3	333.3	366.7
RS treated, unincorporated	280.0	273.3	306.7	340.0	346.7
RS-treated, incorporated	273.3	296.7	343.3	363.3	353.3
$LSD_{P=0.05}$	NS	35.8	41.1	46.3	39.9
CV %	7.7	8.6	9	9.7	7.5

The microbial biomass carbon in soil was affected by rice stubble addition only at 42 and 63 days after incubation [Table-3]. Incorporation of rice stubble, irrespective of yogurt treatment, significantly increased the microbial biomass carbon in soil compared to leaving the residues on the surface. The easily oxidizable carbon in soil increased significantly due to incorporation of rice stubble after 14 days till the 105 days of incubation.

Table-3 *Microbial biomass carbon in soil at different days after treatment*

Treatments	Days after treatment (mg/kg soil)				
	21	42	63	84	105
Without rice straw (RS)	169.8	175.9	172.8	180.2	180.7
RS unincorporated	174.4	177.7	171.6	188.2	180.6
RS incorporated	173.3	194.8	194.8	196.5	185.8
RS treated, unincorporated	179.9	178.3	182.9	186.3	184.9
RS-treated, incorporated	177.8	197.2	204.9	191.3	188.4
$LSD_{P=0.05}$	NS	15.6	17.7	NS	NS
CV %	8.3	6.7	6.3	6.9	7.6

Increase in soil organic carbon and microbial biomass carbon after 15 days of 1.5 % rice straw addition was reported [31], irrespective of soil moisture content. The relatively delayed effect on soil organic carbon in the present investigation was due to much lower rate (0.2 %) of rice stubble addition. Soil organic carbon deposition in the plough layer to the tune of 0.36 t/ha, through addition of 4.5 t/ha rice straw each season, was possible in short-term experiment [39, 40]. Similarly, increase in soil microbial biomass carbon with straw application under aerobic condition was observed [41]. Higher microbial biomass carbon assimilation from added organic residues was observed in low fertility soil than in medium or high fertility soil [42]. The fertility status of the soil used in the present investigation was medium in available potassium, marginally medium in nitrogen and low in phosphorous. Thus, the microbial biomass carbon was significantly affected up to 63 days of incubation without any effect thereafter possibly due to improvement in available nutrient status and increase in organic carbon content beyond this period. During the initial period (up to 63 days) of incubation, growth of microorganisms, specialized in utilizing fresh organic matter, increased and used rice stubble as substrate [43, 44], while beyond this period the population growth reduced with increase in soil organic matter and available nutrient contents [(45].

Conclusion

Incorporation enhanced decomposition of rice stubble under constant moisture regime. The effect of treating rice stubble with cellulose degrading microbe culture (commercial yogurt) was not significant, except for microbial biomass carbon during first two months and easily oxidizable carbon in soil.

Application of research: Study of paddy soil as influenced by rice stubble management

Research Category: Soil Science

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Study area / Sample Collection: Assam Agricultural University, Jorhat, 785013, Assam, India

Cultivar / Variety / Breed name: Rice

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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